International Journal of Engineering Research & Management Technology
ISSN: 2348-4039
July-August 2023 Volume-10, Issue-4
Email: editor@ijermt.org www.ijermt.org

STUDY ON EFFECTS OF STRESS-ADAPTED ENDOPHYTES ON THE GROWTH OF *Andrographis paniculata* UNDER ABIOTIC STRESS

Santosh Kumar Pandey,

Research Scholar, Glocal School of Science,

Glocal University, Mirzapure Pole Saharanpur (U.P).

Prof.(Dr.) Krishan Pal,

Research Supervisor, Glocal School of Science,

Glocal University, Mirzapure Pole Saharanpur(U.P).

ABSTRACT

The present study focuses on the effect of endophytic bacteria on the growth of *Andographic paniculata* grown at an altitude of 786ft under salt and heavy metal stress. The seeds were inoculated with the two endophytic bacteria *Solibacillus silvestris DL3R2 and Pelomonas aquatica AIS1S* isolated from *Abutilon indicum* and *Hermocallis fulva*. The seeds were inoculated with the two endophytic bacteria *Solibacillus silvestris DL3R2 and Pelomonas aquatica AIS1S* isolated from *Abutilon indicum* and *Hermocallis fulva*. The seeds were sown in pots, and growth was checked. On comparing inoculated seeds with control, it was found that the highest mean germination recorded was 2.33±0.47 at 2% salt concentration. Similarly, the maximum root length recorded is 5.3 ±0.5 at 2% lead concentration and the maximum shoot length recorded is 23.7±2.4 at 2% salt concentration.). The maximum dry root and shoot weights recorded is 1.25±0.1 and 2.2±0.07 by the seeds inoculated with *Solibacillus silvestris DL3R2* at 2% lead concentration. The phytochemicals of the experimental plant were compared with the in-situ plants. HPLC analysis has shown that the amount of quercetin and gallic produced at higher altitudes was 0.11%and 0.21%, which is not very less than a plant grown at lower altitudes, i.e., 0.12% 0.22 %, respectively. The other unknown compounds detected at 254 nm were swertiamarin, sweroside mangiferin, amarogentin, swertisin, and caffeic acid. This study provides a basis that plants can be grown under stress conditions with the help of endophytic bacteria, thus suggesting a new step toward the conservation of plants.

Keywords: *Swertia, endophytes*, Solibacillus, root weight, germination

INTRODUCTION

Andrographis paniculata is a herbaceous medicinal plant found in the Acanthacea family. It is commonly found in Southeast Asian tropical nations like India, Thailand, Myanmar, Indonesia, and southern China. It is a component of several ancient oriental remedies and is referred to as "Kalmegh" in India. Known as the "king of bitterness," it has the power to detoxify and

eliminate heat while also lowering blood pressure and reducing swelling(Li et al., 2022).

Andographic paniculata extracts have recently been the subject of numerous pharmacological research, primarily due to their widespread use in treating the symptoms of various viral diseases and immunological disorders. According to reports, *A. paniculata* is barely hazardous to plants and animals. The primary chemical components thought to be in charge of the biological activity of the plant are diterpenoid lactones and flavonoids. The primary active ingredient, and rographolide, has a bitter taste and is primarily present in the leaves (> 2%, w/w). The aerial portion of the plant has been the subject of most phytochemical studies (Chuaa et al., 2013).

Endophytes play an important role in plant growth and development. They are microorganisms that dwell in the host tissues and secrete various compounds such as IAA, gibberellin, exopolysaccharides, etc., that help in plant growth and produce certain enzymes that prevent pest attacks (Eid et al., 2021). They also help the plant tolerate a wide range of stress. The present research deals with the effect of such stress tolerance endophytic bacteria on *Andographic paniculata* when grown at lower altitudes under different stress conditions.

