Pharmacological perspectives and phytochemical reservoir of *Withania somnifera*: A review

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Abstract
Plants have always been an indispensable part of human life since the beginning of human civilisation. Withania somnifera (L.) DUNAL, popularly known as Wintercherry or Indian Ginseng, is widely considered as Ashwagandha. In Ayurveda, it is classified as a Rasayana (rejuvenation) and projected to promote mental and physical health, rejuvenate the body in debilitated conditions and increase longevity. Due to its beneficial chemical constituents, the herb possesses a broad spectrum of biological activities and is used to treat a variety of diseases affecting human health. These include Cardioprotective activity, Anti-cancer activity, Immunomodulatory activity, Neuroprotective activity, Antimicrobial activity, Anti-stress activity,Anti-diabetic activity and Anti-inflammatory activity. The present review discusses the pharmacological importance of Withania somnifera and its important constituents.

Keywords: ***Withania somnifera,*** **Withanolides, Pharmacological activity, Anti-diabetic, Anti-tumour,**

Introduction

Nature gifted us an extensively diverse repertoire of plants (~250,000). Plants have tremendous importance in all life present in the planet earth. They produce oxygen, maintains water cycle, store carbon, serves as a source of food, habitat and medicine [1], [2], [3]. Since time immemorial human civilization gained the information related to the usage of medicinal plants comes as a source of drugs evolved from many years of struggles against illnesses by which human evolved to pursue drugs in parts of the plants [4],[5]. The oldest written records about the usage of medicinal plants as medicine was found to 5000 years back in India (Nagpur) in Sumerian clay slab which described the prescription for preparation of formulations from 250 plants such as poppy, mandrake and henbane [6] [7]. Ayurveda is considered as oldest traditional system of medicine, it applies experimental, experiential and philosophical approach [8]. It is based on the concept that the whole universe is constituted by integration of the five mother elements also called as "pancha-mahabhutas" (vayu (air), akasha (ether), teja (fire), aap (water) and prithvi (earth)) and are coded in living beings as three vital forces known as vata, kapha and pitta and the interplay and imbalances between these forces generates symptom of disease in a living being [9]. Ancient holy books Vedas which were considered as oldest sacred text contained plant based therapies which were used in ancient times in India. Nutmeg, pepper, clove, coriander etc were described in the texts which were in use in that time as both spices and medicines [10]. Arabians were the founder of traditional Unani system of medicine in their region and introduced various plants for medicine purpose mostly from India. They used mainly plants such as henbane, ginger, aloe, euphorbia, pepper, curcuma and saffron etc. [7], [11], [10].

Due to high cost, side effects, development of resistance in microbes against drugs and lack of curative treatment for chronic diseases scientific interest is renewed in complementary and alternative system of medicines [12]. Ayurveda and Traditional Chinese system of medicine is continuously gaining interest worldwide. As a result more than 1500 herbal preparations were sold in United States under dietary supplements category. Now a day, worldwide approximately one quarter of the total drugs in total usage originated from plants. Two hundred and fifty-two basic and essential drugs were prescribed by the World Health Organization (WHO), 11% comes from plant or plant based derivatives [13], [14]. Plants served as an important resource of pharmaceutically active biomolecules. Drug synthesis is based on the availability of these pharmaceutical molecules. There are a series of drugs currently in use derived from plants examples including digoxigenin (*Digitalis* spp.), vinblastine and vincristrine (*Catharanthus roseus*), atropine (*Atropa belladonna*) quinidine and quinine (*Cinchona* spp.), codeine and morphine (*Papaver somniferum*) etc. Some compounds of the plant can be synthesised chemically (vincristine) but some cannot be reproduced by synthetic methods (digitoxin) [4], [15]. Plants are not directly used for the production of pharmaceuticals but they provide "lead molecules" or "target molecules'' on which synthesis of a future drug is based. Now a day's plants are gaining worldwide popularity for cost effective large scale production of recombinant proteins. Candidate vaccines can be expressed in edible organ of banana to mediate its unprocessed administration in the body [16]. More than 100 recombinant proteins have in production in different species of plants examples include the production of rabies vaccine in tomato, rotavirus VP6 capsid protein in potatoes, recombinant avidin, β-glucuronidase, laccase, trypsin and aprotinin, production in maize [17].

In addition to major primary metabolites such as lipids, carbohydrates, amino acids and nucleotides, etc plant also synthesize an array of low molecular weight secondary metabolites. Plant secondary metabolites are those metabolites which have no fundamental role in processes of development and growth of a plant but are needed for survival and adaptations of plants [18], [19]. Secondary metabolites are the derivatives of primary metabolism and commonly called as secondary metabolites, secondary products or natural products. These organic products have no direct role in plant development but believed to be involved in protecting plants against attack by herbivores, micro-organisms and pests; also they have roles in attracting insects for pollination [20], [21]. They also help to adapt plant against various abiotic stresses like drought, flood and cold. The release of these compounds in plants is very low as < 1 percent of the total dry weight and majorly based on interaction of plants to environment or a particular stress [22]. They accumulate in vacuoles or specific plant cells or organs.

Approximately more than two hundred thousand secondary metabolites possess unique complex structures with most of them possessing building blocks of acetate C2-unit in polyketides, derived C9-unit of phenylpropanoids derived from phenylalanine /tyrosine and the isopentenyl diphosphate C5-unit. Various important secondary metabolite compounds were synthesized naturally by plants [24]. These includes include plant volatiles, sterols and carotenoids etc.), phenolics (lignin, phenolic acids, tannins, flavonoids) and N_2-bearing compounds such as alkaloids alkaloids [15], [18], [21], [24].

The winter cherry plant or *Withania somnifera* (Fig 1.) which is also known as Ashwagandha **(**indicating the odor of horse from roots) possess extensive medicinal value and have been used in ancient systems of medicine like Ayurveda, Siddha and Unani [27]. Earliest description of this plant was found in "Rig-Veda" which was defined as the oldest sacred text [28]. Other than India this plant is widely distributed in Africa, Asia, Middle East and Mediterranean region of the world (Fig 2.).

