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Open Access Review Article

Antibacterial and Antifungal Activity of Ashwagandha (*Withania somnifera* L.): A review

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ABSTRACT

Approaches for studying antimicrobial susceptibility and discovering new antimicrobial agents from the plants and other natural sources have been extensively utilized. *Withania somnifera* (L.) Dunal, commonly known as Ashwagandha or Indian ginseng or winter cherry, is a popular medicinal plant in Ayurvedic medicine. The principal active compounds include several withanolide-type compounds. Various plant parts, like roots and less often leaves and fruits of Ashwagandha, have been used as plant-derived medicines. The plant possesses various pharmacological activities including antimicrobial activity. Many bacterial and fungal species have been used as a test microorganism for the assessment of the antimicrobial activity of extracts and purified compounds of various plant parts of Ashwagandha. In this article, we tried to compile and to discuss the information about the antimicrobial activity of *W. somnifera*. This 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.

Keywords: Antibacterial activity, Antifungal activity, Antimicrobial activity, Ashwagandha, *Withania somnifera*

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INTRODUCTION

At present, microbial infections have turned out to be an important clinical warning, with significant allied sickness and death, which is mainly due to the increase in microbial resistance to the prevailing antimicrobial agents[1,2]. Hence, approaches for antimicrobial susceptibility analysis and discovering new antimicrobial agents have been extensively utilized and continue to be developed[3].

After the revolution in the 'golden era', when nearly all groups of important antibiotics (cephalosporins, tetracyclines, aminoglycosides 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. Currently, its effect is considerable with treatment failures related to the multidrug-resistant bacteria and it has become a worldwide alarm to public health[4]. For this reason, the discovery of new antibiotics is an absolutely essential objective. Natural products, mostly plants are still one of the chief sources of new drug molecules today[5,6]. Microbial and plant products occupy the main part of the antimicrobial compound identified and discovered till date. Plants and other natural resources can deliver an enormous range of complex and structurally different compounds. Recently, many researchers have focused on the investigation of plant and microbial extracts, essential oils, pure secondary metabolites and newly synthesized molecules as probable antimicrobial agents[7-10]. The fact that a plant extract shows antimicrobial activity is of concern, but this primary part of the data should be reliable and let researchers to relate results, avoiding work in which researchers use the antimicrobial activity study only as a supplement to a phytochemical analysis[3].

Various methods are used for the assessment of the antimicrobial potential of plant extracts, essential oils and other antimicrobial agents which include, agar disk-diffusion method, antimicrobial gradient method, agar well diffusion method, agar plug diffusion method, cross streak method, poisoned food method, thin-layer chromatography (TLC)–bioautography (agar diffusion, direct bioautography, agar overlay bioassay), dilution methods (broth dilution method,

agar dilution method), time-kill test (time-kill curve), ATP bioluminescence assay, flow cytofluorometric method[3]. A variety of laboratory methods can be used to screen or evaluate the *in vitro* antimicrobial activity of a pure compound or a plant extract. The widely used and basic methods are the disk/disc diffusion method and broth/agar dilution methods. Other methods are used especially for the testing of antifungal activity, like, poisoned food technique[11]. To further study the antimicrobial effect of an agent in depth, Time-kill test and flow cytofluorometric methods are endorsed for further in depth study of the effect of antimicrobial agents, which provide details on the nature of the inhibitory effect (bacteriostatic or bactericidal) (concentration-dependent or time-dependent) and the cell damage imposed to the test microorganism[3].

ASHWAGANDHA: AN IMPORTANT MEDICINAL PLANT

Withania somnifera (L.) Dunal, commonly known as Ashwagandha or Indian ginseng or winter cherry, is a renowned medicinal plant in Ayurvedic medicine[12]. The principal active compounds include several withanolide-type compounds[13,14]. Due to the nonhazardous and great medicinal value, it is commonly used all over the world. Roots, and less often leaves and fruits, have been used as phytomedicines in the form of decoction, infusions, ointment, powder, and syrup[13-15]. These days, it is cultivated as a crop to maintain the high demand of biomass and a sustainable eminence for the requirements of pharmaceutical industry[16].

