Chemical Analysis Of Ashwagandha With Its Applications Especially As Antioxidant & Anti Stress Agent.

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ABSTRACT

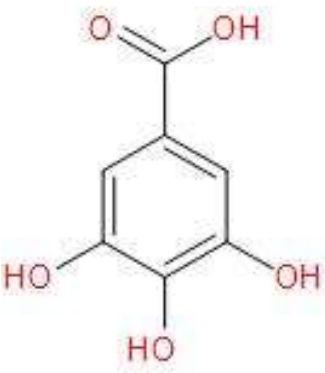
Withania somnifera (Ashwagandha) is a plant used in medicine from the time of Ayurveda , the ancient system of Indian medicine. The dried roots of the plant are used in the treatment of nervous disorders & in boosting immune system . Ashwagandha's chemical analysis reports it's chemical composition which includes alkaloids, tannins, flavonoids , vitamin and phenolic compounds etc .These results are very encouraging and indicate this herb should be studied more extensively to confirm these results and reveal other potential therapeutic effects.

Introduction

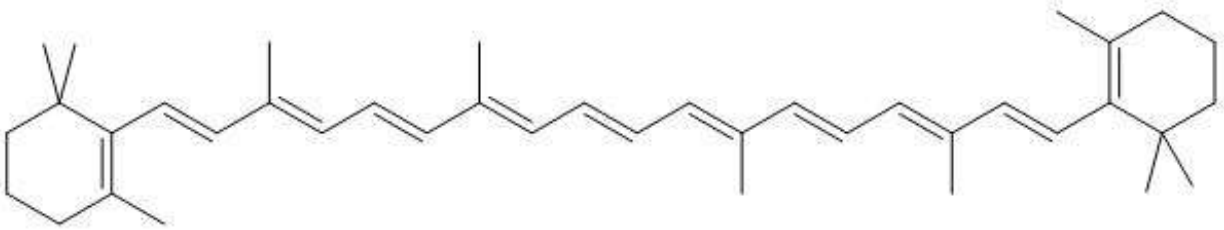
Ashwagandha (*Withania somnifera)* is a small evergreen shrub. It grows in India, the Middle East, and parts of Africa. Plant-based traditional medicine system continues to play a vital role in the health care system with about 60% of the world inhabitants relying mainly on traditional medicines for their primary health care. Ashwagandha Can Help Restore Our Immune System.The medicinal plant products, which are derived from plant parts such as stem, bark, leaves, fruits and seeds have been part of phytomedicine that produce a definite physiological action like treatment of nervous disorderness. The most important of these natural bioactive constituents of plants are alkaloids, tannins, flavonoids and phenolic compounds. Medicinal plants also contain large amounts of antioxidants, such as polyphenols, vitamin C, vitamin E, selenium, βcarotene carotenoids etc. Antioxidants have been reported to prevent oxidative damage by free radicals & ROS and may prevent the occurrence of disease, cancer and aging. Antioxidants are compounds that inhibit or delay the oxidation of other molecules by inhibiting the initiation or propagation of oxidizing chain reactions. There are two basic categories of antioxidants, namely, synthetic and natural. In general, synthetic antioxidants are compounds with phenolic structures of various degrees of alkyl substitution, whereas natural antioxidants can be phenolic compounds (tocopherols), flavonoids and phenolic acids), nitrogen compounds (alkaloids, chlorophyll derivatives, amino acids and amines), or carotenoids as well as ascorbic acid. Synthetic antioxidants such as butylated hydroxyl anisole (BHA) and butylated hydroxyl toluene (BHT) have been used as antioxidants since the beginning of this century. Phytoconstituents present in medicinal plant are important source of antioxidant and capable to terminate free radical chain reaction. Plant-derived antioxidants such as vitamin E, vitamin C, polyphenols including phenolic acids, flavonoids, catechins, procyanidins and anthocyanins are becoming increasingly important as dietary factors. Various studies indicate that medicinal plants contain a wide variety of natural antioxidants, such as phenolics, flavonoids, and tannins, which possess more potent antioxidant activity than common dietary plants. Compounds responsible for such antioxidant activity can be isolated and used for prevention and treatment of free radical-related disorders.

