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ORIGINAL ARTICLE

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Stress-induced impacts on anti-oxidant potential and production of photosynthetic pigments of *Gymnema sylvestre* (Retz.) R. Br. ex Sm.

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Gymnema sylvestre is a perennial woody climber belonging to the family Apocynaceae. The leaves and roots of the plant have medicinal properties and are used in the Ayurveda system of medicine to treat various diseases. It is an important medicinal plant mainly for its antidiabetic properties and also it possess anti-inflammatory, anti-microbial and anti-oxidant activities. In this study the effect of stress signals in the pigment production and anti-oxidant potential assessed. The experiment was conducted in a poly house with twelve replications of each treatment. The different stress signals were Zinc sulphate, Sodium nitroprusside, Salicylic acid, Jasmonic acid and Squalene. The present study reveals an idea about the effect of stress signals in antioxidant activity and pigment production. The fresh leaf samples of treated plants were collected for pigment estimation. The leaf samples of treated plants were collected, dried and extracted in methanol and this methanolic extracts were used for analysis of anti-oxidant activity. A variation in both the parameters observed in different treatments.

Key words: Anti-oxidant, Gymnema silvestre, Medicinal plant, Photosynthetic pigments, Stress signals

Gymnema sylvestre is slow slow-growing plant belonging to the family Apocynaceae. It is found ideally in tropical and subtropical humid climates and is common in hills of evergreen forests. It is a perennial woody climber and generally requires support for growth. Historically, the leaves of *Gymnema sylvestre* were used to treat diabetes and other diseases, while the flowers and bark were used for diseases related to tuberculosis (Kirtikar and Basu, 1975). It is used to lower blood pressure, tachycardia or arrhythmias, hypolipidemia, weight loss, stomach problems, constipation, water retention, and liver diseases. It can also be utilized to lower serum cholesterol, triglycerides, and blood sugar levels (hypoglycemic or antihyperglycemic), as well as to treat eye problems. It has anti-inflammatory properties, smooth muscle relaxant, cataract prevention, dental caries and as anti-cancer agent. (Thakur *et al.*, 2012).

MATERIALS AND METHODS

The vegetatively propagated plants of *Gymnema sylvestre* (Retz.) R.Br. ex Sm. for the experiments were collected from Kottakkal Arya Vaidyasala Medicinal Garden. It was maintained in a polyhouse. Stress signals of three different categories included mineral salts -Zinc sulphate and Sodium nitroprusside, elicitors - Salicylic acid and Jasmonic acid and mineral feeder-squalene. The concentrations of the stress chemicals were, Sodium nitroprusside- SNP 0.1mM (SNP1), SNP0.3mM (SNP2) and SNP 0.5mM (SNP 3), Zinc sulphate -ZS 1mM (ZS 1), ZS 5mM (ZS 2) and ZS 9mM (ZS 3), Salicylic acid – SA 1mM (SA1), SA 5mM (SA2) and SA 9Mm (SA3) and Squalene –SQ 1µM (SQ1), SQ 3µM (SQ2), SQ 5µM (SQ3). Methanol was used as the extraction solvent. For pigment estimation, Di Methyl Sulphoxide (DMSO) was the solvent.

Application of stress

The mode of application is foliar spray in the early morning and the frequency of application was once in a week. After a period of 90 days the leaves were collected and shade dried and made powder.

Extraction

Methanolic extracts were prepared using soxhlet extraction method 2g of dried powder of *Gymnema sylvestre* leaf was placed in a thimble, connected to a flask containing the extraction solvent. The powder was dissolved in 200 ml methanol. After complete extraction it is concentrated in a water bath and the final volume is 50ml.

