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Antioxidant activity of *Withania somnifera* L. *In vitro* and *In vivo* grown explants

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Abstract
To probe the antioxidant potential of *Withania somnifera* due to the presence of secondary metabolites, it possesses considerable importance in various pharmacological activities. In addition, these metabolites are often associated with defense against pathogen, response against stresses. To enhance or to control the metabolism of these secondary metabolites, transcription factors (TF) came into play a very important role. In the present study different plants of *Withania* (R1, R2, R3 and R4) were successfully propagated and grown in *in vitro* and *in vivo condition* and their comparative propagation rates were measured. There antioxidant activity was also measured by spectroscopic method. Reaction was carried out in 4 replicates in *in vitro* and *in vivo* condition.

Keywords: *Withania somnifera, In vitro* and *In vivo,* ascorbic acid, chlorophyll content, total phenolic content

1. Introduction
Withania somnifera Dunal L. is one of the most important herb in Ayurvedic and indigenous medical systems in India. It is commonly known as Ashwagandha, Indian ginseng, winter cherry and it belongs to family solanaceae. Plants have been therapeutic sources for curing diseases from the time immemorial and in recent times, these plants occupy an important position as the paramount sources of drug discoveries. Due to their pharmaceutical and biological activities, plant secondary metabolites have been used for centuries in traditional medicine. Their curative properties are due to the presence of chemical substances of complex composition known as secondary metabolites (Rana *et al*., 2014) [7]. These compounds are regarded as "luxury items" that are not required for growth and development of plants in normal.

Conditions but are required when a plant encounters any type of stress or pathogenic attack. Thus, these compounds play important role in defense and adaptation to certain environmental conditions. It has been revealed that plants produce more than 100,000 secondary metabolites, which might increase to 200,000 once they are characterized structurally (Rana *et al.,* 2014) [7].

In recent years most of the drug researches are on herbal medications. Pharmaceutical companies largely depend upon material produced from naturally occurring plants which are being depleted rapidly, thus raising concern about possible extinction of the species. Hence, interest in *in-vitro* culture technique has increased in last few years. As these techniques are viable tools for mass multiplication, large-scale propagation and germplasm conservation of rare, endangered and threatened medicinal plant (Rana *et al.,* 2014, Bhuria *et al.,* 2014, Siddique *et al.,* 2016) [7, 1, 8].

Antioxidants are either enzymatic or non-enzymatic which protect the cell or the organism from the damage caused by ROS. The condition of the cell in which the concentration of ROS goes beyond the limit which can be stabilized by the action of antioxidants is called oxidative stress (Karuppanapandian *et. al.,* 2011) [5]. Simple concept of oxidative stress is when balance is maintained between ROS production and antioxidant defense. The result is 'oxidative stress' which deregulate the cellular function and cause many different diseases in case of plant programmed cell death like 1-Damage of DNA, 2-Oxidation of polyunsaturated fatty acid in LPO and amino acids in proteins, 3-It damages the macromolecules like carbohydrates and in human body such as again arthritis, asthma, carcinogenesis, diabetes, rheumatism and various neural degenerative disease. ROS basically are free radical such as singlet oxygen, superoxide,

peroxyl radicals, hydroxyl radicals. Every plant contains antioxidant compounds, which protect the plant cells against the ROS (Karuppanapandian *et al.*, 2011) [5], Shrivastava and Sahu., 2013 [9]. Antioxidant neutralized free radical reaction in the plant cells. For controlled level of ROS and protect those cells injury under stress condition, it's important that ROS should be scavenged by the antioxidants. In plant secondary metabolism very efficient enzymatic and non-enzymatic defense system, which form versatile and flexible unit in modulating ROS level. Major ROS scavenging enzymatic antioxidants are catalase (CAT), ascorbate peroxidase (APX), guaiacol peroxidase (GPX), superoxide dismutase (SOD), monodehydroascorbate reductase (MDHAR), dehydroascorbate reductase (DHAR), glutathione reductase (GR), and non-enzymatic antioxidants are ascorbic acid, glutathione, tocopherols, carotenoids, phenolics compounds (Karuppanapandian *et al.*, 2011) [5]. The antioxidant activity of *Withania* is associated with free radical oxidative damage had result in treatment of many diseases. The active principles of *Withania* like sitoinosides and with farin A (Glycowithanolides) in equimolar concentration was found to increase SOD, CAT, and GPX levels in rat brain frontal cortex and striatum. An increase in these enzymes results in increased antioxidant activity and decrease in enzymes represent decreased antioxidant activity. Active withanolides were tested experimentally on animals, mice and rabbits in dose related manner which implies that dose related increase in all enzymes were observed showing antioxidant effect in the brain may be responsible for its diverse pharmacological properties (Goil *et al.*, 2012) [3].