Ashwagandha is an important herb in the Ayurvedic and indigenous medicine system for over 3000 years. It belongs to the family *Solanaceae* and possess a chromosome number of 2n=48. In the India, only two species of *Withania* are found which includes *W. somnifera* and *W. coagulans*[17]. This plant has been used as a home remedy for numerous diseases in the India and many parts of the world. It is found in the wild form in many parts of the India and in the Mediterranean region of North Africa. In the India, it is grown in Rajasthan, Madhya Pradesh, Himachal Pradesh, Punjab and Uttar Pradesh[17]. It is designated as an herbal tonic and health food in the Vedas and is considered as 'Indian ginseng' in the conventional Indian medicine. It is utilized as a liver tonic, anti-inflammatory, antioxidant, antimicrobial agent and cure for asthma[18]. Withaferin-A has been receiving a good deal of attention because of its antibiotic and antitumor activity[19].

In Unani system of medicine, roots of *W. somnifera* usually known as Asgand are utilized for the medicinal properties[20]. In Ayurveda, Ashwagandha is claimed to have effective aphrodisiac rejuvenating and life extending properties. It has overall animating and regenerative abilities and is used among others, for the treatment of nervous exhaustion, insomnia, memory related conditions, skin problems, tiredness potency issues and coughing. It also increases learning capability and memory capacity. The traditional use of Ashwagandha was to escalate energy, youthful vigor, strength, endurance, health, increase vital fluids, nurture the time elements of the body, muscle fat, lymph, blood, cell production and semen. It helps counteract chronic fatigue, dehydration, weakness, loose teeth, bone weakness, impotency, thirst, premature aging emaciation, muscle tension, debility and convalescence. It aids invigorate the body by rejuvenating the reproductive organs, just as a tree is invigorated by feeding the roots[21].

CHEMICAL CONSTITUENTS

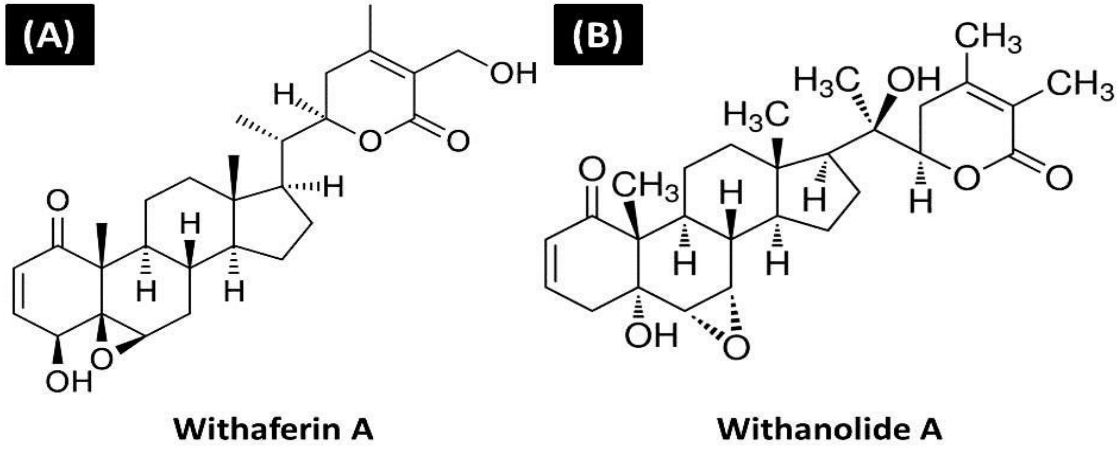

Figure 1: Chemical structure of (A) Withaferin A and (B) Withaolide A

The chemical constituents of *W. somnifera* have always been of great interest to the scientific community. The biologically active chemical constituents are alkaloids (ashwagandhine, anahygrine, cuscohygrine, tropine etc), steroidal compounds i.e. withaferin A, withasomniferin A, ergostane-type steroidal lactones, withanolides A-Y, withasomniferols A-C, withasomidienone, withanone etc[22]. Withaferin A (Figure 1A), and withanolide A (Figure 1B) are the chief withanolidal active components isolated from the plant. These compounds are chemically similar but varied in their chemical constituents[23].