Leaf pigment estimation

Leaf pigment contents estimated according to the method of Hiscox and Israelstm (1978). Fresh leaf samples were washed in distilled water and blotted with filter papers. 0.25 g fresh leaf sample was weighed and put it in a tube containing 7 ml DMSO. Incubated for 1 hour at 600 C and the supernatant were collected. The absorbance was read at 470, 646, 663 and 750 nm against the solvent blank DMSO using UV- VIS Spectrophotometer. Pigments present in the extract was calculated as microgram pigment per gram fresh weight using the formula

$$\textbf{Chlorophyll a} = \frac{20.12\{A_{663} - A_{750}\} - 2.69\{A_{646} - A_{750}\}}{\text{fresh weight of the sample}} \times \text{volume}$$

$$\textbf{Chlorophyll b} = \frac{22.9\{A_{646} - A_{750}\} - 4.68\{A_{663} - A_{750}\}}{\text{fresh weight of the sample}} \times \text{volume}$$

$$\textbf{Carotenoids} = \frac{1000\{A_{470}\} + 3.27\{\text{Chl a} - \text{Chl b}\}}{\text{fresh weight of the sample} \times 229} \times \text{volume}$$

$$\textbf{Chlorophyll a + b} = \frac{20.12\{A_{646} - A_{750}\}\ 8.02\{A_{663} - A_{750}\}}{\text{fresh weight of the sample}} \times \text{volume}$$

Anti-oxidant activity

The DPPH radical quenching activities of methanolic extract of *Gymnema sylvestre* leaf was assessed using the method developed by Chang *et al.* (2002).180 µl of different samples of methanolic extracts were taken. This was added to 60 µl of DPPH (0.1 mM) solution and the system was kept for 10 minutes in dark. After incubation, the absorbance of the samples at 517 nm was measured using methanol as a blank using UV- VIS Spectrophotometer. In the same way, standard samples were also run with control being the DPPH in methanol and standard as known concentration (50µl) of antioxidant ascorbic acid. The DPPH radical inhibition percentage was calculated as stated in the below formula. % Inhibition = (ODc − ODt / ODc) × 100 Wherein, ODc = control optical density and ODt = test sample optical density.

RESULTS AND DISCUSSION

Results of Leaf pigment estimation

In the sodium nitroprusside treated samples SNP

0.1mM and SNP 0.5mM there is considerable increase in pigment production (Figure 1). In the samples treated with Zinc sulphate none of them showed improved pigment production (Figure 2). In the leaves of plants treated with different concentration of Salicylic acid show increased concentrations of all the pigments when compare with control (Figure 3). In the case of Jasmonic acid treatment lower concentration 50µm concentration showed an increase in all pigment contents and 100µm treatment given the maximum result in total pigments (Figure 4). When squalene is applied, its lower concentration of concentration 1µM and higher concentration 5µM shows increased pigment concentration. The result obtained for 5µM was similar to that of control (Figure 5).

Of the all obtained data Jasmonic acid of 100µM gave a total pigment content of 3.1630 which is recorded highest in this study.

Results of Anti-oxidant activity

While assessing the anti-oxidant activity in the present study maximum percentage of inhibition of free radicals observed in samples treated with Sodium nitroprusside 0.5mM concentration that is 79.81± 0.44 (Figure 6). Salicylic acid 5mM treated samples are coming next to this with percentage of inhibition 66.26 ±.54 (Figure 8). All other treatments under the study also showed increased anti-oxidant activity. More than 50% of inhibition observed in SA 1 mM (51.68±0.6137), SA 5mM 66.26±0.54, JA 100 µM 63.91±0.44, JA 150 µM 57.96±0.49, SQ 50.74±0.41.

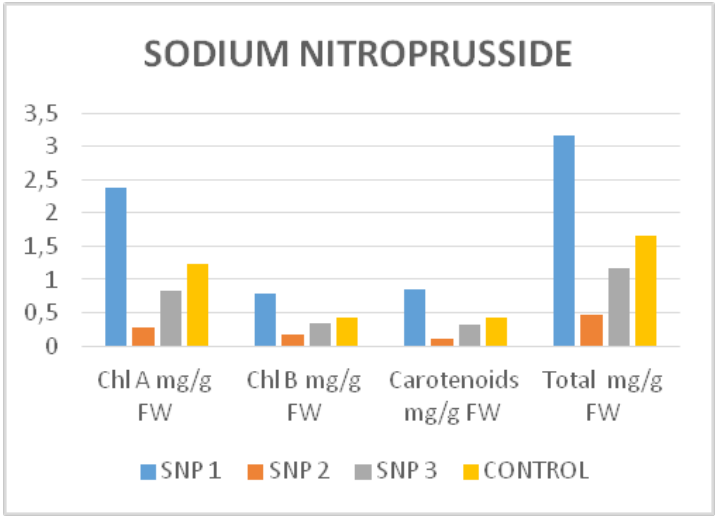

Figure 1: Sodium nitroprusside- SNP 0.1mM (SNP1), SNP0.3mM (SNP2) and SNP 0.5mM (SNP 3)