MATERIALS AND METHODS

Seed collection and sterilization

Authentic seeds of *Andographic paniculata* were procured from the Office of Herbal Garden Saharanpur Tehsil. Behat District Saharanpur UP. Seeds were cleaned with distilled water, and surface sterilization was done with 70% ethanol for 1 min. They were further sterilized with 3%Sodium hypochlorite and were again washed with autoclaved water to remove the chemical (Dubeyet al., 2021, Madhaiyan et al., 2015). Sterilized seeds were kept on sterile filter paper in autoclaved Petri plates.

Inoculation of seeds

Seeds were inoculated with the two-stress tolerant endophytic bacteria *Pelomonas aquatica AIS1S and Solibacillus silvestris DL3R2* isolated from *Abutilon indicum* and *Hermocallis fulva*. Seeds were sown in triplicate at a depth of 2 cm in pots with 3:1 of sterile moist soil with farmyard manure. The soil was treated with sodium chloride (2-10%) and heavy metal cadmium and lead (2-6%) concentrations. The pots were kept in a semi-shady area with a temperature range of 30-35^{0}C, and growth was observed for 3 months.

Extract preparation

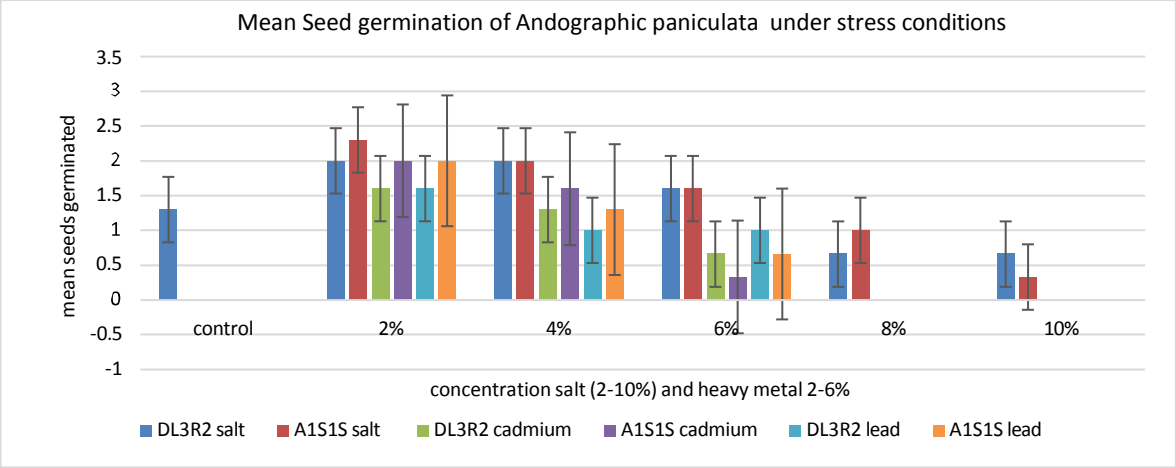
Fresh roots of plants were collected from the Office of Herbal Garden Saharanpur Tehsil. Behat District Saharanpur UP.and from plants grown in labs for comparing phytochemical constituents. Roots were washed twice to remove the dirt, air dried, and were converted into powder. 2 g of fine powder was further subjected to extraction in 250 mL of methanol by the soxhlet extraction method. A rotary evaporator was used to evaporate the liquid to obtain semi-solid material, which was used further to analyze phytochemicals (Singh et al., 2018).

HPLC analysis

Chromatographic analysis was carried out through a reverse phase HPLC system. C18 column (25 cm × 4.6 mm, 5 µm) was used to separate compounds. The column temperature and flow rate were kept at 35 °C and 1 mL/min. 10 µLof samples were injected, and the wavelength was detected at 350,420 and 254 nm. The mobile phase with a gradient elution system comprises 0.5% acetic acid in water at 0 min, 70% at 30 min (Lee et al., 2020). Quercetin and gallic acid were used as the standard solution (Tabin et al., 2016).