The plant is an erect, evergreen and perennial, grows with optimal temperature range of 20–32 °C with 500–750 mm rainfall as optimal. Leaves and Roots of this plant are mainly used in herbal formulations [30]. Ashwagandha is also known as Indian Ginseng, Winter cherry and Common Indian names includes: Asgandh in Hindi, Asgundh, Kanchuki, Askandha in Gujrati, Ashvagandha, Balada, Gandhpatri, Kamrupini, Vajini in Sanskrit, Ashvagandh in Bengali, Asgand in Punjabi, Asuragandi in Tamil, Asvagandhi, Penneru in Telgu and Asgandanagaori in Urdu [31]. Due to its immense importance in Ayurveda *W. somnifera* is also known as "Rasayana" which metabolites have been reported from plants [23]. These pharmaceuticals (atropine, digoxin, taxol, vinblastine, vincristine, morphine, artemisinin etc), some industrially important biochemicals and free chemicals like physostigmine, yohimbine, colchicines and phorbol esters, muscarine, cannabinoids, forskolin, colchicines etc [25], [26]. Secondary metabolites are grouped under three classes: terpenes (which means that it possess rejuvenating properties. *Withania somnifera* is one of the most important medicinal plants and is also known as ashwagandha, winter cherry and Indian ginseng [32]. It is one of the most prestigious medicinal plant and important herb in the Ayurveda and indigenous medical systems for over past 3000 years. The roots of this plant plays a very important role due to this reason it is categorized under the group rasyanas i.e., the tonics which are helps to increase health , vigor and longevity of an organism by enhancing the defense mechanisms to fight against infectious diseases [33] .This herbs have other effects such as revitalization of the body under debilitated conditions, increasing the immunity of the individual and development of resistance against adverse environmental conditions .The genus *Withania* consists of 23 species, numerous of which are distributed in the east Mediterranean region and South Asia and especially occurred in India [34]. *Withania somnifera* is the most important, commonly and widely used medicinal species in this genus. Group of unique steroidal lactones present in few species of Withania, and they also consist of highest amount of withanolides [34]. This species posses highest medicinal value due to the presence of important metabolites. *Withania somnifera* is used for the treatment of various ailments [35], [36], [37].

Plant Profile

Kingdom - Plantae
Subkingdom - Tracheobionta
Superdivision - Spermatophyta
Division - Magnoliophyta
Class - Magnoliopsida
Subclass - Rosidae
Order - Solanales
Family - Solanaceae
Genus - *Withania*
Species - *somnifera*

Botanical description and climatic conditions for growth

Late rainy season is the best season for the growth of *Withania somnifera*. This crop is regarded as a kharif crop. Dry region area with approximately 500 to 750 mm rainfall is suitable for cultivation of *Withania somnifera*. Relatively dry season is the suitable for the proper growth of this crop [38]. Temperature range of 21 to 38°C is optimum for growth of this plant. *Withania somnifera* is a small herbaceous and woody in appearance of approximately two feet in height. Leaves of the plant has shiny and smooth in appearance and floral region are smaller and opposite .The berries of the plant are globose and small, red in colour after maturation (Fig 1.) [39].

Fig.1. *Withania somnifera*- The Indian Ginseng

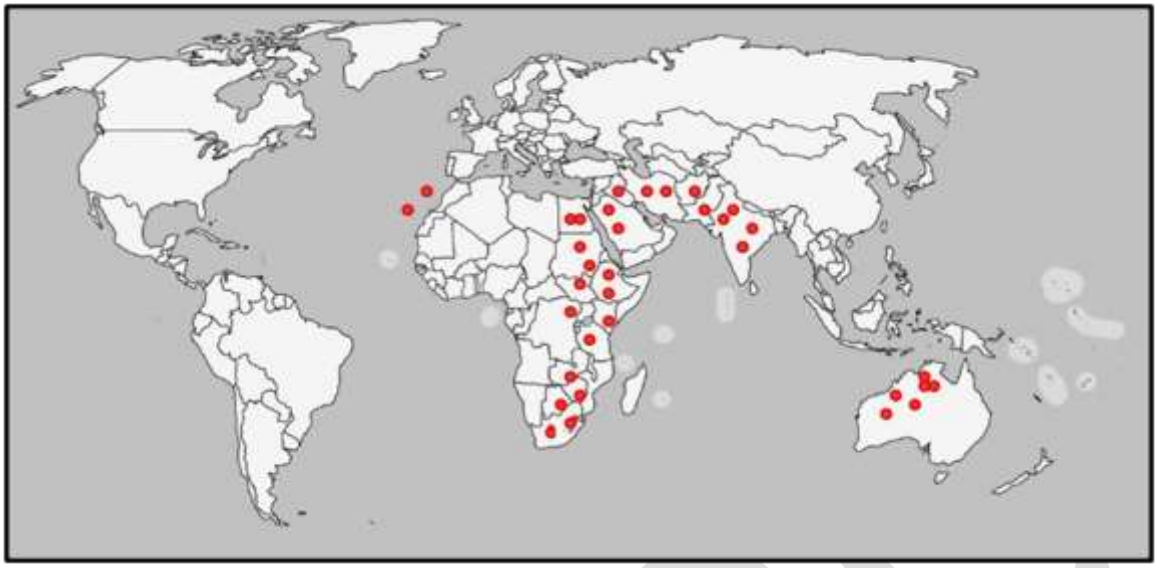

Fig. 2 Geographical distribution of *W. somnifera*

Chemical constituents

Withania somnifera is composed of various diverse groups of chemicals constituents such as, tannins, flavonoids, steroidal lactones alkaloids, etc. More than 12 alkaloids, 40 withanolides and various sitoindosides have been extracted from aerial parts, berries and roots of Withania plant. The priority chemical constituents of these plants are withanolides mostly found in leaves and root (Fig 3.). The chemical constituents were described in the table (S1.)[33], [40]

