IJARSCT
International Journal of Advanced Research in Science, Communication and Technology
International Open-Access, Double-Blind, Peer-Reviewed, Refereed, Multidisciplinary Online Journal
ISSN: 2581-9429
Volume 6, Issue 10, March 2026
Impact Factor: 8.2

Development and Optimization of an Efficient In Vitro Regeneration Protocol for Hemidesmus indicus (L.) R. Br.

Dr. Amanulla Khan and Ayesha Pangarkar
Anjuman Islam Janjira, Degree College of Science, Murud-Janjira Maharashtra, India
dramanullak@gmail.com

Abstract: *Hemidesmus indicus (L.) R. Br., commonly known as Indian sarsaparilla (Anantmool), is a medicinally important plant widely distributed across various regions of India. The species holds a prominent place in traditional Indian systems of medicine due to its broad spectrum of therapeutic properties. Previous studies have reported its aromatic, antimicrobial, anticancer, antiviral, anti-inflammatory, antipyretic, and antidysenteric activities, along with significant antioxidant and hepatoprotective potential. Considering the medicinal importance and the presence of valuable phenolic compounds, the present study focuses on the development of an efficient in vitro tissue culture protocol for H. indicus. Various explants were cultured under controlled laboratory conditions using different concentrations of plant growth regulators in both solid and liquid culture media to induce multiple shoot and root formation. The results demonstrated a marked enhancement in shoot proliferation and root induction when appropriate combinations of growth hormones, nutrients, and culture media were employed. The optimized protocol provides a reliable method for rapid propagation and conservation of this valuable medicinal plant.*

.**Keywords:**Hemidesmus indicus, tissue culture, plant growth regulators, culture medium.

I. INTRODUCTION

Hemidesmus indicus (L.) R. Br. is a perennial twining shrub belonging to the family **Periplocaceae** (formerly Asclepiadaceae), commonly known as **Indian sarsaparilla** or *Anantmool*. In India, the species is widely distributed across most regions, extending from the upper Gangetic plains eastward to Assam and occurring throughout central, western, and southern parts of the country (1-2). Beyond India, its natural distribution includes Sri Lanka and the Moluccas (3-5). The plant occupies a significant position in traditional Indian systems of medicine, and its extracted plant materials are extensively utilized in the preparation of Ayurvedic, Unani, and Homeopathic formulations.

H. indicus is traditionally employed in the treatment of various ailments such as inflammatory conditions, fever, rheumatism, leprosy, and liver disorders (6). The roots possess a wide range of pharmacological properties, including aromatic, antimicrobial, anticancer, antiviral, anti-inflammatory, antipyretic, and antidysenteric activities (7), along with notable antioxidant and hepatoprotective effects (8-9). Additionally, the roots are used as an adjunct therapy in the treatment of snakebite and scorpion sting (10-11). Owing to its extensive medicinal applications, *Anantmool* holds a prestigious place across all traditional medical systems in India (12). However, indiscriminate harvesting from natural habitats has resulted in severe depletion of wild populations, rendering the species increasingly rare due to overexploitation (13). Ethnic communities traditionally administer the roots internally for conditions such as premature greying of hair, jaundice, and various eye-related disorders.

Morphologically, the roots are long, rigid, cylindrical, and sparsely branched, comprising a lignified central core surrounded by a brownish corky bark marked with furrows and annular cracks. The stems and branches are elongated, narrow, and twine in an anticlockwise direction (14-15). They are profusely laticiferous, woody, and exhibit a deep purple to purplish-brown coloration, with slightly ridged surfaces at the nodes (16-19). Leaves are simple, petiolate,

DOI: 10.48175/IJARSCT-35008

IJARSCT
International Journal of Advanced Research in Science, Communication and Technology
International Open-Access, Double-Blind, Peer-Reviewed, Refereed, Multidisciplinary Online Journal
ISSN: 2581-9429 Volume 6, Issue 10, March 2026 Impact Factor: 8.2

exstipulate, opposite, and entire, with acute to obtuse apices. They are dark green on the upper surface and comparatively paler, occasionally pubescent, on the lower surface. Basal leaves are linear to lanceolate in shape (15).