RESULTS AND DISCUSSION

All the seeds grown under stress conditions were monitored for their mean germination and growth under salt (2-10%) and heavy metal (lead, cadmium 2-6%) stress (**Fig. 1, Table 1, Graph 1).**



Graph 1: Ggermination % of *Andographic paniculata* seeds under salt and heavy metal (cadmium and lead) stress at different concentrations

Fig 1(a) *Andographic paniculata* seeds (b) Seeds sown under stress (c) Plant growth after three months (d) Roots of *Andographic paniculata*

Table 1: Seed Germination of *Andographic paniculata* under stress conditions

Stress	Isolates	Concentrations (%)					
		control	2%	4%	6%	8%	10%
Salt	*Solibacillus silvestris DL3R2*	1.3±0.47	2±0	2±0.81	1.6±1.24	0.6±0.47	0.6±0.47
	Pelomonas aquatica AIS1S		2.3±0.4	2±0.81	1.6±0.47	1±0	0.3±0.47
Cadmium	*Solibacillus silvestris DL3R2*		1.6±0.4	1.3±0.4	0.6±0.47		
	Pelomonas aquatica AIS1S		2±0.8	1.6±1.24	0.3±0.47		
Lead	*Solibacillus silvestris DL3R2*		1.6±0.4	1±0.81	0.66±0.47		
	Pelomonas aquatica AIS1S		2±0.8	1.3±0.94	0.66±0.47		

From above graph, it can be seen that the maximum seed germination is shown at 2% salt concentration i.e., on 2.3±0.4 on inoculation with *Pelomonas aquatica AIS1S* under 2% salt stress whereas least mean germination recorded was 0.3±0.47 at 10% salt concentration in seeds inoculated with *Pelomonas aquatica AIS1S.* LSD post hoc test shows that significance difference($p<0.05$) exists among seed germination at different salt concentration whereas at heavy metal concentration no significance existed.

The maximum root length recorded is 5.3 ±0.5 by the seeds inoculated with *Pelomonas aquatica* at 2% lead concentration whereas least root length is 0.06 ±0.09 at 10% salt concentration by the seeds inoculated with *Pelomonas aquatica* AIS1S (Table 2). The maximum shoot length recorded is 23.7±2.4 by the seeds inoculated with *Pelomonas aquatica* at 2% salt concentration whereas least shoot length recorded is 2.4 ±3.4 at 10% salt concentration by the seeds inoculated with *Pelomonas aquatica AIS1S*. LSD post hoc test revealed that significance difference exists among the root length at different salt concentration, cadmium and lead concentration ($p<0.05$) (Table 3). The test also revealed that significance difference exists among the shoot length at different salt concentration, ($p<0.05$).

The maximum dry root weight recorded is 1.25±0.1 by the seeds inoculated with *Solibacillus silvestris DL3R2* at 2% lead concentration whereas least dry root weight recorded is 0.14±0.1 at 10% salt concentration by the seeds inoculated with *Solibacillus silvestris DL3R2.* LSD post hoc test shows that significance difference exists among the dry root weight at different salt and cadmium concentration ($p<0.05$) whereas no significant difference exists at different lead concentration (Table 4). The maximum dry shoot weight recorded is 2.2±0.07 by the seeds inoculated with *Solibacillus silvestris DL3R2* at 2% lead concentration whereas least short weight recorded is 0.2±0.1 at 10% salt concentration by the seeds inoculated with *Pelomonas aquatica AIS1S*.LSD post hoc test revealed that the dry root weight is significantly different at different salt and cadmium concentrations ($p<0.05$) whereas no significant difference exists at different lead concentrations.