2. Materials and Methods

2.1 Replication of plants

In the present study *Withania somnifera* L. plants used to know the antioxidant effect in different concentrations *in vitro* and *in vivo* Grown Explants.

2.2 Chlorophyll content: Tissue were ground with the 12 ml 70% acetone. Homogenate was centrifuged at 10,000 rpm for 10 minutes and supernatant collected in another eppendrof tube. Extraction procedure was repeated on until the residue were colorless. The extracts Pooled and made up to 10ml. with 70% acetone. Absorbance were taken at 645 and 663 nm.

Calculation

$$\text{Chlorophyll a (mg g}^{-1}\text{ fw)} = \frac{\{12.7(A663)-2.63(A645)\}V}{1000\times W\,(g)}$$

$$\text{Chlorophyll b (mg g-1 fw)} = \frac{\{22.9(A645)-4.68(A663)\}\,V}{1000\times W\,(g)}$$

$$\text{Total chlorophyll (mg g-1fw)} = \frac{[20.2(A645)+8.02(A663)]\,V}{1000\times W\,(g)}$$

Where: A_{510} and A_{480}=absorption at these wavelengths
V=Final extract volume (ml)
W=Weight of sample (g)

2.3 DPPH radical – scavenging activity: *Scavenging'' radical capacity* of samples was determined using DPPH radical. Reaction mixtures (0.001 to 1 mg/ml) of samples were prepared by mixing appropriate amounts of extract, 2.5ml of DPPH and methanol to a total volume of 10ml. Prepared solutions were left in the dark for 60 minutes and then the absorbance was measured at 515 nm. All determinations were performed in triplicate. Methanol was used to zero spectrophotometer methanolic solutions pure compounds (gallic acid, BHT, Trolox, ascorbic acid, rutin and quercethin) were tested too at different concentration. DPPH radicals" scavenging "capacity was expressed by applying the following equation DPPH – *RSC (%) = 100 × {A0-A1}* Ao-absorbance of blank solution, A1- absorbance of solution in the present of active components.

2.4 Ascorbic Acid

Plant tissue ground with 10% trichloroacetic acid (TCA) and centrifuged at 10,000 rpm for 10 minutes 2 times. Collect the supernatant made up to 10ml. with 10% TCA. 10 ml supernatant, 2.0ml. Of 2,4-Dinitrophenyl hydrazine-thiourea-$CuSO_4$ (DTC) was added and incubated for 3 hours at 37^0C. After incubation added 0.80 ml of ice cold 65% H_2SO_4 add in the solution and mixed carefully incubate it for half an hour at 30^0C. Absorbance were taken at 520 nm.

2.5 Estimation of total phenolic content

Total phenol content in the samples was estimated by the method of Hossain *et al*., 2013 Reagents: 80% methanol, Folin-Ciocalteu reagent (FCR), 20% Na_2CO_3, Standard (100 mg catechol in 100 ml of water).

2.5.1 Procedure: Tissue (1g) was finely chopped into small pieces and refluxed in 80% methanol for 10 min. After cooling, the tissue was homogenized with mortar and pestle. The homogenate was filtered and centrifuged at 10000 rpm for 10 min. The supernatant was used for the estimation of total phenols. An aliquot of the sample was pipetted out and made up to 3 ml with 80% methanol. Folin-ciocalteau reagent (0.5 ml) was added and kept for 3 min. 2 ml, 20% Na_2 CO_3 was added to the mixture and kept in boiling water bath for 1 min. The white precipitate was removed by centrifuging for 10 min and the absorbance of the clear light blue solution was recorded at 650 nm against the reagent blank containing 3 ml 80% methanol, 0.5 ml Folin's reagent and 2 ml 20% Na_2 CO_3. A standard graph of phenols was constructed with pyrocatechol by taking absorbance against different concentration. Calculation of total phenols g-1 tissue was calculated from the standard.