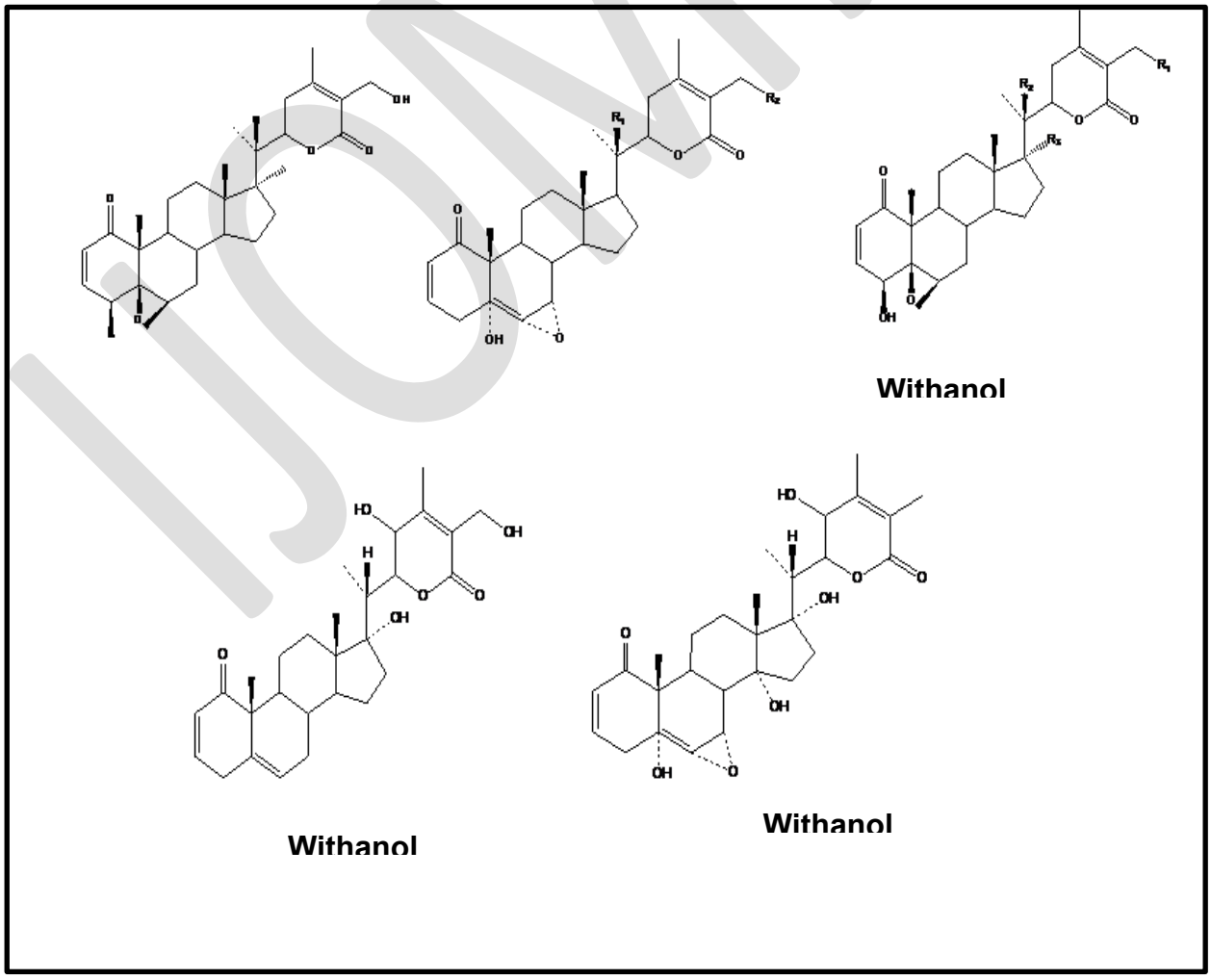

Fig 3. Structures of some important withanolides

Biosynthesis of withanolides

Withanolide biogenesis and accumulation is limited to specific genera of Solanaceae family, among them *Withania* shows maximum production of withanolide in more than 200 diversified forms, with or without functional groups. The biosynthetic pathway of withanolides, their function in *W. somnifera* and metabolic steps leading to their glyco-transformation are unknown [41]. However, it has been demonstrated that precursor molecules governing withanolide biosynthesis could be isoprenoids. Isoprenoids produced during isoprenogenesis, is governed by 2 independent pathways; the classical cytosolic mevalonate (MVA) pathway, which ultimately leads to biosynthesis of 24-methylene cholesterol [41].

It was proved that the biosynthetic pathway upstream of 2,3-oxidosqualene is combination of classical mevalonate pathway (MVA) in cytosol and non mevalonate or deoxy xylulose pathway (DOXP) in plastids are involved in the biosynthesis of Withanolides. Mevalonate pathway starts from Acetyl Co-A that form mevalonic acid after some chemical reaction and this mevalonic acid is converted into isopentenyl pyrophosphate (IPP) through the loss of one carbon atom while DOXP pathway starts with the combination of pyruvate and glyceraldehydes [42]. After the formation of 2, 3 oxidosqualene, cycloartenol is formed by the activity of cycloartenol synthase and then series of methylation, oxidation, reduction, demethylation reaction precede the synthesis of 24-methylene cholesterol. After biosynthesis of 24-methylene cholesterol, specific enzymes like CyP450s and glucosyltransferases play role in secondary conversion with respect to transfer of different moieties or redox reactions leading to biosynthesis of specific withanolides in tissue-specific manner [43], [44].

Pharmacological activities of *W. somnifera*

W. somnifera is one of the most important plant of Ayurveda and several pharmacological activities are reported from its molecules, extracts, plant parts and preparations [44]. Reports suggested that not only the raw material but also extracts and isolated fractions of the plants possess a wide spectrum of pharmaceutical activities (Fig 4.). The pharmacological potential of this herb makes it a suitable candidate plant to be used in drug development and hereby it is documented as an official drug in Indian Pharmacopoeia [45].

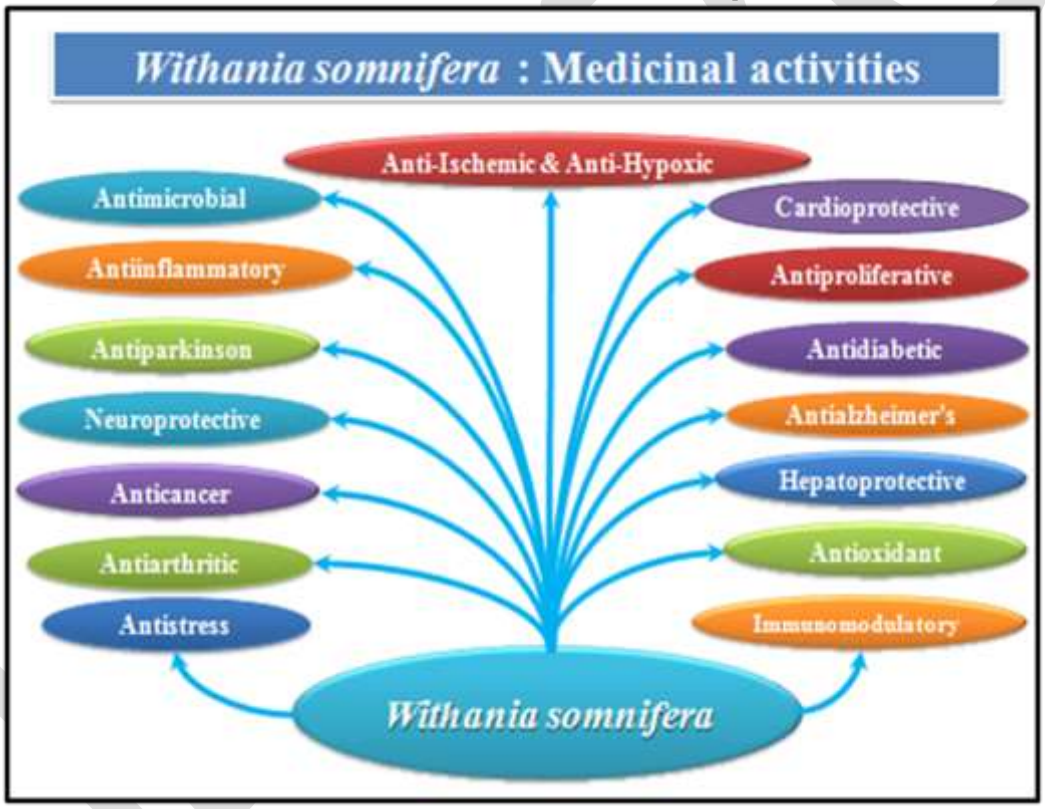

Fig 4 . Pharmacological properties of *W. somnifera*

Anti-inflammatory properties

Various studies had proven that *W. somnifera* possess a strong anti-inflammatory activity. The leaf extracts of *W. somnifera* showed to reduce implant induced inflammation in model animals [46]. Root extracts were also reported to reduce the inflammation caused by trinitro benzyl sulfonic Acid (TNBS) [47]. Very potent activity was recorded when the root powder (upto 600mg kg $^{-1}$) was tested against collagen induced arthritic rats [48]. Alcoholic leaf extracts were tested against acute and sub acute inflammation in rats and the activity of the extract was found to be comparable with drug phenylbutazone [49]. The effect of root powder of *W. somnifera* was found to be effective on gouty arthiritis without causing any gastric damage, when tested against standard drug indomethacin [50]. In Ashwagandha leaves treated animals, inhibition of reactive gliosis, inflammatory cytokines production and expression of nitro-oxidative stress enzymes showed that active phytochemicals from the plant might come out as suitable candidates for neuro-inflammation associated with various neuropathologies [51]. The root extract of *W. somnifera* has also shown to reduce pro-inflammatory cytokine levels and increase in cancerous cell death that might lowered the progression of cancer and cachexia. *W. somnifera* root extract may therefore be effective in cancer cachexia [52]. The plant can also be used as a dietary supplement for treatment of sleep deprivation (SD) induced stress and associated functional impairments [53].

