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# Introduction

Since the beginning of time, human societies have had a close relationship with plants because of their magical ability to treat illness. According to Winter and Tang, 2012, Yuan *et al.,* 2016, about 80% of the world's population uses plants and substances derived from plants for their fundamental healthcare needs. The quantity and quality of plants, however, have started to pose a significant obstacle to the development of herbal-based formulations. Secondary metabolites are unique sources of therapeutic benefits in medicinal plants. However, in response to numerous biotic and abiotic stressors, plants accumulate secondary metabolites. So, the secondary metabolites of traditionally farmed plants show significant divergence as a result of environmental stress factors, and it even negatively affects the biosynthesis pathway of pharmacological actives (Atanasov *et al.,* 2015, Ochoa-Villarreal *et al*., 2015). The generation of secondary metabolites is impacted by UV radiation, wounding, nutritional shortages, and herbicide content in the soil. Field cultivation is challenging because growth rates are slow, overexploitation is possible, heavy metals accumulate, and frequent microbial attacks. It has taken a revolutionary approach to create plants that are disease-resistant, contaminant-free, and capable of rapid multiplication (Debnarh *et al.,* 2006, Altpeter *et al.,* 2016). So, *in vitro* plants grown under controlled conditions provide a solid foundation for the homogeneous synthesis of major therapeutic actives, and metabolites free from seasonal and environmental variance. The *in vitro* cultivation of plants showed greater productivity over the course of the growing season compared to field cultivated plants (Sangeetha *et al.,* 2022).

*W. somnifera*, or Indian ginseng, is one of the most illustrious and consistently fascinating medicinal plants for researchers due to its innumerable therapeutic benefits. These ethnomedicinal herbs are notable for their usage in indigenous medical systems to treat age related illnesses and

as good adaptogens to reduce bodily stress (Day *et al.,* 2017). Beyond its rejuvenating properties, it has neuroprotective effects (Kurapati *et al.,* 2013, Nagashayana *et al.,* 2000), and it improves the functioning of muscarinic receptors to revamp the cognitive ability of the brain. It has outstanding antioxidant activity both *in vivo* and *in vitro* its capacity to associate and collaborate with a variety of antioxidant enzymes such as glutathione peroxidase, monodehydroginase reductase, and peroxidase (Ahmed *et al.,* 2018, Mishra *et al.,* 2014, Sabina *et al.,* 2013). Furthermore, it has also been claimed to increase ATP synthesis by reducing mitochondrial damage (Vidyashankar *et al.,* 2014).

This herb has been used as a Rasayana for its wide range of health benefits in Ayurveda, the traditional medical system in India for millennia. The 'Rasayana' is a type of herbal formulation that promotes mental and physical state and brings happiness. As well as being given to children as tonics, it is also common for middle-aged and elderly individuals to take these types of remedies in order to prolong their lives. The herb *W. somnifera* holds the top position among Rasayana herbs in Ayurveda. It is known as 'Sattvic Kapha Rasayana' Herb (Changhadi, 1938). As a botanical medicine, it is used throughout the world in a variety of forms like infusions, decoctions, ointments (external application), powder, tonic and syrups (Davis and Kuttan, 2001, *Kumar et al.,* 2007) and used as the dominant ingredient over a hundred of formulations in Ayurveda (Sangwan *et al.,* 2007). Among the 98 reported genera, the family Solanaceae contains the genus *Withania* which is very important for its therapeutic usage. In addition to the twenty-six known species of this genus, *W. coagulans, W. somnifera, W. adunensis, and W. riebeckii* exist in several parts of the world (Atal and Kapoor, 1989, Sirkar, 1989).

A steroidal lactone, specifically withanolides, is what gives *W. somnifera* its medical relevance (Jayaprakasam *et al.,* 2003), Choudhary *et al.,* 2010), Kaileh *et al.,* 2007). Withanolide A (WA) and withaferin A (WFA), the two most prevalent withanolides, are being explored more and more for their potential medicinal value. It is due to the high lipid solubility of WFA that it is able to cross the blood-brain barrier (BBB) in such a remarkable manner

(Swarup *et al.,* 2011). Additionally, WFA has received attention for its effectiveness in the promotion of autophagy, the ubiquitin-proteasome pathway (UPS) function, neuroprotection, anti-inflammatory effects, antioxidant benefits, and neurodegeneration (Zhang *et al.,* 2017, Livne-Bar *et al.,* 2016). Several brain diseases, including amyloid pathology of AD, neuritis regeneration, recovery of injured synapses, reanimation of neuritis, protuberance of axonal rejoins, etc., have also been linked to WA (Zhao *et al.,* 2002; Kuboyama *et al.,* 2002, Kuboyama *et al.,* 2005, Baitharu *et al.,* 2014).