The flowers are small, axillary, and arranged in sessile racemes, greenish-yellow to greenish-purple externally and dull yellow to light purplish internally, accompanied by imbricate, scale-like bracts. The fruit consists of two long, slender, spreading follicles, while the seeds are numerous, flat, oblong, and bear a tuft of silky white hairs (20-22).

Anatomically, transverse sections of the fresh root are circular with a fairly regular outline, containing a slightly porous woody strand at the center (23). The cork region comprises 3–15 layers of thick-walled, reddish-brown cells, followed by 2–3 layers of colorless phellogen and 1–2 layers of narrow, thin-walled phelloderm cells. Beneath this, 2–3 layers of thick-walled polygonal parenchymatous cells containing starch grains and calcium oxalate crystals are observed (24-25). The cortex is broad and composed of thin-walled, tangentially elongated cells rich in simple and compound starch grains and calcium oxalate prisms. The phloem is narrow and consists of sieve tubes, phloem parenchyma, companion cells, and uniseriate medullary rays (2,4,26). The cambium is distinct and narrow. The xylem contains scattered vessels with pitted walls, tracheids, thick-walled fibers, and uniseriate medullary rays (27).

II. MATERIAL AND METHODS

Healthy plants were collected from Sinhagad Fort, Pune, Maharashtra, located at 18°21′49.12″ N latitude and 73°45′17.99″ E longitude. The collected specimens were transported to the laboratory for further experimental procedures (28-29).

Preparation and Surface Sterilization of Explants: The collected plant material was initially washed thoroughly under running tap water with the addition of Tween-20 to remove surface contaminants (30). Stem nodal segments measuring 2–3 cm were excised and washed in running tap water for 30 minutes. The explants were subsequently treated with 1% (w/v) antifungal solution for 30 minutes under continuous shaking, followed by three washes with sterile autoclaved distilled water. Surface sterilization was carried out by treating the explants with mercuric chloride (HgCl□) for 5–6 minutes, followed by a brief immersion in 70% ethanol for 15–20 seconds. The explants were then rinsed five times with sterile distilled water. All sterilization procedures were performed under aseptic conditions in a laminar air flow cabinet. The sterilized explants were subsequently inoculated onto the culture medium (31-34).

Preparation of Murashige and Skoog (MS) Medium: Murashige and Skoog (MS) medium was prepared using standard protocols. For full-strength MS medium, 3% sucrose (30 g L^{-1}) and 0.8% agar (8 g L^{-1}) were incorporated. The medium consisted of 50 ml macro-nutrient stock, 5 ml micro-nutrient stock, 1 ml organic supplement, and 5 ml iron (Fe) stock, with the final volume adjusted to 1000 ml using distilled water. The pH of the medium was adjusted to 5.8 prior to autoclaving (35).

For half-strength MS (MS½) medium, 25 ml macro-nutrient stock, 2.5 ml micro-nutrient stock, 0.5 ml organic supplement, and 2.5 ml Fe stock were used along with 1.5% sucrose (15 g L^{-1}) and 0.8% agar (4 g L^{-1}). The final volume was adjusted to 1000 ml, and the pH was maintained at 5.8.

Hormonal Treatments: Different concentrations of plant growth regulators (PGRs) were incorporated into both MS and MS½ media to study their effects on shoot proliferation and root induction (36-37).

Inoculation Procedure: Under aseptic conditions in a laminar air flow cabinet, surface-sterilized explants of 2–3 cm length were placed on sterile Petri plates. Both ends of the explants were trimmed using a sterile surgical blade, and the explants were inoculated onto autoclaved slanted culture tubes containing the prepared medium (38-39).

Root Culture: Well-developed roots obtained from subcultured plantlets, measuring 1–1.5 cm, were excised and thoroughly washed to remove adhering solid medium. Three to four root segments were transferred to 500 ml conical flasks containing 50 ml of liquid MS½ medium supplemented with varying concentrations of plant growth regulators, along with a control (40). The cultures were maintained under dark conditions on a rotary shaker at 70 rpm.