Anti-cancer properties

Studies from the experiments on animal system and cell culture revealed that *W. somnifera* plant extracts also have anti cancerous properties. In addition to having therapeutic potential

against cancer it also have cancer preventive properties [54]. Different parts of this plant especially the root system have been proved to be effective against various kinds of cancers [55], [56] due to the presence of unique phytochemical. The active principles of roots are withanolides such as withaferin A along with withanone, withanolide D and withanosides are found to be effective against cancer cell lines [57]. In comparison to ovary and lungs cancer the leaf extract was more effectual against breast cancer [58]. In addition to having anti cancer properties, this report also suggested the ability of *W. somnifera*, to recover from fatigue induced by cancer, in cancer patients which may be helpful for improvement of their life expectancies. The root extract of this plant is found to be effective against cervical cancer [59]. Ingredients of *W. somnifera* activate apoptosis in inhibited delayed type hypersensitivity model (Balb/c) mice [63]. The extracts have anti-allergic properties when tested in IgE-mediated anaphylactic immuno-inflammatory system [64]. *W. somnifera* root powder possessed immunosuppressive properties and it is reported to be a potent inhibitor of complement system, delayed-type hypersensitivity and lymphocyte proliferation. Thus it can be used to create a candidate immunosuppressive drug for inflammatory diseases [65]. This herb reduced the toxicity caused by cancerous cells when investigated against cyclophosphamide treated rats by increasing WBC, ALC and platelets counts [66]. When azoxymethane (potential carcinogen) cancerous cells. A plethora of *in-vitro* evidences exists that reported about the stimulation of apoptosis by withanolides [60], [61].

Neuroprotective properties

In ancient Ayurvedic literature *W. somnifera* is regarded as "Medharasayana" –a memory enhancing tonic. Root extracts of the plants were found to reduce degeneration of brain region [69], [70]. The extracts of *W. somnifera* had profound effect on Parkinson's disease rat model i.e. 6-Hydroxydopamine rats [71]. The extracts showed anti-Parkinson effect in the Parkinson's induced (MPTP induced) mice models [72]. The neuroprotective effects of plant may be related to the antioxidant effect and lipid peroxidation actions [73], [74]. Withanolides were showed to increase catalase and peroxidase levels in the frontal cortex part in rat models, thus regulating oxidative damage of brain [75]. Phenolic compounds of the roots were found to be involved in the antioxidant activity of *W. somnifera* [76]. Extract of *W. somnifera* were shown to down regulate the level of neurodegenerative agent nitric oxide [77]. Root extract was also shown to possess anti-parkinson effect in induced mouse models [78]. Water extract of the plant was also effective against glutamate neurotoxicity [79]. Root extracts were also shown to possess profound effects against β-amyloid and hydrogen peroxide toxicity in brain tissues [80].

Heart diseases like myocardial infarction and ischemia-reperfusion injury are in the rising trend in today's world [81]. Owing to the presence of chemical constituents in this plant it is regarded as a potent cardio tonic [82]. The level of cardiac enzymes, antioxidant enzymes and cardiac cycle was improved by *W. somnifera*. It possessed a strong cardioprotective activity as shown by a study in which the cardioprotective effects of *W. somnifera* were compared with the known cardioprotective antioxidant vitamin E [81]. Also when the extracts of *W. somnifera* were tested against vitamin E in Wistar rats to check the effect of the herb in myocardial injury, the level of myocardial enzymes indicated that by withanolide treatment, the level of myocardial injury was reduced as compared to vitamin E [83].

Salbutamol induced rabbit model of myocardial necrosis showed the increase in the levels of antioxidant enzymes in *W. somnifera* treated models as compared to control [84]. *W. somnifera* activated Nuclear factor (erythroid-derived 2)-like 2 (Nrf2) and hence acted as an inducer of phase II antioxidant enzymes which were involved in protection of cardiovascular diseases [82]. *W. somnifera* extracts were reported to act as a preventive agent in cardiotoxicity induced by doxorubicin [85]. Preventive treatment by *W. somnifera* restored myocardial oxidant/anti-oxidant, pro-apoptotic/ anti-apoptotic effects and decreased the deterioration of myocardium cells in animal model [86].

Anti-diabetic properties

W. somnifera extracts (root and leaf) were tested on alloxan induced diabetic rat for hypoglycemic effect [87].

Immunomodulatory properties

W. somnifera acts as an immuomodulator checked against cisplatin-treated *Leishmania donovani*-infected BALB/c mice. Also enhanced antileishmanial activity was reported when *W. somnifera* was applied in combination with cisplatin [62]. *W. somnifera* extract increased total antibody forming cells as well as stimulate these cells much before in normal condition in mice. Administration of *W. somnifera* extract also induced colon cancer in mice was administered by *W. somnifera* extract, the level of immune cells was altered. Immunomodulatory properties (neutrophils count, hypersensitivity and humoral antibody response) were investigated in mice after administration of ethanolic root extracts of *W. somnifera* exhibiting an enhanced neutrophil counts and humoral antibody response [67]. *W. somnifera* root powder has changed the immune response against *Aeromonas hydrophila* infection in *Oreochromis niloticus* and hence it may be used as immunotherapeutic agent against various fish bacterial diseases [68].

Cardioprotective properties

Root powder of *W. somnifera* was reported to have profound effect in reducing sugar levels in humans when administered orally that was comparable to antidiabetic drug [88]. *W. somnifera* was reported to possess the ability to enhance the insulin sensitivity index in rat models [89]. Extracts were reported to influence the glucose uptake potential in skeletal myotubes and adipocytes with leaf extract possessing more profound effects when compared with root extract [87]. The extracts of the plant was also reported to be regulator of blood glucose, urine sugar and tissue glycogen levels in alloxan-induced diabetes mellitus rat [90].

Anti-microbial properties

W. somnifera was used as a folk medicine for the management of infections. The methanolic extracts of the plant showed activity against a variety of bacterium including *Escherichia coli*, *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas fluorescens*, *Xanthomonas axonopodis* [91]. The methanolic extracts of root and leaf tissues were also active against gram-positive *Staphylococcus aureus* and *Enterococcus spp*[92]. Aqueous extracts of root was also showed marked biological activity against methicillin resistant *Staphylococcus aureus* (MRSA) [93]. Organic extracts of *W. somnifera* showed both antibacterial activity against *Staphylococcus aureus*, clinical isolates, methicilin resistant *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Antifungal activity was also reported against *Candida albicans*, clinical isolates of *Cryptococcus neoformans*, *Microsporum gypseum*, and *Trichophyton mentagrophytes* [94]. Additionally, antifungal activity was reported against *Fusarium verticillioides*, *Dreschlera turcic*a and *Aspergillus flavus* [91]. Administration of *W. somnifera* extracts were also reported to increase survival rate with decreased bacterial load on vital organs of mice with salmonellosis

[95].