3. Result and discussion

3.1 chlorophyll a, Chlorophyll b and total chlorophyll content (m mol $min^{-1}g^{-1}$ fw) in *in vivo* Condition:

The concentration scrutiny of the data demonstrated decrease in chlorophyll a, b, and total chlorophyll content in the leaves, The maximum decrease in R_4 Chl.a (0.440±0.002), Chl.b is(0.373±0.002) and total chlorophyll (0.732±0.015). Then R_2 Chl.a (0.630±0.005), Chl.b (0.410±0.004), total chlorophyll (0.790±0.009). In R_1 Chl.a (0.739±0.005), Chl.b (0.459±0.006), total chlorophyll (0.730±0.010). Highest concentration of chlorophyll was observed in R_3 Chl.a (0.670±0.008), Chl.b (0.670±0.008), total chl. (0.859±0.009). The reduction were observed in R_1 95.8% in chl.a,65% in Chl.b and total chlorophyll 84%, R_2 86% in chl.a,68% in Chl.b and total chlorophyll 89%, R_3 97% in chl.a,95% in Chl.b and total chlorophyll 92%, R_4 84% in chl.a,65% in Chl.b and total chlorophyll 85% Fig.1,table1. This observation was also supported by Chandra and Alam, (2013) [2] in the effect on chlorophyll in seedlings of *Brassica juncea* (L.) The decrease in chlorophyll content may be due to reduced chlorophyll biosynthesis by inhibiting δ-amino levulinic dehydrogenase and protochlorophyllide reductase

activities and breakdown of pigments or their precursor as reported by Teramura and Sullivan in 1994.

3.2 Activity Ascorbic Acid in *in vitro* and *in vivo* condition:

The concentration wise analysis of the result of ascorbic acid activity in the leaves showed maximum content were observed in *in vitro* condition R_1 82%, R_2 79.5%, R_3 95.5%,R_4 65% and in Case of *in vivo* Condition R_1 70%, R_2 65%, R_3 79%, R_4 50%. Observation were taken in *in vitro* R_4 (0.47±.0.009), R_1 (0.29±0.008), $_{R2}$ (0.34±0.008) and highest concentration were noted in R_3 (0.78±0.010). In *in vivo* R_4 (0.39±0.004), R_1 (0.19±0.003), R_2 (0.22±0.003) and highest concentration were noted in R_3 (0.65±0.005) Fig.2, table2. There were significant increase level retinol, α- pherol and ascorbic acid Cd-treated seedlings stated in the experiment effects of some heavy metals on content of chlorophyll, proline and some antioxidant chemicals in bean (*phaseolus vulgaris* l.) seedlings.Ascorbic acid (AA) is a key antioxidant, and involved in protection of plant cells against oxidative damage catalyzed by ROS. It acts as a chain breaking antioxidant impairs with the formation of free radicals in the process of formation of intracellular substances throughout the plants body (Prakash *et al*., 2009) [6]. It is essentially required in scavenging of H_2O_2. by acorbates –glutathione cycle, ii) elimination of ROS, iii) used maintenance of α-tocopherol in reduced form, and iv) utilized as a cofactor in xanthophylls cycle to protect chloroplast against photooxidative damage (Smirnoff, 2000) [10].

3.3 Total phenol content in *in vitro* and *in vivo* condition

The concentration wise analysis of the result of total phenolic content in leaves observation were taken in *in vitro* R_4 (0.110±0.005), R_2 (0.165±0.006), R_1 (0.180±0.007) and highest concentration were noted in R_3 (0.195±0.010). In *in vivo* R_4 (0.105±.0.003), R_2 (0.135±0.005), R_1 (0.129±0.007) and highest concentration were noted in R_3 (0.140±0.009). Phenolic compound can be involved in the H_2O_2 scavenging cascade in the plant's cells. (Takahama and Oniki, 1997) [11]. the total phenolic content was observed in *in vitro* condition R_1 92%, R_2 90%, R_3 97%, R_4 67% and in Case of *in vivo* Condition R_1 70%, R_2 65%, R_3 75%, R_4 45% Fig.3, table3. Effect of abiotic stress on plants correlated with total free phenols. Phenolic compounds are recognized as class of antioxidant which act as a in the form of free radicals scavengers. Phenolics have various functions in plants. An enhancement of phenylpropanoid metabolism and the number of phenolic compounds can be observed under different environmental factors and stress conditions. The induction of phenolic compound biosynthesis was observed in wheat in response to nickel toxicity.