Anatomical Studies: Comparative anatomical analyses were performed on in vivo and in vitro raised plants. Samples included the second leaf, stem segment from the second nodal region, and newly formed root tips (7,9,12). Transverse sections of leaf, stem, and root tissues were prepared and stained with safranin and light green. The stained sections

IJARSCT
International Journal of Advanced Research in Science, Communication and Technology
International Open-Access, Double-Blind, Peer-Reviewed, Refereed, Multidisciplinary Online Journal
ISSN: 2581-9429
Volume 6, Issue 10, March 2026
Impact Factor: 8.2

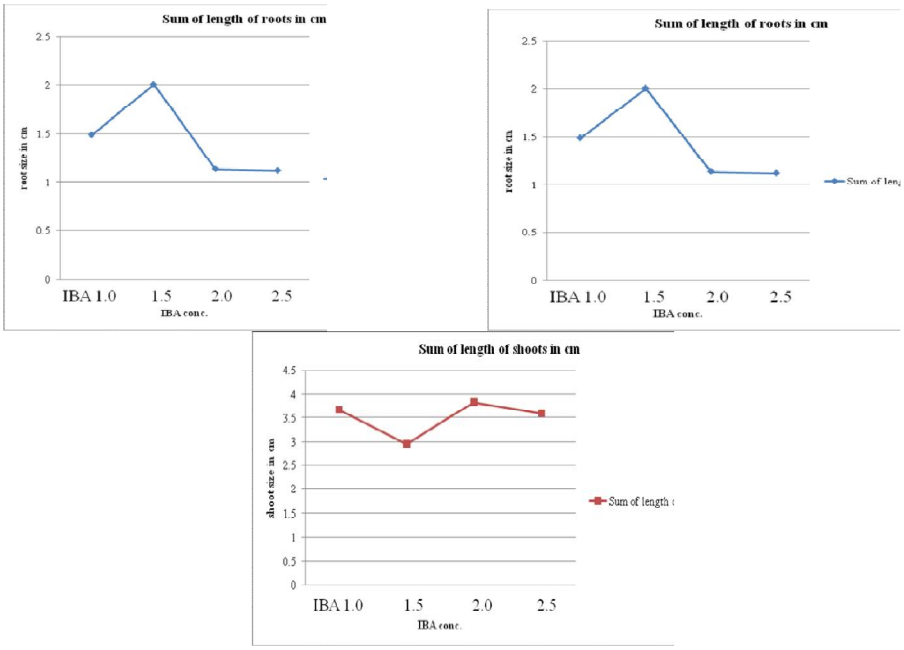
were mounted in 50% glycerin for microscopic examination. Observations focused on the presence and density of leaf and stem trichomes, with counts recorded across 20 microscopic fields for both in vivo and in vitro samples (25).

III. RESULT AND DISCUSSION

Statistically analysis of rooting and shooting observation

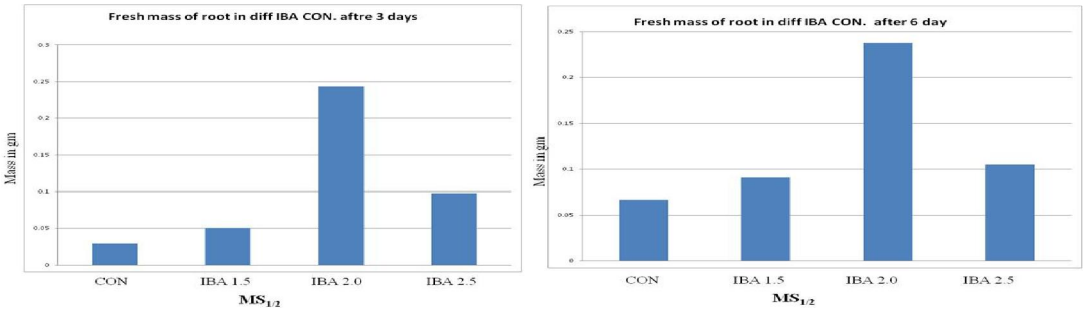
Sr. No	Medium +PGR mg/lit	Number of Replicates for observation	Sum of length of roots in cm	Number of Roots/replicate	Sum of length of shoots in cm	Tot no. of shoots/replicate
1	MS CON	20	0.912.9±0.21	0.970±1.21	2.514±2.80	1.025±0.254
2	MS +KN	60	1.10548±0.253	2.101±1.53	**5.575±3.1319**	1.75±0.8660
3	$MS_{1/2}$ CON	40	0.8±05.1072	1.5±2.010	1.9±2.45	1.3±2.234
4	$MS_{1/2}$+IBA 1.0	100	1.489±0.9222	2±1.1094	3.1875±2.7861	0.75±0.1670
5	$MS_{1/2}$+IBA 1.5	100	**2.009±1.4171**	2.0153±1.260	2.96153±1.5380	2.30±0.5201
6	$MS_{1/2}$+IBA 2.0	100	1.139±0.81135	**3.13636±1.5787**	3.8421±2.193	**3.01±2.3012**
7	$MS_{1/2}$+IBA 2.5	100	1.12246±0.8275	2.66667±1.4505	3.2021±1.6235	2.61±1.2540