Table 2: Root length and shoot length of the plant under stress condition

Stress	Isolates	Control		2%		4%		6%		8%		10%	
		Root length (cm)	Shoot length(cm)	Root length (cm)	Shoot length(cm)	Root length (cm)	Shoot length (cm)	Root length (cm)	Shoot length (cm)	Root length (cm)	Shoot length (cm)	Root length (cm)	Shoot length (cm)
Salt	*Solibacillus silvestris DL3R2*	0.39±0.04	4.2±0.2	5.2 ±0.5	19.6 ±0.7	4.1 ±0.3	15.3 ±0.3	2.2 ±1.5	9.9 ±7.0	1.5 ±1.1	8.1 ±5.9	0.3 ±0.2	5.1 ±3.6
	Pelomonas aquatica AIS1S			5.7 ±0.2	23.7 ±2.4	4.5 ±0.2	18.0 ±0.4	3.7 ±0.1	15.5 ±0.4	3 ±0.1	14.5 ±0.4	0.06 ±0.09	2.4 ±3.4
Cadmium	*Solibacillus silvestris DL3R2*			5.0 ±0.2	16.9 ±0.2	3.7 ±0.3	15.1 ±0.2	1.7 ±1.2	7.5 ±5.3	-			
	Pelomonas aquatica AIS1S			5.3 ±0.5	18.4 ±0.5	3.8 ±2.1	13.7 ±0.2	0.8 ±1.2	7.7 ±5.4	-			
Lead	*Solibacillus silvestris DL3R2*			5.2 ±0.3	17.5 ±0.4	3.5 ±1.7	15.8 ±0.3	1.5 ±1	9.5 ±6.7	-			
	Pelomonas aquatica AIS1S			5.2 ±0.1	18.1 ±0.3	3.4 ±1.7	16.7 ±0.2	1.4 ±1	8.6 ±6.1	-			

Table 3: Dry weight of roots under stress condition

Isolate	Stress	Concentrations					
		Control	2%	4%	6%	8%	10%
		Mean	Mean	Mean	Mean	Mean	Mean
Solibacillus silvestris DL3R2	Salt	0.6±0.03	1.16±0.08	1.01±0.02	0.6±0.4	0.4±0.3	0.14±0.1
Pelomonas aquatica AIS1S	Salt	0.6±0.03	1.19±0.07	1.1±0.03	0.5±0.04	0.7±0.05	0.18±0.1
Solibacillus silvestris DL3R2	Cadmium	0.6±0.03	1.0±0.3	0.6±0.1	0.3±0.2	-	-
Pelomonas aquatica AIS1S	Cadmium	0.6±0.03	1.15±0.2	0.5±0.3	0.14±0.1	-	-
Solibacillus silvestris DL3R2	Lead	0.6±0.03	1.25±0.1	0.5±0.3	0.2±0.1	-	-
Pelomonas aquatica AIS1S	Lead	0.6±0.03	1.17±0.3	0.5±0.3	0.2±0.2	-	-

Table 4: Dry weight of shoot under stress condition

Isolate	Stress	Concentrations					
		control	2%	4%	6%	8%	10%
		Mean	Mean	Mean	Mean	Mean	Mean
Solibacillus silvestris DL3R2	Salt	0.6±0.08	2.2±0.07	1.1±0.04	0.5±0.4	0.5±0.3	0.45±0.3
Pelomonas aquatica AIS1S	Salt	0.6±0.08	2.3±0.2	1.4±0.15	0.6±0.4	0.7±0.04	0.2±0.1
Solibacillus silvestris DL3R2	Cadmium	0.6±0.08	2.0±0.09	1.2±0.02	0.5±0.4	-	-
Pelomonas aquatica AIS1S	Cadmium	0.6±0.08	2.2±0.1	1.2±0.22	0.6±0.4	-	-

Solibacillus silvestris DL3R2	Lead	0.6±0.08	1.8±0.1	1.0±0.11	0.6±0.4	-	-
Pelomonas aquatica AIS1S	Lead	0.6±0.08	1.7±0.3	1.3±0.1	0.7±0.5	-	-

HPLC analysis

High-performance liquid chromatography detected quercetin and gallic acid in *Andographic paniculata* (Fig 2). *The* highest peak was observed at 254 nm. Comparative analysis has shown that the amount of quercetin and gallic produced at higher altitudes was 0.11%and 0.21%, which is not very less than a plant grown at lower altitudes, i.e., 0.12% and 0.22 %, respectively (Fig 3 and 4). The other unknown compounds detected at 254 nm were andrographolide, neoandrographolided, ehydroandrographolide and deoxyandrographolide.