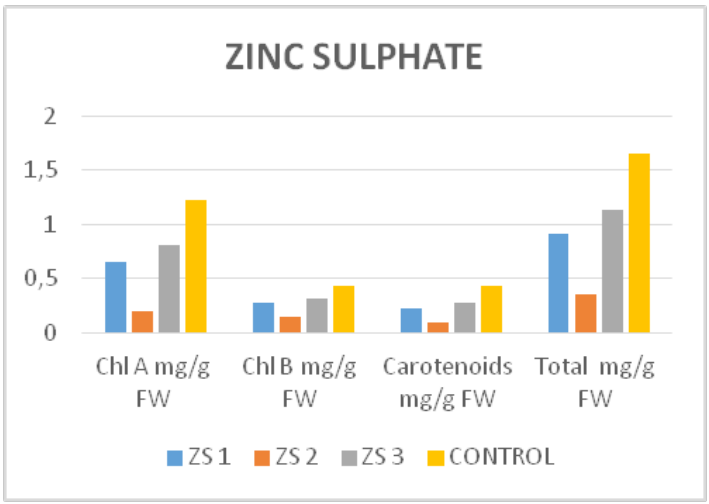

Figure 2: Zinc sulphate -ZS 1mM (ZS 1), ZS 5mM (ZS 2) and ZS 9mM (ZS 3)

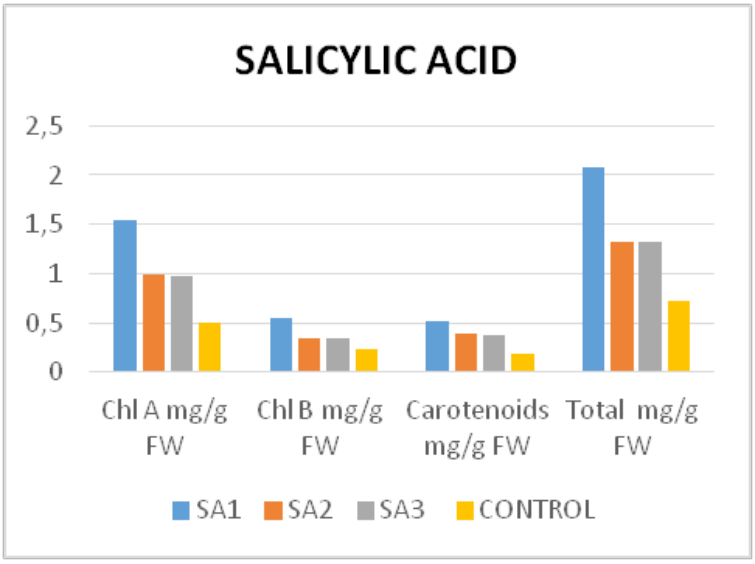

Figure 3: Salicylic acid - SA1 mM (SA1), SA 5mM (SA2) and SA 9Mm (SA3)