Graphical representation of rooting and shooting



IJARSCT
International Journal of Advanced Research in Science, Communication and Technology
International Open-Access, Double-Blind, Peer-Reviewed, Refereed, Multidisciplinary Online Journal
ISSN: 2581-9429
Volume 6, Issue 10, March 2026
Impact Factor: 8.2

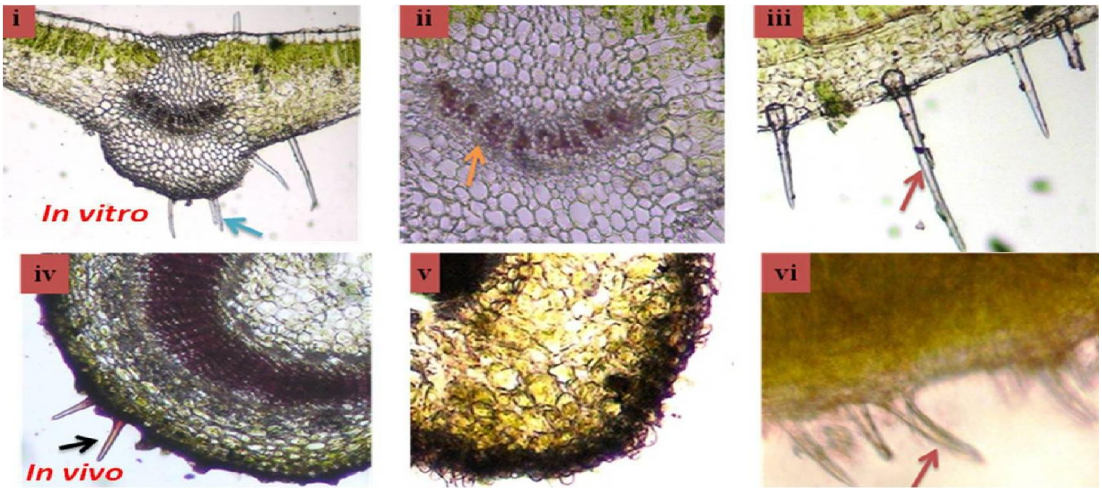
Fresh mass of root in different IBA concentration



Comparison of number of trichomes *in vivo* and *in vitro*:-

No. of Field	Number of *In vivo* trichomes	No. of Field	Number of *In vivo* trichomes	No. of Field	Number of *In vivo* trichomes	No. of Field	Number of *In vivo* trichomes
1	11	1	7	11	8	11	5
2	12	2	8	12	10	12	6
3	12	3	9	13	9	13	5
4	14	4	6	14	10	14	6
5	9	5	4	15	10	15	5
6	9	6	7	16	8	16	7
7	10	7	4	17	7	17	6
8	9	8	5	18	10	18	5
9	6	9	9	19	12	19	6
10	12	10	5	20	8	20	6
				Tot.	196		121
				Mean	9.8		6.05