Anti-stress properties

Ashwagandha is a known rejuvenating herb and its vitalizing potential was comparable to Korean and Siberian Ginseng [32]. Various studies were performed to find the role of *W. somnifera* in stress tolerance or adaptogenic activities [97], [98]. These studies had shown that *W. somnifera* was involved in the production of improved stamina, combat stress produced during hepatotoxicity and carbon tetrachloride induced mortality in rat models. When root powder of *W. somnifera* was administered with water by oral route in rats, the increased level of plasma corticosterone level was recorded along with phagocytic and avidity index in untreated rats but the herb treated shown the values closer to control values [99]. Thus these results suggested that ashwagandha possess a strong antistress activity and may be used in the formulations or as a whole drug to combat stress conditions [97], [32], [98], [99].

Conclusion

Plants are regarded as green factories due to their ability to produce natural products beneficial to mankind. Traditional and ethnobotanical evidences are the basis of dugs discovery from plants. The traditional system of Medicines claims the therapeutic use of numerous plants. Plant-based drugs are favoured because they have fewer or no side effects. Extensive research has been done on this plant related to its scent, but there is brief knowledge available about its pharmacological profile. This review is an attempt to compile the information on pharmacological effects of *W. somnifera* in a comprehensive manner. The presence of various bioactive constituents with low toxicity profiles and the new mechanism of action make Aswagandha, an effective drug candidate for the treatment of different diseases. The diverse bioactive agents present in *W. somnifera* participate in treating various ailments of the human body. Reports suggested that *W. somnifera* is pharmacologically active as an anticancer, antifungal, antibacterial and antioxidant agent. However, extensive researches related to the Phytochemistry, pharmacology and biotechnology are needed to develop a drug from *W. somnifera*.

Future Directions

Since *W. somnifera* is well known as a possible source of various chemical constituents, this herb holds a prominent position among many significant medicinal plants. There exist different bioactivities of *W. somnifera*; hence more research is required to expose the underlying mechanisms of drug compound action. If the correlation of particular metabolite (chemical constituent) of *W. somnifera* were scrutinized, it would provide better opportunities for future researchers to develop a potential drug from this plant. The large scale consumption of *W. somnifera* by humans as a remedy of healthcare in traditional systems of medicines, further screening and investigation of various formulations for different bioactivities *in-vivo* and *in-vitro* is essential.

Associated Content

Supporting Information

Table S1. Chemical constituents of *W. somnifera*

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Notes: The authors declare no competing financial interest

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References:

[1] Woods, D., Epistemic Things in Charles and Ray Eames's Powers of Ten. In Scale in Literature and Culture, Springer: 2017; pp 61-92.
[2] Hayat, S.; Ali, B.; Ahmad, A., Salicylic acid: biosynthesis, metabolism and physiological role in plants. In Salicylic acid: A plant hormone, Springer: 2007; pp 1-14.
[3] Koutsospyros, A. D.; Koutsospyros, D. A.; Strigul, N.; Braida, W.; Christodoulatos, C., Tungsten: Environmental pollution and health effects. In Encyclopedia of environmental health, Elsevier: 2019; pp 161-169.
[4] Kong, J.-M.; Goh, N.-K.; Chia, L.-S.; Chia, T.-F., Recent advances in traditional plant drugs and orchids. Acta Pharmacologica Sinica 2003, 24 (1), 7-21.
[5] Petrovska, B. B., Historical review of medicinal plants' usage. Pharmacognosy reviews 2012, 6 (11), 1.
[6] Sangwan, N. S.; Tripathi, S.; Srivastava, Y.; Mishra, B.; Pandey, N., Phytochemical genomics of Ashwagandha. In Science of Ashwagandha: Preventive and Therapeutic Potentials, Springer: 2017; pp 3-36
[7] Rates, S. M. K., Plants as source of drugs. Toxicon 2001, 39 (5), 603-613.
[8] Mukherjee, P. K.; Harwansh, R. K.; Bahadur, S.; Banerjee, S.; Kar, A.; Chanda, J.; Biswas, S.; Ahmmed, S. M.; Katiyar, C., Development of Ayurveda–tradition to trend. Journal of ethnopharmacology 2017, 197, 10-24.
[9] Patwardhan, B.; Warude, D.; Pushpangadan, P.; Bhatt, N., Ayurveda and traditional Chinese medicine: a comparative overview. Evidence-Based Complementary and Alternative Medicine 2005, 2.
[10] Tiwari, S., Plants: A rich source of herbal medicine. Journal of natural products 2008, 1 (0), 27-35.
[11] Yesilada, E., Past and future contributions to traditional medicine in the health care system of the Middle-East. Journal of Ethnopharmacology 2005, 100 (1-2), 135-137.
[12] Ackerknecht, E. H.; Haushofer, L., A short history of medicine. JHU Press: 2016.
[13] Rai, A.; Rai, A. C., Microbial endophytes and their intellectual property rights. In Microbial Endophytes, Elsevier: 2020; pp 349-387.
[14] Dutfield, G.; Suthersanen, U., Traditional Knowledge and Genetic Resources: Observing Legal Protection through the Lens of Historical Geography and Human Rights. Washburn LJ 2019, 58, 399.
[15] Alamgir, A., Secondary Metabolites: Secondary Metabolic Products Consisting of C and H; C, H, and O; N, S, and P Elements; and O/N Heterocycles. In Therapeutic Use of Medicinal Plants and their Extracts: Volume 2, Springer: 2018; pp 165-309
[16] Fischer, R.; Stoger, E.; Schillberg, S.; Christou, P.; Twyman, R. M., Plant-based production of biopharmaceuticals. Current opinion in plant biology 2004, 7 (2), 152-158.
[17] Twyman, R. M.; Stoger, E.; Schillberg, S.; Christou, P.; Fischer, R., Molecular farming in plants: host