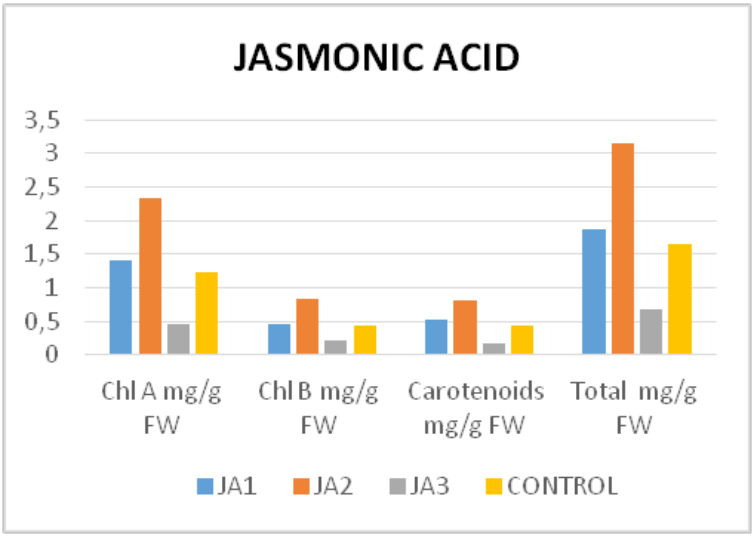

Figure 4: Jasmonic acid – JA50 µM (JA1), JA100 µM (JA2) and JA 150 µM (SJ3)

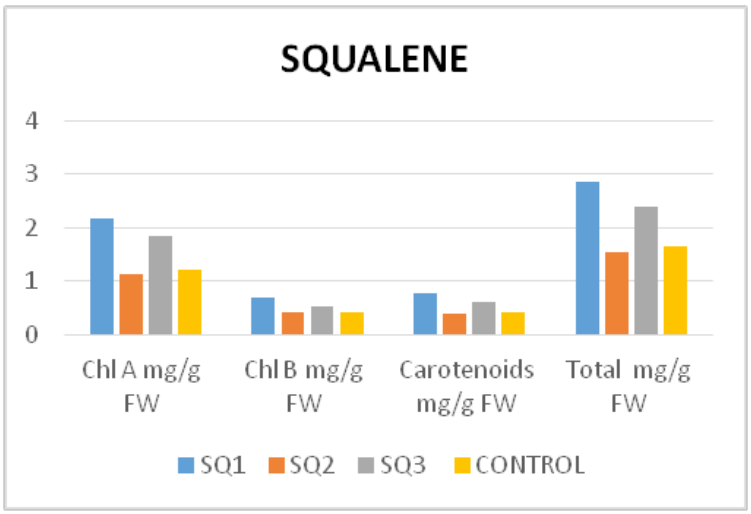

Figure 5: Squalene – SQ 1µM (SQ1), SQ 3µM (SQ2), SQ 5µM (SQ3).

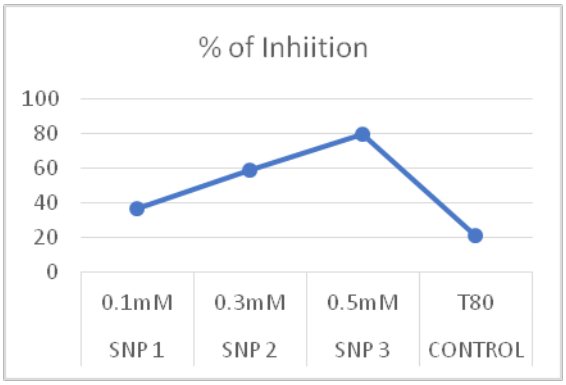

Figure 6: Sodium nitroprusside- SNP 0.1mM (SNP1), SNP0.3mM (SNP2) and SNP 0.5mM (SNP 3)

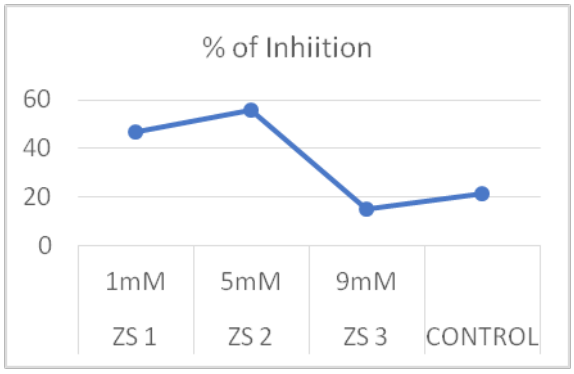

Figure 7: Zinc sulphate -ZS 1mM (ZS 1), ZS 5mM (ZS 2) and ZS 9mM (ZS 3)