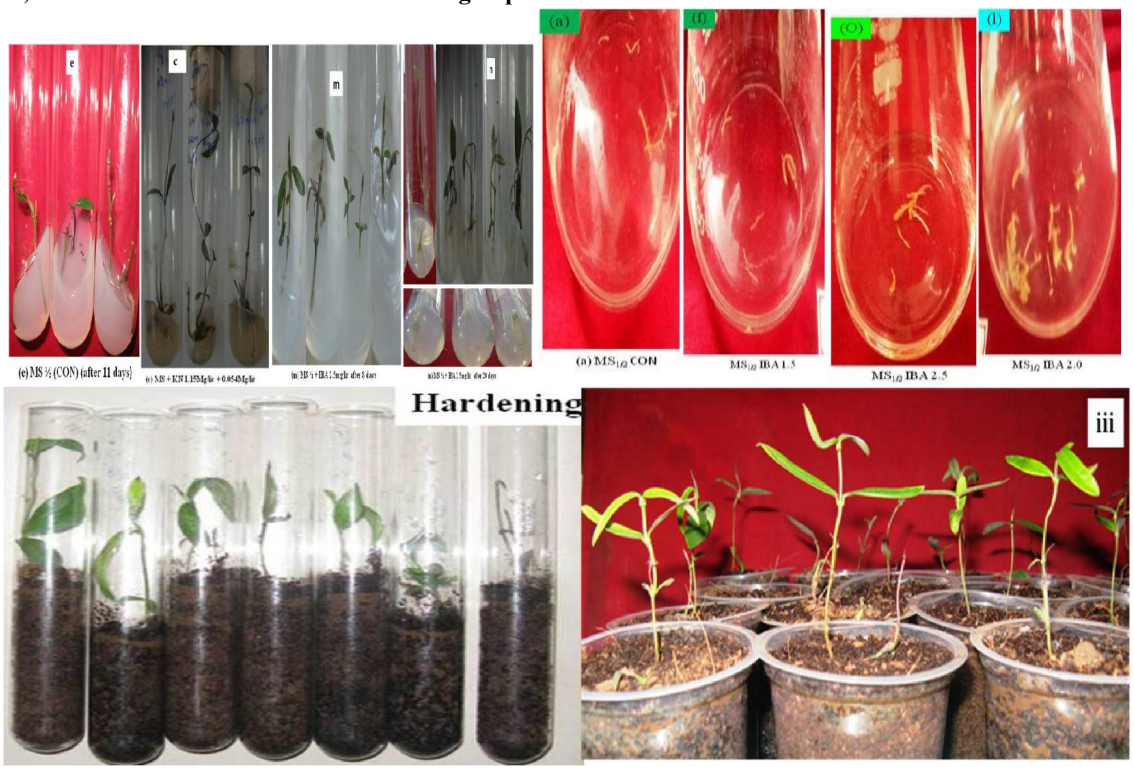
***In vivo* and *in vitro* anatomical observation**



DOI: 10.48175/IJARSCT-35008

IJARSCT
International Journal of Advanced Research in Science, Communication and Technology
International Open-Access, Double-Blind, Peer-Reviewed, Refereed, Multidisciplinary Online Journal
ISSN: 2581-9429
Volume 6, Issue 10, March 2026
Impact Factor: 8.2

Shoot, Root culture observation and Hardening of plant



IV. CONCLUSION

In vitro shoot multiplication exhibited a maximum response on Murashige and Skoog (MS) medium supplemented with kinetin and NAA, where a higher mean number of shoots per explant was recorded. However, the mean shoot length was significantly greater on half-strength MS (MS½) medium supplemented with IBA at 2.0 mg L^{-1}. These observations are in agreement with the findings reported by Sreekumar and Seeni (1998). Root induction studies revealed that MS½ medium supplemented with IBA at 1.5 mg L^{-1} resulted in the highest rooting response and maximum number of roots per explant. Furthermore, root proliferation in liquid culture was most pronounced on MS½ medium containing IBA at 2.0 mg L^{-1}, which showed a significant increase in fresh root biomass. Comparative anatomical analysis of in vivo and in vitro raised plantlets indicated a noticeably higher density of trichomes in in vitro cultured plants compared to those grown under natural conditions.