systems and expression technology. TRENDS in Biotechnology 2003, 21 (12), 570-578.
[18] Verpoorte, R., Exploration of nature's chemodiversity: the role of secondary metabolites as leads in drug development. Drug Discovery Today 1998, 3 (5), 232-238.
[19] Tripathi, S.; Srivastava, Y.; Sangwan, R. S.; Sangwan, N. S., In silico mining and functional analysis of AP2/ERF gene in Withania somnifera. Scientific reports 2020, 10 (1), 1-1
[20] Tripathi, S.; Sangwan, R. S.; Narnoliya, L. K.; Srivastava, Y.; Mishra, B.; Sangwan, N. S., Transcription factor repertoire in Ashwagandha (Withania somnifera) through analytics of transcriptomic resources: Insights into regulation of development and withanolide metabolism. Scientific reports 2017, 7 (1), 1-17.
[21] Edreva, A.; Velikova, V.; Tsonev, T.; Dagnon, S.; Gürel, A.; Aktaş, L.; Gesheva, E., Stress-protective role of secondary metabolites: diversity of functions and mechanisms. Gen Appl Plant Physiol 2008, 34 (1-2), 67-78.
[22] Srivastava, Y.; Sangwan, N. S., Chapter 17 - Improving medicinal crops through phytochemical perspective: Withania somnifera (Ashwagandha). In Advancement in Crop Improvement Techniques, Tuteja, N.; Tuteja, R.; Passricha, N.; Saifi, S. K., Eds. Woodhead Publishing: 2020; pp 275-295.
[23] Aharoni, A.; Galili, G., Metabolic engineering of the plant primary–secondary metabolism interface. Current opinion in biotechnology 2011, 22 (2), 239-244.
[24] Pichersky, E.; Gang, D. R., Genetics and biochemistry of secondary metabolites in plants: an evolutionary perspective. Trends in plant science 2000, 5 (10), 439-445.
[25] Phillipson, J. D., Phytochemistry and medicinal plants. Phytochemistry 2001, 56 (3), 237-243.
[26] Bourgaud, F.; Gravot, A.; Milesi, S.; Gontier, E., Production of plant secondary metabolites: a historical perspective. Plant science 2001, 161 (5), 839-851.
[27] Achar, G. P. K.; Prabhakar, B.; Rao, S.; George, T.; Abraham, S.; Sequeira, N.; Baliga, M. S., Scientific Validation of the Usefulness of Withania somnifera Dunal in the Prevention and Treatment of Cancer. In Anticancer Plants: Properties and Application, Springer: 2018; pp 285-301.
[28] Gurib-Fakim, A., Medicinal plants: traditions of yesterday and drugs of tomorrow. Molecular aspects of Medicine 2006, 27 (1), 1-93.
[29] Almoulah, N. F. Phytochemical study and biological evaluation of five plants belonging to the family Solanaceae. Université de Lorraine, 2017.
[30] Kumar, A.; Kaul, M.; Bhan, M.; Khanna, P. K.; Suri, K., Morphological and chemical variation in 25 collections of the Indian medicinal plant, Withania somnifera (L.) Dunal (Solanaceae). Genetic Resources and Crop Evolution 2007, 54 (3), 655-660.
[31] Rajeswara Rao, B., Opportunities and challenges in the cultivation of Ashwagandha {Withania somnifera (L.) Du-nal}. Journal of Pharmacognosy, ISSN 2012.
[32] Singh, N.; Bhalla, M.; de Jager, P.; Gilca, M., An overview on ashwagandha: A Rasayana (Rejuvenator) of Ayurveda. African Journal of Traditional, Complementary and Alternative Medicines 2011, 8 (5S).
[33] Mirjalili, M. H.; Moyano, E.; Bonfill, M.; Cusido, R. M.; Palazón, J., Steroidal lactones from Withania somnifera, an ancient plant for novel medicine. Molecules 2009, 14 (7), 2373-2393.
[34] Wasnik, N. G.; Muthusamy, M.; Chellappan, S.; Vaidhyanathan, V.; Pulla, R.; Senthil, K.; Yang, D. C., Establishment of in vitro root cultures and analysis of secondary metabolites in Indian Ginseng-Withania somnifera. Korean J Plant Res 2009, 22, 584-91.
[35] Khan, J.; Srivastava, P.; Singh, S., Sensitive detection of a phytoplasma associated with little leaf symptoms in Withania somnifera. European journal of plant pathology 2006, 115 (4), 401-408.
[36] Martín-Herrera, D.; Abdala, S.; Benjumea, D.; Pérez-Paz, P., Diuretic activity of Withania aristata: An endemic Canary Island species. Journal of ethnopharmacology 2007, 113 (3), 487-491.
[37] Mir, B. A.; Khazir, J.; Mir, N. A.; Hasan, T.-u.; Koul, S., Botanical, chemical and pharmacological review of Withania somnifera (Indian ginseng): an ayurvedic medicinal plant. Indian J Drugs Dis 2012, 1, 147-60.
[38] Shah, S.; Saravanan, R.; Gajbhiye, N., Leaf gas exchange, chlorophyll fluorescence, growth and root yield of Ashwagandha (Withania somnifera Dunal.) under soil moisture stress. Indian Journal of Plant Physiology 2010, 15 (2), 117-124.
[39] Singh, P. K.; Sharma, Y. K., Withania somnifera (Ashwagandha): A wonder herb with multiple medicinal properties. Asian Journal of Pharmacy and Pharmacology 2018, 4 (2), 123-130.
[40] Kumar, V.; Dey, A.; Hadimani, M. B.; Emeraldjavascript, M., Chemistry and pharmacology of Withania somnifera: An update. Tang 2015, 5 (1), 1.1-1.13.
[41] Sharada, M.; Ahuja, A.; Suri, K.; Vij, S.; Khajuria, R.; Verma, V.; Kumar, A., Withanolide production by in vitro cultures of Withania somnifera and its association with differentiation. Biologia Plantarum 2007, 51 (1), 161-164.
[42] Nagella, P.; Murthy, H. N., Establishment of cell suspension cultures of Withania somnifera for the production of withanolide A. Bioresource Technology 2010, 101 (17), 6735-6739.
[43] Gupta, P.; Goel, R.; Pathak, S.; Srivastava, A.; Singh, S. P.; Sangwan, R. S.; Asif, M. H.; Trivedi, P. K., De novo assembly, functional annotation and comparative analysis of Withania somnifera leaf and root transcriptomes to identify putative genes involved in the withanolides biosynthesis. PLoS One 2013, 8 (5), e62714.
[44] Dhar, N.; Razdan, S.; Rana, S.; Bhat, W. W.; Vishwakarma, R.; Lattoo, S. K., A decade of molecular understanding of withanolide biosynthesis and in vitro studies in Withania somnifera (L.) Dunal: prospects and perspectives for pathway engineering. Frontiers in plant science 2015, 6, 1031.
[45] Singh, A. K.; Varshney, R.; Sharma, M.; Agarwal, S. S.; Bansal, K. C., Regeneration of plants from alginate-encapsulated shoot tips of Withania somnifera (L.) Dunal, a medicinally important plant species. Journal of plant physiology 2006, 163 (2), 220-223.
[46] Sivamani, S.; Joseph, B.; Kar, B., Anti-inflammatory activity of Withania somnifera leaf extract in stainless steel implant induced inflammation in adult zebrafish. Journal of Genetic Engineering and Biotechnology 2014, 12 (1), 1-6.
[47] Pawar, P.; Gilda, S.; Sharma, S.; Jagtap, S.; Paradkar, A.; Mahadik, K.; Ranjekar, P.; Harsulkar, A., Rectal gel application of Withania somnifera root extract expounds anti-inflammatory and muco-restorative activity in TNBS-induced Inflammatory Bowel Disease. BMC complementary and alternative medicine 2011, 11 (1), 34.
[48] Gupta, A.; Singh, S., Evaluation of anti-inflammatory effect of Withania somnifera root on collagen-induced