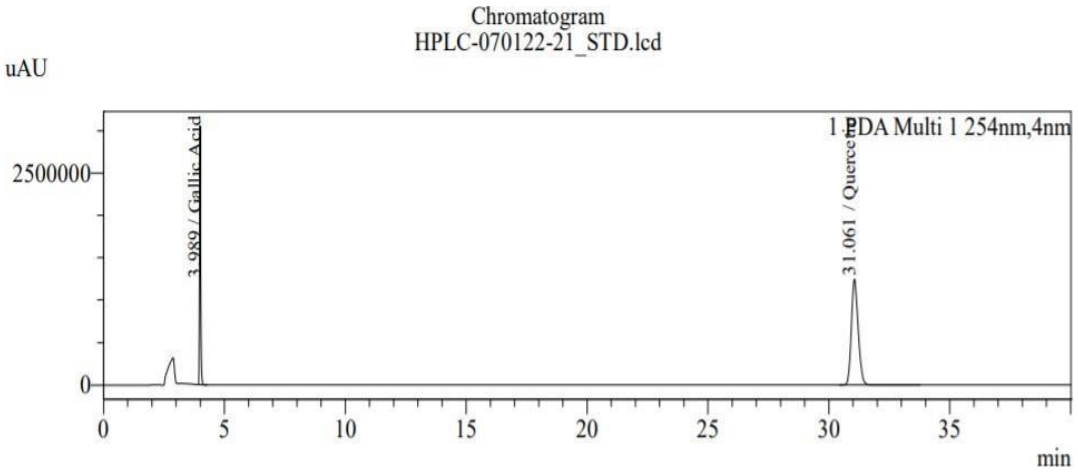


Fig 2: HPLC chromatograms of standard quercetin and gallic acid

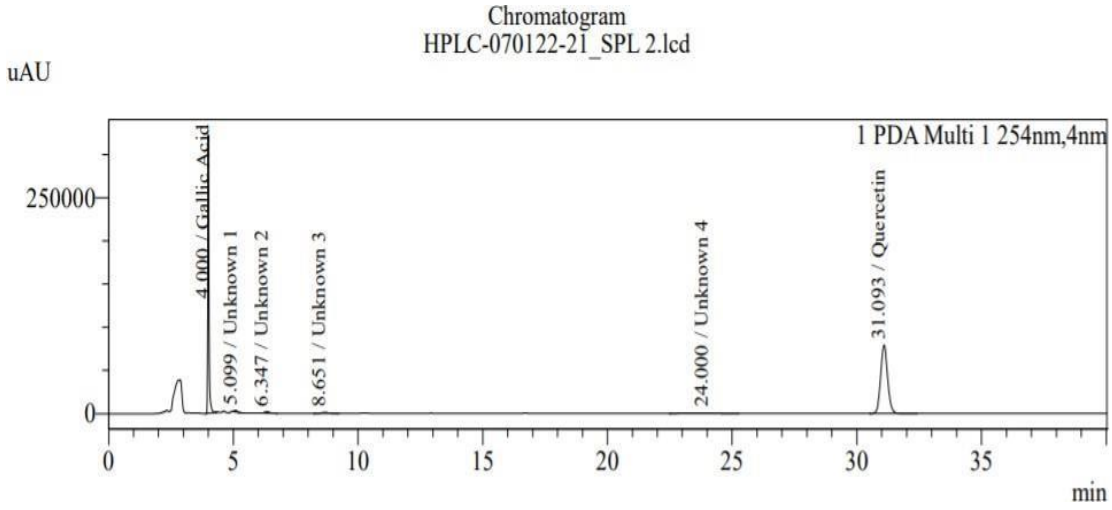


Fig 3: HPLC chromatogram of root sample collected from high altitude (in-situ)