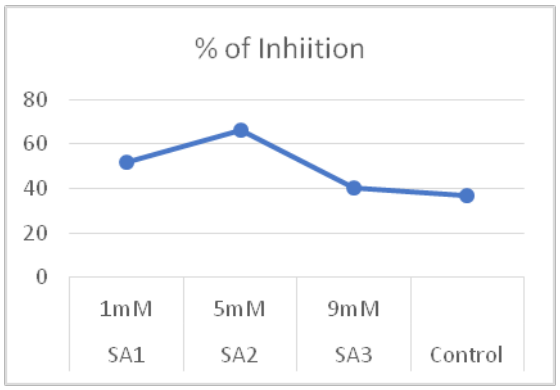

Figure 8: Salicylic acid - SA1 mM (SA1), SA 5mM (SA2) and SA 9Mm (SA3)

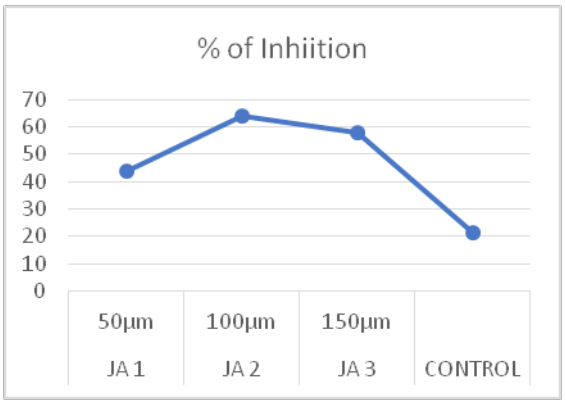

Figure 9: Jasmonic acid – JA50 µM (JA1), JA100 µM (JA2) and JA 150 µM (SJ3)

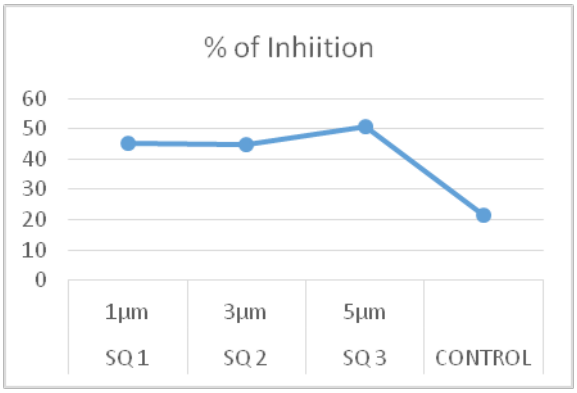

Figure 10: Squalene – SQ 1µM (SQ1), SQ 3µM (SQ2), SQ 5µM (SQ3).

CONCLUSION

Stress signal of various concentrations was applied to the selected medicinal plant *Gymnema sylvestre* and its effects on the antioxidant potential and pigment production were analysed. In the Jasmonic acid treatment, 50µm concentration showed more pigment contents and 100µm treatment shows an increase in all the pigments except chlorophyll. The leaves of plants treated with different concentration of Salicylic acid show increased concentrations of all the pigments when compare with control. Leaf samples treated with Sodium nitroprusside of the concentration 0.1mM shows an increase in all the pigment content. The pigment content in leaf samples treated with squalene of concentration 1µM and 5µM shows increased pigment concentration. Increased concentration of pigments correlates with the higher absorption of light energy and enhanced photosynthetic rate of the plants.

In the present study maximum percentage of inhibition of free radicals observed in samples treated with Sodium nitroprusside 0.5mM concentration that is 79.81± 0.44. Salicylic acid 5mM treated samples are coming next to this with a percentage of inhibition 66.26 ± 0.54. All other treatments under the study also showed increased anti-oxidant activity. In the concentration of 0.5mM Sodium nitroprusside treated samples showed maximum effect in the anti-oxidant activity. *Gymnema sylvestre* is popularly considers as a medicinal plant for its anti-diabetic properties. Present study reveals that applying specific concentrations of stress signals its anti-oxidant potential can be enhanced. Antioxidants help treat a wide range of illnesses in humans, such as cancer, heart disease, diabetes, and inflammatory conditions by lowering oxidative stress in cells.

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CONFLICTS OF INTEREST

The authors declare that they have no potential conflicts of interest.

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