PHARMACOLOGICAL ACTIVITIES OF ASHWAGANDHA

W. somnifera possesses various pharmacological activities (Figure 2) viz., anti-inflammatory activity, antibacterial activity, antifungal activity, antiviral activity, antitumour activity, immunomodulatory activity, anti-stress/adaptogenic activity, anticonvulsant activity, neuropharmacological activity, musculotropic activity, anti-oxidant activity, anti-ageing effect, anti-hyperglycaemic effect, macrophage-activating effect, hepatoprotective activity, morphine tolerance and dependence-inhibiting effect[20].

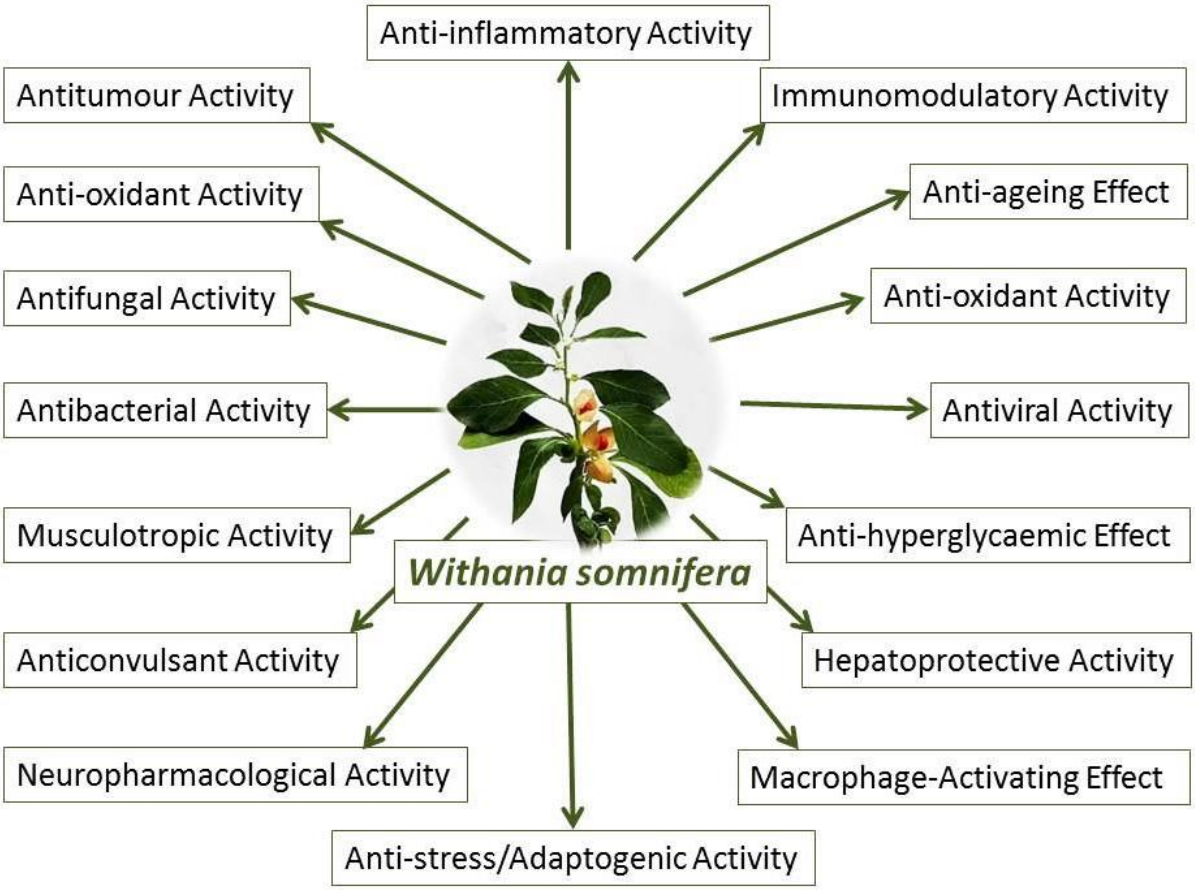

Figure 2: Pharmacological activities of Ashwagandha (*W. somnifera*)

Antibacterial activity of Ashwagandha

Many bacterial species have been used as a test microorganism for the assessment of the antimicrobial activity of extracts and purified compounds of *W. somnifera*. These bacterial strains were *Acinetobacter baylyi, Agerobacterium tumefaciens, Bacillus cereus, Bacillus subtilis, Bacillus thuringiensis, Chlamydophila pneumonia, Citrobacter freundii, Corynebacterium diphtheriae, Enterobacter aerogens, Enterococcus feacalis, Escherichia coli, Klebsiella pnemoniae,* Lactic acid bacterial (LAB) strains, Methicillin Resistance *Staphylococcus aureus, Micrococcus luteus, Neisseria gonorrhea, Proteus mirabilis, Proteus solanacearum, Proteus vulgaris, Pseudomonas aeruginosa, Pseudomonas fluorescens, Raoultella planticola, Salmonella typhi, Salmonella typhimurium, Serratia marcescens, Staphylococcus aureus, Staphylococcus epidermis, Streptococcus pyogenes, Xanthomonas axonopodis* pv. *malvacearum, Yersinia enterocolitica* and few others. The detailed compilation of information regarding the antibacterial activity of *W. somnifera* is given in Table 1.

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	*Salmonella typhimurium*, *Escherichia coli*	24