ACKNOWLEDGEMENTS

My sincere gratitude is extended to all the teaching and non-teaching staff members of the Department of Botany, AIJ DC College, Murud, for their constant support, cooperation, and encouragement throughout the course of this work. I am also thankful to the department for providing me with excellent laboratory facilities, equipment, and a conducive research environment, which greatly facilitated the successful completion of this study.

IJARSCT
International Journal of Advanced Research in Science, Communication and Technology
International Open-Access, Double-Blind, Peer-Reviewed, Refereed, Multidisciplinary Online Journal
ISSN: 2581-9429
Volume 6, Issue 10, March 2026
Impact Factor: 8.2

REFERENCES

1. Abdul Manap, A. S., Vijayabalan, S., Madhavan, P., Chia, Y. Y., Arya, A., Wong, E. H., Rizwan, F., Bindal, U., & Koshy, S. (2019). Bacopa monnieri , a Neuroprotective Lead in Alzheimer Disease: A Review on Its Properties, Mechanisms of Action, and Preclinical and Clinical Studies. Drug Target Insights, 13, 117739281986641. https://doi.org/10.1177/1177392819866412
2. Abdullah, A.-S., Mohammed, A., Rasedee, A., & Mirghani, M. (2015). Oxidative Stress-Mediated Apoptosis Induced by Ethanolic Mango Seed Extract in Cultured Estrogen Receptor Positive Breast Cancer MCF-7 Cells. International Journal of Molecular Sciences, 16(2), 3528–3536. https://doi.org/10.3390/ijms16023528
3. Abou Hashish, E. A., Al Najjar, H., Alharbi, M., Alotaibi, M., & Alqahtany, M. M. (2024). Faculty and students perspectives towards game-based learning in health sciences higher education. Heliyon, 10(12), e32898. https://doi.org/10.1016/j.heliyon.2024.e32898
4. Amanulla Khan, R. L. Pawar, M.H. Mali, Kamlakar More, & Satish V. Deore. (2023). Proteomics: An Emerging Tools, Database and Technique for Understanding Biological System. Biological Forum – an International Journal, 15(3), 511–523. https://doi.org/10.5281/zenodo.7837890
5. Amanulla, K., Rahim, B., Javed, K., Namrata, M., Manojkumar, C., & Abhay, S. (2025). GC-MS-based phytochemical profiling and anti-diabetic efficacy of Sargassum tenerrimum-mediated silver nanoparticles. Plant Science Today. https://doi.org/10.14719/pst.8697
6. Archana U, Khan A, Sudarshanam A, Sathya C, Koshariya AK, Krishnamoorthy R. Plant disease detection using ResNet. In: Proceedings of the International Conference on Inventive Computation Technologies (ICICT). Lalitpur, Nepal; 2023 Apr 26–28. p. 614–618. https://ieeexplore.ieee.org/document/10133938
7. Bhojwani SS, Dantu PK. Plant Tissue Culture: An Introductory Text. New Delhi: Springer; 2013. p. 1–309.
8. Cakir O, Ari S. Defensive and secondary metabolism in Astragalus chrysochlorus cell cultures in response to yeast extract stress. J Environ Biol. 2009;30(1):51–55.
9. Debnath M, Malik CP, Bisen PS. Micropropagation: a tool for the production of high quality plant-based medicines. Curr Pharm Biotechnol. 2006;7(1):33–49.
10. Faisal M, Anis M. Rapid mass propagation of Hemidesmus indicus (L.) R. Br. through shoot tip and nodal explants. Biol Plant. 2003;47(4):563–566.
11. George EF, Hall MA, De Klerk GJ. Plant Propagation by Tissue Culture. 3rd ed. Dordrecht: Springer; 2008. p. 1–501.
12. George S, Tusar KV, Umrikrishna KP, Ilastim KM, Balachandran I. Hemidesmus indicus (L.) R. Br.: a review. J Plant Sci. 2008;3(2):146–156.
13. Gopiesh Khanna SV, Kannabiran K. Larvicidal effect of Hemidesmus indicus, Gymnema sylvestre and Eclipta prostrata against Culex quinquefasciatus. Afr J Biotechnol. 2007;6(3):307–311.
14. J, Jeba Sonia, D. J. Joel Devadass Daniel, Dr. R. Sabin Begum, Dr. A K N K Pathan, Dr. Veera Talukdar, and Vivek Dadasaheb Solavande. (2022) 2023. “AI Techniques for Efficient Healthcare Systems in ECG Wave Based Cardiac Disease Detection by High Performance Modelling”. International Journal of Communication Networks and Information Security (IJCNIS) 14 (3):290-302 https://www.ijcnis.org/index.php/ijcnis/article/view/5629
15. Karabournitis G, Easses C. Defensive indumentum with polyphenol content may replace the protective role of the epidermis in some young xeromorphic leaves. Can J Bot. 1996;74:347–351.
16. Karwasara V, Jain R, Tomar P, Dixit VK. Elicitation as a yield enhancement strategy for glycyrrhizine production by cell cultures of Abrus precatorius Linn. In Vitro Cell Dev Biol Plant. 2010;46:354–362.
17. Khan A, More KC, Mali MH, Deore SV, Patil MB. Phytochemical screening and gas chromatography–mass spectrometry analysis of Ischaemum pilosum (Klein ex Willd.). Plant Sci Today. 2023;10(4):88–96. https://doi.org/10.14719/pst.2349