Chemical constituent of Ashwagandha

Laboratory analysis has revealed more than 30 chemical constituents contained in the roots of Withania somnifera . The biologically active chemical constituents are alkaloids (isopellertierine, anferine), steroidal lactones (withanolides, withaferins), saponins containing an additional acyl group (sitoindoside VII and VIII), and withanoloides with a glucose at carbon 27 (sitonidoside XI and X). Withania somnifera is also rich in iron. The roots of Withania somnifera consist primarily of compounds known as withanolides, which are believed to account for its extraordinary medicinal properties. Withanolides are steroidal and bear a resemblance, both in their action and appearance, to the active constituents of Asian ginseng (Panax ginseng) known as ginsenosides. Ashwagandha's withanolides have been researched in a variety of animal studies examining their effect on numerous conditions, including immune function and even cancer. Viji et al. (2013) assessed main active principles (alkaloids, flavonoids, glycosides, phenols, phytosterols, reducing sugars, saponins, tannins, terpenoids and anthraquinones) in in vitro and in vivo regenerated leaf, stem and root of Withania somnifera and found that phenols content was highest under in vitro conditions i.e. 1.27 ± 0.04 mg/g than in vivo conditions i.e. 0.94 ± 0.01 mg/g in roots of Withania somnifera.Chemical analysis of Ashwagandha show its main constituents to be alkaloids and steroidal lactones. Among the various alkaloids, withanine is the main constituent. The other alkaloids are somniferine, somnine, somniferinine, withananine, pseudo-withanine, tropine, pseudo-tropine, 3-a-gloyloxytropane, choline, cuscohygrine, isopelletierine, anaferine andanahydrine. Two acyl steryl glucoside viz. sitoindoside VII and sitoindoside VIII have been isolated from root. The leaves contain steroidal lactones, which are commonly called withanolides. The withanolides have C28 steroidal nucleus with C9 side chain, with a six membered lactone ring . Twelve alkaloids, 35 withanolides, and several sitoindosides from Withania somnifera have been isolated and studied. Asitoindoside is a withanolide containing a glucose molecule at carbon 27. Much of Ashwaganda's pharmacological activity has been attributed to two main withanolides, withaferin A and withanolide D. Further chemical analysis has shown the presence of the following: Anaferine (Alkaloid), Anahygrine (Alkaloid), Beta-Sisterol, Chlorogenic acid (in leaf only), Cysteine (in fruit), Cuscohygrine (Alkaloid), Iron, Pseudotropine (Alkaloid), Scopoletin, Somniferinine (Alkaloid), Somniferiene (Alkaloid), Tropanol (Alkaloid), Withanine (Alkaloid), Withananine (Alkaloid) and Withanolides A-Y(Steroidal lactones) . Prakash et al. (2007) reported that Withania somnifera leaves, fruits, stem and roots have 18.9, 16.1, 4.7 and 2.8 mg/g gallic acid equivalent total phenolic content, respectively and exhibited 37.9, 45.7, 33.1 and 45.1 % antioxidant activity, respectively as assayed by auto-oxidation of β-carotene and linoleic acid.



GALLIC ACID



B-carotene

CLASSIFICATION OF ANTIOXIDANT

(i) **Primary antioxidants or chain breaking antioxidants are those compounds, mainly phenolic substances that terminate the free radical chains in lipid oxidation and function as hydrogen and electron donors. In addition, primary antioxidants chelate transition metals acting as catalyst in lipid oxidation.**

(ii) **Oxygen scavengers are those substances which react with oxygen and can thus remove it in a closed system, e.g., ascorbic acid (vitamin C).**

(iii) **Secondary antioxidants are those compounds which function by decomposing the lipid hydroperoxides into stable end products.**