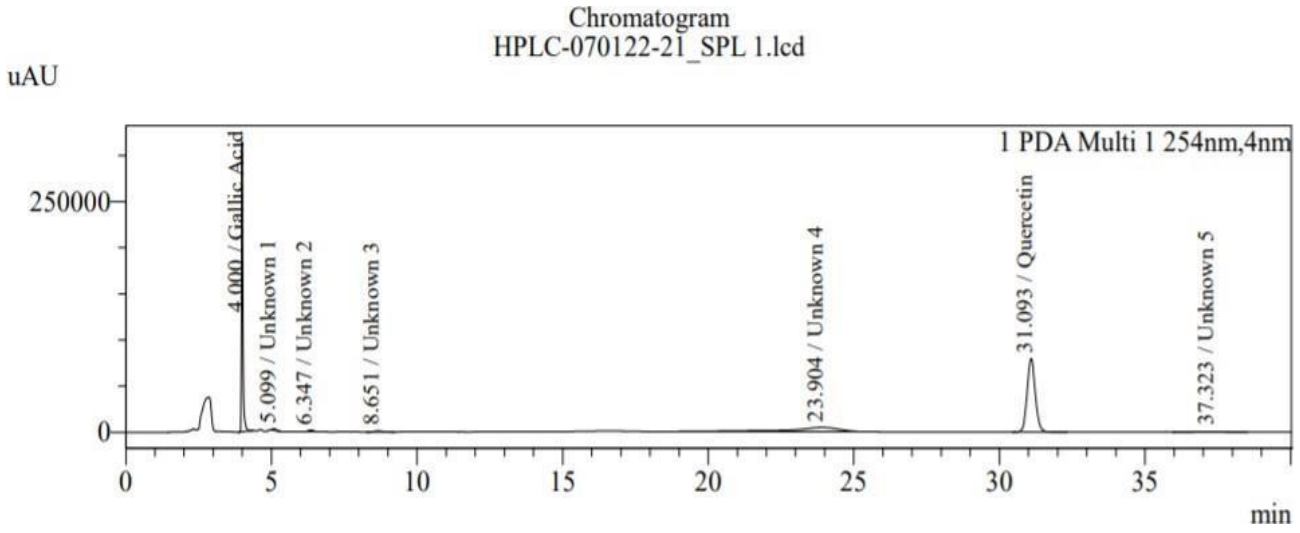


Fig 4: HPLC chromatograms of root sample collected from lower altitude (experimental site)

DISCUSSION

Stress tolerance endophytic bacteria play a significant role in plant growth promotion and make them resilient to stress. They secrete various compounds that help in plant growth but also help maintain osmotic balance and increase nutrient uptake (Yaish et al., 2015). *Andographic paniculata* has pharmaceutical value. The present study was conducted to study the effect of stress tolerance endophytic bacteria on the growth of *Andographis paniculata* lower altitudes. The plant growth is decreased with an increase in salt and heavy metal concentration though the plant was able to tolerate salt stress by upto10% and heavy metal stresses up to 6%. Results show that bacterial inoculated seeds have the highest mean germination compared to control.

The highest germination was recorded under 2% salt concentration, higher than the control mean germination, i.e., 1.3±0.47. Similarly, the maximum root length recorded was 5.3 ±0.5, and the shoot length recorded was 2.4 ±3.4. Studies have shown the effect of salt concentration on the growth, isoenzymes, secondary metabolites, and proline content of the A.paniculata. It was found that growth traits decreased, and the expression level of superoxide dismutase, cytochrome oxidase, peroxidase, and isoenzyme in roots and leaves increased with the increase in salt concentrations. (Shao et al., 2015).

The study was conducted to determine how elicitors such as metal salts, amino acids, and growth enhancers might cause *A. paniculata* seedling cultures to produce excessive amounts of andrographolide. The andrographolide level(25.88 2.72 mg g1 dry weight, 7.09 fold greater than control) was increased by exposing the seeds to elicitors. It also showed significant biomass increment on treating with aspartic acid and casein acid hydrolysate(Das et al., 2019). This study provides evidence that *Andographic paniculata* can be grown at low altitudes suggesting an alternative method to conserve plant species. Comparative analysis of phytochemical constituents' quercetin and gallic acid of in-situ root extract with the experimental plant has shown little difference in production. The amount of quercetin and gallic produced by the plants at higher altitudes

was 0.11%and 0.21%, whereas plants grown at lower altitudes were 0.12% and 0.22 %, respectively. Kurzawa et al. detected caffeine, theophylline, theobromine, indole harmane, harmol, brucine, and strychnine by HPLC. The total alkaloid was found tolie in the 50.71 ± 0.36 mg/g d.m. to 78.71 ± 0.48 mg/g d.m.