IJARSCT
International Journal of Advanced Research in Science, Communication and Technology
International Open-Access, Double-Blind, Peer-Reviewed, Refereed, Multidisciplinary Online Journal
ISSN: 2581-9429
Volume 6, Issue 10, March 2026
Impact Factor: 8.2

18. Khan A, Pawar RL, Mali MH, More K, Deore SV. Proteomics: an emerging tool, databases and techniques for understanding biological systems. Biological Forum – Int J. 2023;15(3):511–523. https://doi.org/10.5281/zenodo.7837890
19. Khan, A. (2026). Bacopa monnieri as a Potential Natural Acetylcholinesterase Inhibitor for Neurodegenerative Disease Management. Research Journal of Biotechnology, 4(21), 280. https://doi.org/10.25303/214rjbt2800284
20. Marathe S. A, Khan A. Study of Soil Health in Irrigated and Non-Irrigated Bt Cotton in North Maharashtra Region of India. Biotech Res Asia 2026;23(1). Available from: https://bit.ly/46NB7jd
21. Misra N, Misra P, Datta SK, Mehrotra S. Improvement in clonal propagation of Hemidesmus indicus R. Br. through adenine sulphate. J Plant Biotechnol. 2003;5(4):239–244.
22. Mohana Rao GM, Venkateswararao C, Rawat AKS, Pushpangadan P, Shirwaikar A. Antioxidant and antihepatotoxic activities of Hemidesmus indicus R. Br. Acta Pharm Turc. 2005;47:107–113.
23. Morandim AA, Kato MJ, Cavalheiro AJ, Furlan M. Intraspecific variability of dihydrochalcone, chromenes and benzoic acid derivatives in leaves of Piper aduncum L. (Piperaceae). Afr J Biotechnol. 2009;8(10):2157–2162.
24. Murali A, Ashok P, Madhavan V. Effect of leaf of Hemidesmus indicus (L.) R. Br. var. pubescens (W. & A.) Hook. f. (Periplocaceae): an in vitro analysis. Int J Drug Formul Res. 2010;1(2):162–175.
25. Murashige T, Skoog F. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol Plant. 1962;15:473–497.
26. Muthukumar K, Selvin Samu A. Traditional herbal medicines of the coastal diversity in Tuticorin District, Tamil Nadu, India. J Phytol. 2010;2(8):38–46.
27. Nagarajan S, Rao LJM, Gurudutt KN. Chemical composition of volatiles of Hemidesmus indicus (L.) R. Br. Flavour Fragr J. 2001;16:212–214.
28. Patil MB, Khan PA (2017). Ethnobotanical, phytochemical and Fourier Transform Infrared Spectrophotometer (FTIR) studies of Catunaregam spinosa (Thunb.) Tirven. Journal of Chemical and Pharmaceutical Sciences. Vol. 10 Issue 2 PP 950-955 https://doi.org/10.5281/zenodo.7562415
29. Patil MB, Khan PA. Review: techniques towards plant phytochemical study. Int J Sci Info. 2016;1(3):157–172. https://doi.org/10.5281/zenodo.7559052
30. Prakash E, Sha Valli Khan PS, Rao KR, Meru ES. Regeneration of plants from callus cultures of Hemidesmus indicus (L.) R. Br. Indian J Biotechnol. 2002;1:165–168.
31. Ravishankar MN, Shrivastava N, Padh H, Rajani M. Evaluation of antioxidant properties of root bark of Hemidesmus indicus R. Br. Phytomedicine. 2002;9:153–160.
32. Rout GR, Samantaray S, Das P. In vitro manipulation and propagation of medicinal plants. Biotechnol Adv. 2000;18(2):91–120.
33. Sahu SC, Dhal NK, Mohanty RC. Potential medicinal plants used by the tribals of Deogarh District, Orissa, India. Ethno Med. 2010;4(1):53–61.
34. Sandeep A. Marathe, & Amanulla Khan. (2025). Pest Incidence in Bt Cotton: Evaluating the Impact of Pink Bollworm in Nandurbar, Maharashtra. https://doi.org/10.5281/ZENODO.17605877
35. Sandeep, A. M., & Amanulla, K. (2026). Study of Soil Health in Irrigated and Non-Irrigated Bt Cotton in North Maharashtra Region of India. Biotech Res Asia, 23(1). https://bit.ly/46NB7jd
36. Saravanan N, Nalini N. Hemidesmus indicus protects against ethanol-induced liver toxicity. Cell Mol Biol Lett. 2008;13:20–37.
37. Seeni S, Latha PG. Micropropagation and conservation of rare medicinal plants of Western Ghats. Indian J Exp Biol. 2000;38: 123–129.
38. Shah S, Mukhopadhyay MJ, Mukhopadhyay S. In vitro clonal propagation through bud culture of Hemidesmus indicus (L.) R. Br. J Plant Biochem Biotechnol. 2003;12:61–64.