Table 1: chlorophyll a, Chlorophyll b and total chlorophyll content (m mol $min^{-1}g^{-1}fw$) in *in vivo* Condition of Withania *Somnifera*

Treatment	Chlorophyll a	Chlorophyll b	Total Chlorophyll
R_0 Control	0.789±0.007	0.628±0.004	1.301±0.009
R_1	0.639±0.005	0.459±0.006	0.730±0.010
R_2	0.630±0.005	0.410±0.004	0.790±0.009
R_3	0.770±0.008	0.670±0.008	0.859±0.009
R_4	0.440±0.002	0.373±0.002	0.732±0.015

Table 2: Ascorbic acid content (µ mol min^{-1} $g^{-1}fw$) From *in vitro* and *in vivo* Grown leaves Samples of *W. somnifera*

Treatment	*In vitro*	*In vivo*	*In vitro*	*In vivo*
R_1	0.29±0.008	0.19±0.003	82%	70%
R_2	0.34±0.008	0.22±0.003	79.95%	65%
R_3	0.78±0.010	0.65±0.005	95.5%	79%
R_4	0.47±.0.009	0.39±0.004	65%	50%

Table 3: Total phenol content (µ mol min^{-1} $g^{-1}fw$) From *in vitro* and *in vivo* Grown leaves Samples of *W. somnifera*

Treatment	*In vitro*	*In vivo*	*In vitro*	*In vivo*
R_1	0.180±0.007	0.129±0.007	92.1%	70%
R_2	0.165±0.006	0.135±0.005	90%	65%
R_3	0.195±0.010	0.140±0.009	96.8%	75%
R_4	0.110±0.005	0.105±.0.003	65%	45%

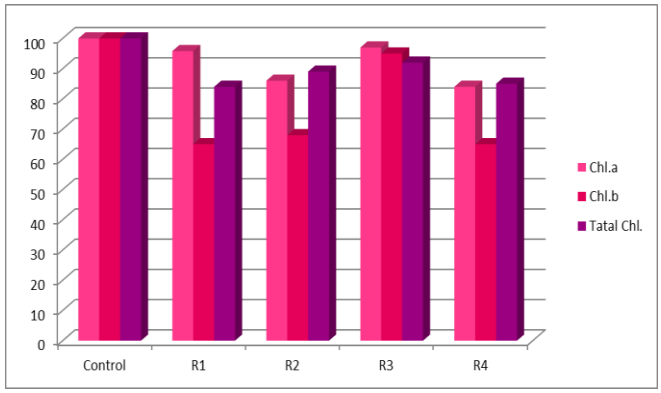

Fig 1: Effect of Chlorophyll a, Chlorophyll b and total chlorophyll content in (m mol min^{-1} g^{-1} fw) *in vivo* Condition of *Withania Somnifera*.

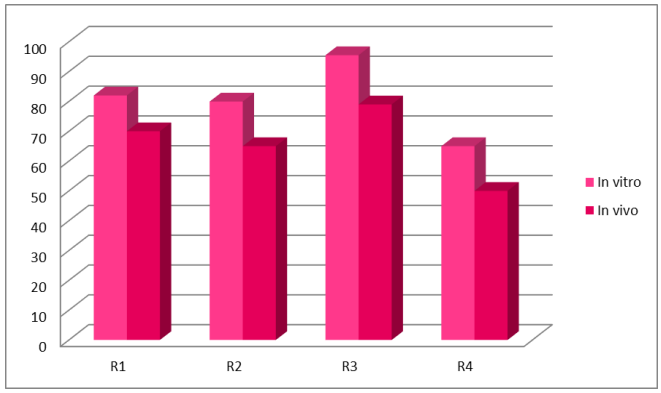

Fig 2: The effect of the ascorbic acid content (µ mol min^{-1} $g^{-1}fw$) from *in vitro* and *in vivo* Grown leaves Samples of *W. somnifera*