2.	Ro	E	AWD	*Salmonella typhimurium*	25
3.	Le	M	AWD	*Neisseria gonorrhea*	26
4.	Le	M	DD	*Bacillus subtilis*, *Escherichia coli*, *Pseudomonas fluorescens*, *Staphylococcus aureus*, *Xanthomonas axonopodis pv. malvacearum*	27
5.	Ro	M	DD	*Escherichia coli*, *Proteus vulgaris*, *Proteus solanacearum*	28
6.	Ro, St, Le, Ca	C	DD	*Escherichia coli*, *Staphylococcus aureus*, *Raoultella planticola*	29
7.	Ro	M, E, BU	AWD	*Staphylococcus aureus*	30
8.	Le	E	AWD	*Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa*	31
9.	Ro, Fr, Le	M	AWD	*Escherichia coli*, *Salmonella typhi*, *Citrobacter freundii*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia*	32
10.	Le, Fl	E, A, IP, T, H	DD	*Escherichia coli*, *Staphylococcus aureus*, *Raoultella planticola*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Enterobacter aerogens*	33
11.	Fr, Ca	B, C, W	DD	*Pseudomonas aeruginosa*, *Bacillus subtilis*, *Enterobacter aerogens*	34
12.	Le	E	AWD	*Escherichia coli*, *Micrococcus luteus*, *Pseudomonas aeruginosa*, *Bacillus cereus*, *Klebsiella pneumonia*, *Staphylococcus aureus*	35
13.	St, Ro, Le	A	DD	*Bacillus subtilis*, Methicillin Resistance *Staphylococcus aureus*, *Streptococcus pyogenes*, *Enterococcus feacalis*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pnemoniae*	36
14.	Ro	H, PE, T, B, IP, C, EA, A, E, GAA, W	DD	*Proteus mirabilis*, *Klebsiella pnemoniae*, *Agerobacterium tumefaciens*	37
15.	Ro	EA	AWD	*Staphylococcus aureus*, Methicillin Resistance *Staphylococcus aureus*	38
16.	Ro	E	DD	*Bacillus subtilis*, *Escherichia coli*, *Klebsiella pnuemoniae*, *Pseudomonas aeruginosa*, *Salmonella typhi*	39
17.	Le	M	AWD	*Staphylococcus aureus*, *Enterococcus spp.*	40
18.	St	EA, H, M, DW	AWD	*Serratia marcescens*, *Bacillus cereus*, *Pseudomonas aeruginosa*,	18