The amount of plant material required to produce withanolide in India is estimated to be 9,127 tonnes, while the amount of withanolide produced is 5,905 tonnes. (Sharadha *et al.,* 2007, Sivanandhan *et al.,* 2012) and most commercial withanolides are derived from field grown plants. In response to the ever-escalating utilization of bioactive withanolides, the plant variety has been exploited haphazardly, putting its wild stock in jeopardy. To address the current demand for therapeutically potent withanolides, the pharmaceutical industry commonly uses field grown plants that are uprooted at randomly (Mir *et al.,* 2014). The extraction of withanolide from plant sources is also hampered by a long gestation period, poor growth rate, and low concentration of active chemicals. withanolides unique accumulation patterns and structural complexity make large-scale extraction and chemical synthesis impossible. So, the therapeutical significance of withanolides is insufficient to meet annual requirements (Senthil *et al.,* 2015). In this scenario, to avoid this chronic shortage of *W. somnifera in vitro* cultivation serves as a possible substitute.

Plant cells and organ can be cultured under *in vitro* conditions, which has enormous potential to complement field cultivation and to achieve valuable bioactive secondary metabolites on an industrial scale (Sangwan *et al.,* 2007). Furthermore, it is possible to obtain novel metabolites in large quantities by manipulating culture conditions and nutrient supplementation during *in vitro* propagation of *Withania* plants. *In vitro* cultures are aimed primarily at obtaining differentiated tissues with the ability to synthesize secondary metabolites as high

as or comparable to the mother plants. Moreover, *in vitro* plants grown in standardized settings provide a well-established platform for producing secondary metabolites in a consistent manner regardless of seasonal or environmental variations. As a result, the development of fast-growing *in vitro* plants would provide new prospects for drug production without relying on field cultivated plants, which are prone to variability. However, the acceptance of this approach is not justifiable without, standardization of herbal formulation developed using *in vitro* grown plant tissue, systematic scientific validation, and appropriate clinical trials including toxicity assessment using animal models.

Herbal medicine standardization consists of prescribing a set of inherent parameters, such as quality, efficacy, safety, and reproducibility criteria. Further, the standardization of herbal medicine begins from harvesting plants to extreme clinical trials. Technical standards are developed and agreed upon through this process. Observation and experimentation lead to the development of specific standards for prescribing medicines based on a set of characteristics that they exhibit. Therefore, standardization is an important tool in the process of quality control (Kunle, 2012). American Herbal Product association defines: Standardization refers to the body of information and controls necessary to produce material of reasonable consistency. Agricultural and manufacturing processes are monitored for quality assurance practices in order to minimize the inherent variation in natural product composition (Waldesch, 2003). The term standardization refers to all measures taken during the manufacturing process and quality control that result in reproducible quality. As well as covering the entire life cycle of a plant, it also encompasses its clinical application. To adjust a herbal drug preparations to a defined level of active constituent or ingredient is also a part of quality control process (Bhutani, 2003). So that, the evaluation refers to the process of verifying identity of a drug, determining its quality and purity, and identifying its adulteration characteristics (Uba *et al.,* 2016).

It is necessary to take into consideration all aspects contributing to the quality of herbal drugs in the standardization process, including the correct identification of the sample, organoleptic evaluation, pharmacognostic evaluation,

volatile matter, quantitative evaluation (ash values, extractive values), phytochemical evaluation, test for xenobiotics, microbial load, toxicity, and biological activity tests. It is particularly important to pay attention to the phytochemical profile of herbal drugs, since it contributes directly to their activity. As a guideline for the phytochemical profile of the drug in order to ensure its quality, fingerprint profiles serve as guidelines, while quantification of the marker compound or compounds shall serve as a further parameter for assessing the quality of a sample. (Nikam *et al.,* 2012). Hence, we attempted to develop a herbal powder using *in vitro* and field grown tissues of *W. somnifera* and standardize the powder using standardized pharmacological method and comprehend the multidimensional animal model system *C. elegans* in order to investigate and compare the efficacy and safety profiles of *in vitro* and field generated *W. somnifera* powder in this work. Further, the study also compared life extension and neuroprotection efficiency and some insights into the underlying genetic mechanism of the *in vitro* and field cultivated *W. somnifera* tissues.

This study was designed to test the following hypothesis:

- **Null hypothesis (H0):** *In vitro* shoot tissues of *W. somnifera* are not bioactive as traditionally used field grown roots.

- **Alternate hypothesis (HA):** *In vitro* shoot tissues of *W. somnifera* as bioactive as traditionally used field grown roots.

**To validate *in vitro* shoot of *W. somnifera* with known withanolide content and study its potential efficacy and prove it is equally bioactive as field roots.**

The present study was formulated with the following objectives:

- To compare pharmacogenetic and physicochemical validation of the *W. somnifera* powders developed using *in vitro* and field grown tissues.

- To compare the phytoconstituent and free radical scavenging activity *in vitro* and field grown *W. somnifera*

- To identify the total metabolites profile of *in vitro* and field grown tissues of *W. somnifera*
- To compare toxicity, life extension, oxidative stress resistance and neuroprotective potential of *in vitro* and field grown *W. somnifera* in *C. elegans* as model.