arthritis in rats. Pharmaceutical biology 2014, 52 (3), 308-320.
[49] Sudhir, S.; Budhiraja, R.; Miglani, G.; Arora, B.; Gupta, L.; Garg, K., Pharmacological studies on leaves of Withania somnifera. Planta medica 1986, 52 (01), 61-63.
[50] Rasool, M.; Varalakshmi, P., Immunomodulatory role of Withania somnifera root powder on experimental induced inflammation: An in vivo and in vitro study. Vascular pharmacology 2006, 44 (6), 406-410.
[51] Gupta, M.; Kaur, G., Withania somnifera as a potential anxiolytic and anti-inflammatory candidate against systemic lipopolysaccharide-induced Neuroinflammation. Neuromolecular medicine 2018, 20 (3), 343-362.
[52] Naidoo, D. B.; Chuturgoon, A. A.; Phulukdaree, A.; Guruprasad, K. P.; Satyamoorthy, K.; Sewram, V., Withania somnifera modulates cancer cachexia associated inflammatory cytokines and cell death in leukaemic THP-1 cells and peripheral blood mononuclear cells (PBMC's). BMC complementary and alternative medicine 2018, 18 (1), 126.
[53] Manchanda, S.; Kaur, G., Withania somnifera leaf alleviates cognitive dysfunction by enhancing hippocampal plasticity in high fat diet induced obesity model. BMC complementary and alternative medicine 2017, 17 (1), 136.
[54] Hahm, E.-R.; Lee, J.; Kim, S.-H.; Sehrawat, A.; Arlotti, J. A.; Shiva, S. S.; Bhargava, R.; Singh, S. V., Metabolic alterations in mammary cancer prevention by withaferin A in a clinically relevant mouse model. Journal of the National Cancer Institute 2013, 105 (15), 1111-1122.
[55] Winters, M., Ancient medicine, modern use: Withania somnifera and its potential role in integrative oncology. Alternative Medicine Review 2006, 11 (4).
[56] Vyas, A. R.; Singh, S. V., Molecular targets and mechanisms of cancer prevention and treatment by withaferin a, a naturally occurring steroidal lactone. The AAPS journal 2014, 16 (1), 1-10.
[57] Rai, M.; Jogee, P. S.; Agarkar, G.; Santos, C. A. d., Anticancer activities of Withania somnifera: Current research, formulations, and future perspectives. Pharmaceutical biology 2016, 54 (2), 189-197.
[58] Nema, R.; Khare, S.; Jain, P.; Pradhan, A., Anticancer activity of Withania somnifera (leaves) flavonoids compound. Int J Pharm Sci Rev Res 2013, 19 (1), 103-106.
[59] Manoharan, S.; Kaur, J., Anticancer, antiviral, antidiabetic, antifungal and phytochemical constituents of medicinal plants. Am J PharmTech Res 2013, 3, 149-69.
[60] Malik, F.; Kumar, A.; Bhushan, S.; Mondhe, D. M.; Pal, H. C.; Sharma, R.; Khajuria, A.; Singh, S.; Singh, G.; Saxena, A. K., Immune modulation and apoptosis induction: Two sides of antitumoural activity of a standardised herbal formulation of Withania somnifera. European Journal of Cancer 2009, 45 (8), 1494-1509.
[61] Srinivasan, S.; Ranga, R. S.; Burikhanov, R.; Han, S.-S.; Chendil, D., Par-4-dependent apoptosis by the dietary compound withaferin A in prostate cancer cells. Cancer research 2007, 67 (1), 246-253.
[62] Sachdeva, H.; Sehgal, R.; Kaur, S., Studies on the protective and immunomodulatory efficacy of Withania somnifera along with cisplatin against experimental visceral leishmaniasis. Parasitology research 2013, 112 (6), 2269-2280.
[63] Davis, L.; Kuttan, G., Immunomodulatory activity of Withania somnifera. Journal of Ethnopharmacology 2000, 71 (1-2), 193-200.
[64] Agarwal, R.; Diwanay, S.; Patki, P.; Patwardhan, B., Studies on immunomodulatory activity of Withania somnifera (Ashwagandha) extracts in experimental immune inflammation. Journal of Ethnopharmacology 1999, 67 (1), 27-35.
[65] Rasool, M.; Varalakshmi, P., Protective effect of Withania somnifera root powder in relation to lipid peroxidation, antioxidant status, glycoproteins and bone collagen on adjuvant-induced arthritis in rats. Fundamental & clinical pharmacology 2007, 21 (2), 157-164.
[66] Ali, K.; Shuaib, M.; Ilyas, M.; Hussain, F.; Hussain, F., Medicinal uses of chemical extracts from Withania somnifera and its antimicrobial activity: a mini-review. PSM Microbiology 2017, 2 (1), 20-23.
[67] Siddiqui, N. A.; Singh, S.; Siddiquei, M. M.; Khan, T. H., Immunomodulatory effect of Withania somnifera, Asparagus racemosus and Picrorhiza kurroa roots. International Journal of Pharmacology 2012, 8 (2), 108-114.
[68] Zahran, E.; Abd El-Gawad, E. A.; Risha, E., Dietary Withania sominefera root confers protective and immunotherapeutic effects against Aeromonas hydrophila infection in Nile tilapia (Oreochromis niloticus). Fish & shellfish immunology 2018, 80, 641-650.
[69] Jain, S.; Shukla, S. D.; Sharma, K.; Bhatnagar, M., Neuroprotective effects of Withania somnifera Dunn. in hippocampal sub-regions of female albino rat. Phytotherapy research 2001, 15 (6), 544-548.
[70] Srivastava, A. N.; Ahmad, R.; Khan, M. A., Evaluation and Comparison of the In Vitro Cytotoxic Activity of Withania somnifera Methanolic and Ethanolic Extracts against MDA-MB-231 and Vero Cell Lines. Sci Pharm 2016, 84 (1), 41-59.
[71] Bano, A.; Sharma, N.; Dhaliwal, H. S.; Sharma, V., A systematic and comprehensive review on Withania somnifera (L.) Dunal-an Indian ginseng. Journal of Pharmaceutical Research International 2015, 63-75.
[72] Sankar, S. R.; Manivasagam, T.; Krishnamurti, A.; Ramanathan, M., The neuroprotective effect of Withania somnifera root extract in MPTP-intoxicated mice: an analysis of behavioral and biochemical variables. Cell Mol Biol Lett 2007, 12 (4), 473-81.
[73] Bhatnagar, M.; Sharma, D.; Salvi, M., Neuroprotective effects of Withania somnifera dunal.: A possible mechanism. Neurochem Res 2009, 34 (11), 1975-83.
[74] Kumar, P.; Kumar, A., Possible neuroprotective effect of Withania somnifera root extract against 3-nitropropionic acid-induced behavioral, biochemical, and mitochondrial dysfunction in an animal model of Huntington's disease. J Med Food 2009, 12 (3), 591-600.
[75] Durg, S.; Dhadde, S. B.; Vandal, R.; Shivakumar, B. S.; Charan, C. S., Withania somnifera (Ashwagandha) in neurobehavioural disorders induced by brain oxidative stress in rodents: a systematic review and meta-analysis. J Pharm Pharmacol 2015, 67 (7), 879-99.
[76] Chatterjee, S.; Srivastava, S.; Khalid, A.; Singh, N.; Sangwan, R. S.; Sidhu, O. P.; Roy, R.; Khetrapal, C. L.; Tuli, R., Comprehensive metabolic fingerprinting of Withania somnifera leaf and root extracts. Phytochemistry 2010, 71 (10), 1085-94.
[77] Minhas, U.; Minz, R.; Das, P.; Bhatnagar, A., Therapeutic effect of Withania somnifera on pristane-induced model of SLE. Inflammopharmacology 2012, 20 (4), 195-205.