				Escherichia coli	
19.	Ro	W	TFSD	*Escherichia coli*	41
20.	Ro	M	AWD	*Staphylococcus aureus,* *Bacillus cereus,* *Micrococcus luteus,* *Escherichia coli,* *Pseudomonas aeruginosa,* *Klebsiella pneumonia*	42
21.	Ro	M	DD	*Bacillus cereus,* *Staphylococcus aureus,* *Staphylococcus epidermis,* *Salmonella typhi,* *Escherichia coli,* *Pseudomonas aeruginosa*	43
22.	Le	E, M, A, W	AWD	*Bacillus cereus,* *Serratia* sp.	44
23.	Le	C	DD	*Corynebacterium diphtheriae,* *Pseudomonas aeruginosa,* *Bacillus thuringiensis,* *Corynebacterium diphtheria,* *Salmonella typhi,* *Chlamydophila pneumoniae*	45
24.	Le	M	AWD	*Bacillus subtilis,* *Escherichia coli,* *Staphaylocuccus aureus,* *Pseudomonas fluorescens*	46
25.	Le	M	AWD	Lactic acid bacterial (LAB) strains	47
26.	Ro	M, DIW, C, H, EA	DD	*Proteus mirabilis,* *Klebsiella pneumonia,* *Acinetobacter baylyi,* *Streptococcus pyogenes,* *Pseudomonas aeruginosa*	48
27.	Le	E, C+H	DD	*Escherichia coli*	49

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, PE: Petroleum Ether, W: Water, AWD: Agar Well Diffusion, DD: Disc Diffusion, TFSD: Two-Fold Serial Dilutions, Ca: Calyx, Fr: Fruit, Le: Leaves, Fl: Flower, Ro: Root, St: Stem

Various plant parts viz., calyx, fruit, leaves, flower, root and stem of Ashwagandha were used by researchers in the past, but leaves and roots were used in most of the studies. Different solvents like, acetone, benzene, butanol, chloroform, chloroform+hexane, deionised water, diethyl ester, distilled water, ethanol, ethyl acetate, glacial acetic acid, hexane, isopropanol, methanol, petroleum ether, toluene and water have been used for the extraction of chemical constituents from various plant parts of Ashwagandha but methanol was the mostly used solvent for the extraction. From the literature survey, we have found that disc diffusion method was the most preferred method for the evaluation of the antimicrobial efficacy of Ashwagandha plant extracts.

Antifungal activity of Ashwagandha

In the past, antifungal activity activity has been evaluated for various extracts of different plant parts of Ashwagandha. The detailed information on the antifungal activity of Ashwagandha is provided in the Table 2. Many test fungal species including, *Alternaria brassica, Aspergillus flavus, Aspergillus fumigatus, Aspergillus niger, Aspergillus oryzae, Candida albicans, Candida kefy, Candida tropicalis, Cryptococcus neoforman, Dreschlera turcica, Fusarium oxysporum* f. sp. *cepae, Fusarium oxysporum, Fusarium verticilloides, Penicillium chrysogenum, Penicillium citrinum* and *Trichoderma viridae* were used for the assessment of the antifungal activity of Ashwagandha.