(i) Antioxidant effect

The brain and nervous system are relatively more susceptible to free radical damage than other tissues because they are rich in lipids and iron, both known to be important in generating reactive oxygen species. Free radical damage of nervous tissue may be involved in normal aging and neurodegenerative diseases, e.g., epilepsy, schizophrenia, Parkinson's, Alzheimer's, and other diseases. The active principles of WS, sitoindosides VII-X and withaferin A (glycowithanolides), have been tested for antioxidant activity using the major free-radical scavenging enzymes, superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPX) levels in the rat brain frontal cortex and striatum. Decreased activity of these enzymes leads to accumulation of toxic oxidative free radicals and resulting degenerative effects. An increase in these enzymes would represent increased antioxidant activity and a protective effect on neuronal tissue. Active glycowithanolides of WS were given once daily for 21 days, dose-related increased in all enzymes were observed; the increases comparable to those seen with deprenyl (a known antioxidant) administration. This implies that WS does have an antioxidant effect in the brain, which may be responsible for its diverse pharmacological properties. In another study, an aqueous suspension of WS root extract was evaluated for its effect on stress-induced lipid peroxidation (LPO) in mice and rabbits. LPO blood levels were increased by lipopolysaccharides (LPS) from Klebsiella pneumoniae and peptidoglycans (PGN) from Staphylococcus aureus. Simultaneous oral administration of WS extract prevented an increase in LPO . Apart from hepatic lipid peroxidation (LPO), the serum enzymes, alanine aminotransferase, aspartate aminotransferase and lactate dehydrogenase, were assessed as indices of hepatotoxicity. Silymarin (20 mg/kg, p.o.) was used for comparison. Iron overload induced marked increase in hepatic LPO and serum levels of the enzymes, which was attenuated by glycowithanolides (WSG) in a dose-related manner, and by silymarin.

(ii) As anti stress agent :-

A study conducted by the Institute of Basic Medical Sciences at Calcutta University examined the effects of Ashwagandha on chronic stress in rodents. For a period of 21 days, the animals received a mild electric shock to their feet. The resulting stress on the animals produced hyperglycemia, glucose intolerance, increase in plasma corticosterone levels, gastric ulcerations, male sexual dysfunction, cognitive deficits, immunosuppression and mental depression. Researchers using Withania somnifera discovered the animals given the herb an hour before the foot shock experienced a significantly reduced level of stress. This research confirms the theory that Ashwagandha has a significant anti-stress adaptogenic effect. Research conducted at the Department of Pharmacology, University of Texas Health Science Center indicated that extracts of Ashwagandha produce GABA-like activity, which may account for the herb's

anti-anxiety effects. GABA (Gamma Amino-butyric acid) is an inhibitory neurotransmitter in the brain. Its function is to decrease neuron activity and inhibit nerve cells from over firing. This action produces a calming effect. Excessive neuronal activity can lead to restlessness and insomnia, but GABA inhibits the number of nerve cells that fire in the brain, and helps to induce sleep, uplift mood, and reduce anxiety. Ashwagandha has traditionally been used to stabilize mood in patients with behavioral disturbances. Research has revealed that the herb produces an antidepressant and anti-anxiety effect in rodents comparable to the anti-depressant drug imipramine and the anti-anxiety drug lorazepam (Ativan). In fact, Ashwagandha is one of the most widespread tranquillizers used in India, where it holds a position of importance similar to ginseng in China. It acts mainly on the reproductive and nervous systems, having a rejuvenative effect on the body, and is used to improve vitality and aid recovery after chronic illness. Chronic stress can cause conditions such as cognitive deficit, immunosuppression, sexual dysfunction, gastric ulceration, irregularities in glucose homeostasis, and changes in plasma corticosterone levels. In a rat model of chronic stress syndrome, Withania somnifera and Panax ginseng extracts were compared and contrasted for their abilities to relieve some of the adverse effects of chronic stress. Research results showed that both Ashwagandha decreased the frequency and severity of stress-induced ulcers, reversed stress-induced inhibition of male sexual behavior, and inhibited the effects of chronic stress on retention of learned tasks. Both botanicals also reversed stress-induced immunosuppression, but only the Withania extract increased peritoneal macrophage activity. The activity of the Withania extract was about the same as the activity of the ginseng extract. Withania somnifera, however, has an advantage over Panax ginseng in that it does not appear to result in .ginseng-abuse syndrome., a condition characterized by high blood pressure, water retention, muscle tension, and insomnia.