[78] Prakash, J.; Yadav, S. K.; Chouhan, S.; Singh, S. P., Neuroprotective role of Withania somnifera root extract in maneb-paraquat induced mouse model of parkinsonism. Neurochem Res 2013, 38 (5), 972-80.

[79] Kataria, H.; Wadhwa, R.; Kaul, S. C.; Kaur, G., Water extract from the leaves of Withania somnifera protect RA differentiated C6 and IMR-32 cells against glutamate-induced excitotoxicity. PLoS One 2012, 7 (5), e37080.

[80] Kumar, S.; Harris, R. J.; Seal, C. J.; Okello, E. J., An aqueous extract of Withania somnifera root inhibits amyloid β fibril formation in vitro. Phytotherapy research 2012, 26 (1), 113-117.

[81] Mohanty, I.; Gupta, S. K.; Talwar, K. K.; Dinda, A.; Joshi, S.; Bansal, P.; Saxena, A.; Arya, D. S., Cardioprotection from ischemia and reperfusion injury by Withania somnifera: a hemodynamic, biochemical and histopathological assessment. Molecular and cellular biochemistry 2004, 260 (1), 39-47.

[82] Reuland, D. J.; Khademi, S.; Castle, C. J.; Irwin, D. C.; McCord, J. M.; Miller, B. F.; Hamilton, K. L., Upregulation of phase II enzymes through phytochemical activation of Nrf2 protects cardiomyocytes against oxidant stress. Free Radical Biology and Medicine 2013, 56, 102-111.

[83] Mohanty, I.; Arya, D. S.; Dinda, A.; Talwar, K. K.; Joshi, S.; Gupta, S. K., Mechanisms of cardioprotective effect of Withania somnifera in experimentally induced myocardial infarction. Basic & clinical pharmacology & toxicology 2004, 94 (4), 184-190.

[84] Hina, S.; Rehman, K.; Dogar, Z.-u.-H.; Jahan, N.; Hameed, M.; Khan, Z. I.; Ahmad, K.; Mukhtar, K.; Valeem, E. E., Cardioprotective effect of gemmotherapeutically treated Withania somnifera against chemically induced myocardial injury. Pak J Bot 2010, 42 (2), 1487-1499.

[85] Hamza, A.; Amin, A.; Daoud, S., The protective effect of a purified extract of Withania somnifera against doxorubicin-induced cardiac toxicity in rats. Cell biology and toxicology 2008, 24 (1), 63-73.

[86] Mohanty, I. R.; Arya, D. S.; Gupta, S. K., Withania somnifera provides cardioprotection and attenuates ischemia–reperfusion induced apoptosis. Clinical Nutrition 2008, 27 (4), 635-642.

[87] Gorelick, J.; Rosenberg, R.; Smotrich, A.; Hanuš, L.; Bernstein, N., Hypoglycemic activity of withanolides and elicitated Withania somnifera. Phytochemistry 2015, 116, 283-289.

[88] Andallu, B.; Radhika, B., Hypoglycemic, diuretic and hypocholesterolemic effect of winter cherry (Withania somnifera, Dunal) root. 2000.

[89] Anwer, T.; Sharma, M.; Pillai, K. K.; Iqbal, M., Effect of Withania somnifera on insulin sensitivity in non-insulin-dependent diabetes mellitus rats. Basic & clinical pharmacology & toxicology 2008, 102 (6), 498-503.

[90] Udayakumar, R.; Kasthurirengan, S.; Mariashibu, T. S.; Rajesh, M.; Anbazhagan, V. R.; Kim, S. C.; Ganapathi, A.; Choi, C. W., Hypoglycaemic and hypolipidaemic effects of Withania somnifera root and leaf extracts on alloxan-induced diabetic rats. International journal of molecular sciences 2009, 10 (5), 2367-2382.

[91] Anup, K.; Mohan, K.; Suraj, S.; Sandip, F.; Bhushan, F.; Prashant, W., Antimicrobial Activity of Some Important Medicinal Plants of India against Some Plant and Human Pathogens. Research Journal of Pharmacy and Technology 2010, 3 (3), 924-926.

[92] Bisht, P.; Rawat, V., Antibacterial activity of Withania somnifera against Gram-positive isolates from pus samples. Ayu 2014, 35 (3), 330.

[93] Al-Ani, N.; Hadi, S. A.; Nazar, R., Antimicrobial activities of Withania somnifera Crude extract. Sci Agri 2013, 4, 74-76.

[94] Mwitari, P. G.; Ayeka, P. A.; Ondicho, J.; Matu, E. N.; Bii, C. C., Antimicrobial activity and probable mechanisms of action of medicinal plants of Kenya: Withania somnifera, Warbugia ugandensis, Prunus africana and Plectrunthus barbatus. PloS one 2013, 8 (6), e65619.

[95] Owais, M.; Sharad, K.; Shehbaz, A.; Saleemuddin, M., Antibacterial efficacy of Withania somnifera (ashwagandha) an indigenous medicinal plant against experimental murine salmonellosis. Phytomedicine 2005, 12 (3), 229-235.

[96] Singh, N.; Bhalla, M.; de Jager, P.; Gilca, M., An overview on ashwagandha: A Rasayana (Rejuvenator) of Ayurveda. African Journal of Traditional, Complementary and Alternative Medicines 2011, 8 (5S).

[97] Kaur, K.; Rani, G.; Widodo, N.; Nagpal, A.; Taira, K.; Kaul, S.; Wadhwa, R., Evaluation of the anti-proliferative and anti-oxidative activities of leaf extract from in vivo and in vitro raised Ashwagandha. Food and chemical toxicology 2004, 42 (12), 2015-2020.

[98] Mahdi, A. A.; Shukla, K. K.; Ahmad, M. K.; Rajender, S.; Shankhwar, S. N.; Singh, V.; Dalela, D., Withania somnifera improves semen quality in stress-related male fertility. Evidence-Based Complementary and Alternative Medicine 2011, 2011.

[99] Baitharu, I.; Jain, V.; Deep, S. N.; Hota, K. B.; Hota, S. K.; Prasad, D.; Ilavazhagan, G., Withania somnifera root extract ameliorates hypobaric hypoxia induced memory impairment in rats. Journal of ethnopharmacology 2013, 145 (2), 431-441.

Supplementary Information