Table 2: Antifungal activity of Ashwagandha (*W. somnifera*)

Sr. No.	Plant part used	Solvent used for extraction	Method used	Test microorganisms	References
1	Le	M	DD	*Aspergillus flavus,* *Dreschlera turcica,* *Fusarium verticillioides*	27
2	Le	M	DD	*Aspergillus fumigatus,* *Aspergillus niger*	50
3	Ro	PG	DD	*Fusarium oxysporum*, *Fusarium verticilloides,* *Aspergillus flavus*	51
4	Le, Fl	E, A, IP, T, H	DD	*Candida albicans,* *Aspergillus flavus*	33
5	Fr, Ca	B, C, W	DD	*Candida albicans*	34
6	Le	E, M, EA, A, C, PE, H, W (Hot)	AWD	*Aspergillus niger,* *Candida albicans,* *Candida tropicalis,* *Candida kefy,* *Cryptococcus neoforman*	35
7	St	H, EA, M, W	AWD	*Aspergillus niger,* *Aspergillus flavus,* *Aspergillus oryzae,* *Penicillium chrysogenum,* *Trichoderma viridae*	18
8	Ro	M, W	AWD	*Candida albicans*	26
9	Fr, Ca	B, C, W	DD	*Aspergillus niger*	34
10	Ro	H, PE, T, B, IP, C, EA, A, E, GAA, W	DD	*Aspergillus niger*	37
11	Ro	H	DD	*Fusarium oxysporum,* *Alternaria brassica*	28
12	Le	E, M, A, W (hot & old)	PFT	*Aspergillus flavus,* *Penicillium citrinum*	44
13	Ro	E	DD	*Candida albicans*	52
14	Fr, St, Ro	M	DD	*Fusarium oxysporum* f. sp. *cepae*	53

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

Various plant parts viz., calyx, flower, fruits, leaves, root and stem were used for the antifungal activity assessment. Mostly used plant part was the root of Ashwagandha. Acetone, benzene, chloroform, ethanol, ethyl acetate, glacial acetic acid, hexane, isopropanol, methanol, petroleum ether, toluene and water (hot and cold) were used as a solvent for the extraction procedure to evaluate antifungal activity of various parts of Ashwagandha. However, methanol was the most preferred solvent for the extraction of phytochemicals from parts of Ashwagandha. Like antibacterial activity assessment, most chosen method for the evaluation was the disc diffusion method. However, agar well diffusion method and poison food technique were also used for the evaluation.

Some common concerns must be established to evaluate the antimicrobial activity of plant extracts, essential oils and the isolated/extracted compounds from them. The greatest relevance is the characterizing and defining common factors, such as plant parts used, methods employed, growth medium and test microorganisms evaluated (Rios and Recio, 2005). Systematic standards should be used in the selection and collection of the plant parts/materials. Moreover, to avoid unnecessary exercise, the selection of plants and plant parts should be made from an ethnopharmacological perception. The solvent systems and the extraction procedure may alter the final outcome of the study. The solvents and methods for extraction used in folk medicine should be used as they are most appropriate. The active chemical constituents are more soluble in some solvents, which should be used as it may affect the results. The crude extract or essential oil offers variable results as they contain different phytochemicals present in them. The presence of such active phytochemicals depends on their solubility in the solvents used. Sometimes, the presence of phenolic, carboxylic compounds or other impurities in the extract may affect the activity of the active phytochemicals. The experiments may be carried out with a collection of strains, but additional experiments with isolated pathogens would be of importance in the case of purified compounds or active extracts to evaluate their actual effects. According to the literature the evaluation of medicinal plants as antimicrobial agents, understanding medicinal flora and their real value is important, however the use of a standard technique for the research is also crucial. As the reports suggest, *W. somnifera* possesses significant antimicrobial activity, various types of research on the mechanisms of action, interactions of microorganisms with plant extracts and the pharmacokinetic profile of the extracts should be given main concern (Rios and Recio, 2005).

CONCLUSION:

Ashwagandha (*W. somnifera*) owns a tremendous amount of medicinal properties including antimicrobial activity. Many test microorganisms have been used for the assessment of the antimicrobial activity of extracts and purified compounds of various plant parts of Ashwagandha. 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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