Literature search:-

(i) Shahriar et al. (2013) scrutinized five different extracts of Withania somnifera roots to unfold the antioxidant and free radical scavenging activity and reported that total phenols content varied from 1.4 to 60.9 mg/g, total flavonoids content varied from 44.9 to 122.1 mg/g and total antioxidant capacity varied from 52.7 to 82.9 mg/g ascorbic acid equivalent.

(ii) Pathak et al. (2013) reported that methanolic extract of ashwagandha roots has more total phenolic content (43.77 ± 1.7 mg GAE/g) than aqueous (42.52 ± 0.8 mg GAE/g) extract and they also reported that IC50 value for methanolic and aqueous extracts of ashwagandha roots was 2.9 ± 0.09 and 3.27 ± 0.05 mg/mL, respectively.

(iii) Dhanani et al. (2013) found that ethanol extract of Withania somnifera roots has maximum total phenolic content (35.93 GAE mg/g) followed by water-ethanol (21.15 GAE mg/g) and water (17.63 GAE mg/g) extracts and they also found that in both DPPH and ABTS assays ethanol extract of Withania somnifera roots had lowest IC50 values and varied in following order: ethanol (0.18 mg/mL) < water- ethanol (0.39 mg/mL) < water (0.40 mg/mL).

(iv) Hossain et al. (2015) screened crude methanolic extracts of six medicinal plants of Bangladesh (Ipomoea paniculata, Withania somnifera, Mikania cordata, Abroma augusta, Bombax ceiba and Oxalis corniculata) for in-vitro anti-oxidant activity by using 1,1- diphenyl-2-picrylhydrazyl-hydrate (DPPH) free radical scavenging assay and reported that methanolic extracts of leaves of Withania somnifera and Oxalis corniculata showed strong antioxidant activity (IC50 = 27.8 and 19.98 µg/mL, repectively) in comparison to other plants.

Some other application of Ashwagandha

As immunity booster:- **The antioxidants and withanolides found in Ashwagandha Sealed the presence of various types of bioactive components such as alkaloids, tannins, saponins,** flavonoids**, glycosides, carbohydrate, terpenoids, phenolic compounds, proteins and amino acids. play a major role in its ability to boost our immune system. Studies show that withanolides exhibit antimicrobial, antitumor, and immuno-modulating properties.**

Increases Nitric Oxide Production:

Ashwagandha increases the production of nitric oxide. Nitric oxide is responsible for activating macrophage actions of the immune system and improves the ability to ingest invader cells. Studies show that the body naturally increases nitric oxide production in the face of infection, which accounts for a part of the immune-boosting effects of the herb.

Anti-Inflammatory Effects: **Ashwagandha helps lower inflammation by reducing the number of C-reactive protein in the body. Lowering chronic inflammation helps your immune system to perform efficiently by improving the action of Natural Killer cells. Natural killer cells are a part of the innate immune system, and they play a critical role in host-rejection of both tumors and viral-infected cells. Research shows that Ashwagandha helps to increase the natural killer cells in humans studies.**

Ashwagandha has many beneficial compounds such as flavonoids, antioxidants like glutathione, catalase, and superoxide dismutase which help enhance the body's immune resistance. It also contains amino acids like tryptophan, other favorable compounds like lignans, triterpenes, and neurotransmitters all of which contribute to its therapeutic effects. Studies also show that Ashwagandha's variety of therapeutic effects come with little or no toxic effects. So it is a safe herb to be taken as a supplement.

If we have an under-functioning immune system, taking Ashwagandha supplements alongside a nutritious meal can be a game changer. It is an easy and effective way to boost your immune system safely. It is also important to make sure your Ashwagandha supplement is 100% natural with no additives like coloring, flavoring or preservatives.

Result & conclusion

Ashwagandha improves the body's defense by improving the cell –mediated immunity .It also possesses potent antioxidants properties that helps protect against cellular damage caused by free radicals . The biologically active chemical constituents of Withania somnifera (WS) include Alkaloids , steroidal lactones , flavonoids, glycosides, phenols, phytosterols, reducing sugars, saponins, tannins, terpenoids and anthraquinones.

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