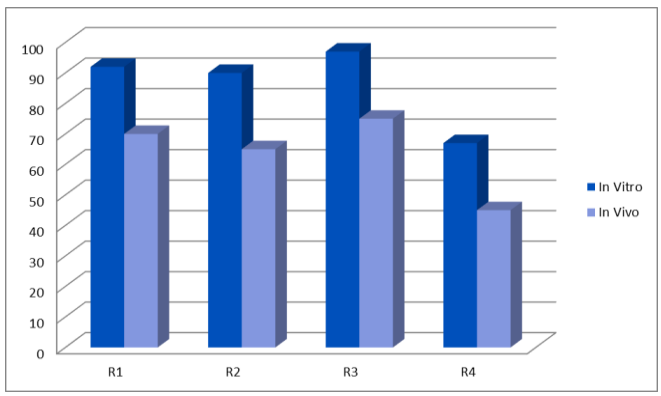

Fig 3: Effect of total phenol content (µ mol min^{-1} g^{-1} fw) from *in vitro* and *in vivo* Grown leaves Samples of *W. somnifera*

4. Conclusion

On the basis of the above results it can be concluded that experiments aimed to obtain 100% germination frequency in *in vitro* condition with minimum chances of infection in *Withania somnifera*. Plant antioxidants give rise to the formation of a vast array of chemically complex compounds, many of which are commercially important. Difference of Antioxidants activity in *In vitro* and *In vivo* condition is totally varese. In *In vitro* condition found to have more ascorbic acid activity and total phenolic activity as comparison to *In vivo* condition of Withania *somnifera.*

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6. References

1. Bhuria P, Singh Y, Tiwari S, Chaturvedi M. Studies on multiple shoot regeneration from nodal explant of ashwagandha (*Withania Somnifera*). Ind. Res. J Genet. & Biotech. 2014; 6:521-525.
2. Chandra R Pantola, Afroz Alam. Correlation between copper induced changes in peroxidase activities and its accumulation in seedling roots of Brassica juncea Mycopath. 2013; 11(2):59-64.
3. Goi1 AH, Fereshteh M, Mehdi R. Phenolic compounds and antioxidant activity from saffron (*Crocus sativus* L.). J of Agricul. Sci. 2012; 10:392-398.
4. Kushwaha AK, Sangwan NS, Trivedi PK, Negi AS, Mishra LN, Sangwan RS. Tropine forming tropinone reductase gene from *Withania somnifera* a (Ashwagandha): biochemical characteristics of the recombinant enzyme and novel physiological overtones of tissue-wide gene expression. Plos. One. 2013; 8:74-90.
5. Karuppanapandian T, Cheol C, Changsoo k, Manoharan K. Reactive oxygen species in plants: their generation, signal transduction, and scavenging mechanisms Afr. J Crop Sci. 2011; 5:709-725.
6. Prakash V, Mishra PK, Mishra M. Screening of medical plant extract for antioxidant activity. J Medicinal Plants Res. 2009; 3:608-612.
7. Rana S, Bhat WW, Dhar N, Vishwakarma R. Molecular characterization of two A-type cyt. P450s, WsCYP98A and WsCYP76A from withanolide accumulation in response to exogenous elicitation. J of Med. Pharm. Sci. 2014; 14:2-17.
8. Siddique A, Joshia P, Mishra L, Sangwan NS, Darokara PK. 5, 6-De-epoxy-5-en-7-one-17-hydroxy With aferin A, a new cytotoxic steroid from *Withania somnifera* L. Dunal leaves. Nat. Pro. res. 2016; 28:392-398.
9. Shrivastava AK, Sahu PK. Economics of yield and production of alkaloid of *Withania somnifera* (L.) Dunal. American J of Plant Sci. 2013; 4:2023-2030.
10. Smirnoff N. Ascorbic acid: metabolism and function of a multifaceted molecule. Curr. Opin. Plant. Biol. 2000; 3:229-235.
11. Takahama U, Oniki T. A peroxide / phenolics / ascorbate system can scavenge hydrogen peroxide in plant cells. Physiol. Plant. 1997; 101:845-473.