IJARSCT
International Journal of Advanced Research in Science, Communication and Technology
International Open-Access, Double-Blind, Peer-Reviewed, Refereed, Multidisciplinary Online Journal
ISSN: 2581-9429
Volume 6, Issue 10, March 2026
Impact Factor: 8.2

39. Shanthi A, Radha R, Jayashree N, Selvaraj R. Pharmacognostic validation of root of Hemidesmus indicus (L.) R. Br. J Chem Pharm Res. 2010;2(5):313–322.
40. Siddique NA, Bari MA, Khatun N, Rahman M, Rahman MH, Huda S. Plant regeneration from nodal segment-derived callus of Hemidesmus indicus (L.) R. Br. Plant Tissue Cult Biotechnol. 2006;16:1158–1163.
41. Singh A, Singh SK, Rai MK. In vitro propagation and conservation of medicinal plants: a review. Int J Biol Technol. 2011;2(2):96–102.
42. Sonia JJ, Daniel DJJD, Begum RS, Pathan AKNK, Talukdar V, Solavande VD. AI techniques for efficient healthcare systems in ECG wave-based cardiac disease detection using high-performance modelling. Int J Commun Netw Inf Secur. 2022;14(3):290–302. https://www.ijcnis.org/index.php/ijcnis/article/view/5629
43. Sreekumar S, Seeni S. In vitro propagation of Hemidesmus indicus (L.) R. Br. through axillary bud culture. Plant Cell Tissue Organ Cult. 1998;55(1):45–51.
44. Udomsuk L, Jarukamjorn K, Tanaka H, Putalun W. Improved isoflavonoid production in Pueraria candollei hairy root cultures using elicitation. Biotechnol Lett. 2011;33:369–374.
45. Wagay NA, Rafiq S, Khan A, Kaloo ZA, Malik AR, Pulate PV. Impact of phenolics on drought stress and expression of phenylpropanoid pathway genes. In: Lone R, Khan S, Al-Sadi AM, editors. Plant Phenolics in Abiotic Stress Management. Singapore: Springer; 2023. p. 281–304. https://doi.org/10.1007/978-981-19-6426-8_13

DOI: 10.48175/IJARSCT-35008