CONCLUSION

Andographic paniculata possess medicinal properties and has become endangered habitat due its over exploitation and destruction of natural habitat. Therefore, strict actions are needed to conserve this plant species. This research provides a new basis for the conservation of plant species with the help of endophytic bacteria having stress tolerance property which not helps in conserving plant species away from their native sites but also help them to cope up with the changing environmental conditions.

ACKNOWLEDGEMENT

I am very thankful to Glocal University,MirzaPur Pole, Saharanpur for their support in conducting my research work.

REFERENCES

Chua, L. S., Yap, K. C., and Jaganath, I. B. 2013. Comparison of total phenolic content, scavenging activity and HPLC-ESI-MS/MS profiles of both young and mature leaves and stems of Andrographis paniculata. Natural Product Communications, 8(12), 1934578X1300801217.

Dubey, A., Saiyam, D., Kumar, A., Hashem, A., Abd_Allah, E. F. and Khan, M. L. 2021. Bacterial root endophytes: characterization of their competence and plant growth promotion in soybean (Glycine max (L.) Merr.) under drought stress. International Journal of Environmental Research and Public Health, 18(3): 931.

Das, D., and Bandyopadhyay, M. 2020. Novel approaches towards over-production of andrographolide in in vitro seedling cultures of Andrographis paniculata. South African Journal of Botany, 128, 77-86.

Eid, A. M., Fouda, A., Abdel-Rahman, M. A., Salem, S. S., Elsaied, A., Oelmüller, R. and Hassan, S. E. D. 2021. Harnessing Bacterial Endophytes for Promotion of Plant Growth and Biotechnological Applications: An Overview. Plants, 10(5): 935.

Kurzawa, M., Filipiak-Szok, A., Kłodzińska, E.,Szłyk, E. 2015. Determination of phytochemicals, antioxidant activity and total phenolic content in Andrographis paniculata using chromatographic methods. Journal of Chromatography B, 995, 101-106.

Liu, Z., Mu, S., Li, S., Liang, J., Deng, Y., Yang, Z., and Xie, H. 2022. Hedyotis diffusae Herba-Andrographis Herba inhibits the cellular proliferation of nasopharyngeal carcinoma and triggers DNA damage through activation of p53 and p21. Cancer Gene Therapy, 29(7), 973-983.

.Madhaiyan, M., Alex, T. H. H., Te Ngoh, S., Prithiviraj, B. and Ji, L. 2015. Leaf-residing Methylobacterium species fix nitrogen and promote biomass and seed production in Jatropha curcas. Biotechnology for biofuels, 8(1):1-14.

Singh, R., Tiwari, T., and Chaturvedi, P. 2017. Rheum emodi Wall ex. meissn (Indian Rhubarb): highly endangered medicinal herb. Journal of Medicinal Plants Studies, 5(4), 13-16.

Shao, Y. H., Gao, J. L., Wu, X. W., Li, Q., Wang, J. G., Ding, P., and Lai, X. P. 2015. Effect of salt treatment on growth, isoenzymes and metabolites of Andrographis paniculata (Burm. f.) Nees. Acta physiologiae plantarum, 37(2), 1-12.

Tabin, S., Gupta, R. C., Bansal, G., and Kamili, A. N. 2016. Comparative HPLC analysis of emodin, aloe emodin and rhein in Rheum emodi of wild and in vitro raised plants. Journal of Pharmacognosy and Phytochemistry, 5(2), 121.

Yaish, M. W., Antony, I. and Glick, B. R. 2015. Isolation and characterization of endophytic plant growth-promoting bacteria from date palm tree (Phoenix dactylifera L.) and their potential role in salinity tolerance. Antonie Van Leeuwenhoek, 107(6): 1519-153..