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TDZ Induced Organogenesis and Phytochemical Profiling of *In Vitro* Raised Plants of Sarpgandha (*Rauvolfia serpentina* L.)- An Endangered Medicinal Plant

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Abstract

This study was conducted during June, 2020–2021 at Department of Biotechnology, COHF, Dr YS Parmar UHF, Neri, Hamirpur, Himachal Pradesh, India for evaluating effect of TDZ on *in vitro* regeneration of *Rauwolfia serpentina* and further phytochemicals screening of regenerants was done. Shoot tips, and leaves explants was collected from field maintained healthy plants of *Rauvolfia serpentina* and cultured on MS medium containing TDZ in combination with auxins. MS medium containing 1.0 mg l^{-1} TDZ with 0.2 mg l^{-1} NAA showed maximum shoot regeneration i.e. 75.3% from shoot tips within 32 days and 85.0% shoot regeneration from leaf explants. Shoots were transferred on half strength MS medium containing different auxin concentrations for *in vitro* rooting and maximum root regeneration percentage (89.00%) was achieved on 0.5 mg l^{-1} NAA. Healthy plantlets were acclimatized further on coco-peat first and then on mixture of 1:1 of sand: FYM. Donor plants as well as *in vitro* raised plants (six month old) were analyzed for different phytochemicals qualitatively and quantitatively. Alkaloids, flavonoids, phytosterols, tannins and phenol compound were present in leaves and roots of mother plant as well as *in vitro* raised plants whereas gums and mucilage was seen in mother plant but absent in *in vitro* raised plants. Also alkaloid, flavonoids, tannin and phenol content of *in vitro* raised plant were comparable higher than donor plant. Presence of important alkaloid reserpine in tissue culture raised plant was also confirmed by thin layer chromatography and Rf value of reserpine was found to be 0.92.

Keywords: *In vitro*, phytochemicals, sarapgandha, thiaduzuron, reserpine, endangered

1. Introduction

Rauvolfia serpentina L. Benth also known as *sarpagandha* or *snakeroo*t plant is an important plant with various medicinal plants. It is a perennial shrub which belongs to Apocynaceae family and it is distributed in different Asian countries including India (Panwar et al., 2011). It grows at 1300–1400 m altitude like in the mountainous region of the Himalayas (Dey and De, 2010). In India, *R. serpentina* is found in the wild forests scattered throughout the lower mountains along the Gangetic plains, which extends from Himachal Pradesh to Nepal, Sikkim, Bhutan and Assam. In the southern territory of India, it is most abundant in the Western Ghats region. Out of 300 species of therapeutic plants available worldwide, *R. serpentina* is an important plant with various medicinal values. From the ancient times, in the Indian system of *Unani* and *Ayurveda*, extracts from this plant were utilized to treat several illness like high blood pressure, anxiety, hypertension etc. (Gantait et al., 2017), and most of the world population still relies on folk medicine and herbal treatment. The root of *R. serpentina* is an affluent source of indole alkaloids (Panwar et al., 2011) and the root bark contains about 90% of the total alkaloids. Among them, reserpine is the most abundant alkaloid (Swain et al., 2023). The other alkaloids are ajmaline, ajmalinine, ajmalicine, reserpine, serpentine, serpentinine, rauwolfinine, iso-ajmaline, and tetraphyllicine. jmaline and ajmalicine have anti-hypertensive qualities (Klyushnichenko et al., 1995). A number of commercial based market products of this important plant are available such as Himalayan serpine medicine, Schwable *Rauwolfia serpentina* 1 X Tablet, Herbal supplemented *Rauwolfia serpentina* Blent (*Snakeroot*) etc.

Plant tissue culture techniques are being used to preserve endangered plants *in vitro* for longer period of time (Cruz-Cruz et al., 2013). Also better quality, true to type and disease free planting material in large quantity could be available throughout the year using plant tissue culture approach. The large-scale cultivation of *R. serpentina* by plant tissue culture techniques and large scale production of secondary metabolites have been made possible by the recent

advancements in the plant tissue culture techniques (Ilahi et al., 2007). These techniques have gained popularity for their ability to generate uniform and high-quality products, allowing for year-round production of secondary compounds (Hasnain et al., 2022; Sharma et al., 2021). Thus this study was focused on induction of shoot organogenesis using TDZ hormone and further phytochemicals profiling of regenerants was done.

2. Materials and Methods

The study was conducted during June 2020–2021 at Department of Biotechnology, COHF, Dr YS Parmar UHF, Neri, Hamirpur, Himachal Pradesh, India.

2.1. Materials and reagents

Chemicals used in this study were of analytical grade purchased from Himedia. All of the standards were also purchased from Himedia.

2.2. Planting material

Young and healthy plants of *Rauwolfia serpentina* L. (*Sarpangandha*) were collected from Sub Tropical Herbal Garden, Neri Hamirpur (H.P.) and maintained further in the Department of Biotechnology, COH&F, Neri Hamirpur (H.P.)

2.3. Plant regeneration and culture conditions

For establishment of *in vitro* cultures, axillary buds and shoot tip explants were excised from maintained plants of *R. serpentina* L and surface sterilized with the treatment of 5% (w/v) Bavistin for 2–3 minutes and 0.1% $HgCl_2$ treatment for 30 s, followed by rinsing with autoclaved distilled water after each treatment. After that explants were inoculated in Murashige and Skoog (MS) medium (Murashige and Skoog, 1962) augmented with a range of TDZ (Thiadiazuron) alone or in combination with auxins. Each culture flasks contained five explants, and there were five culture flasks for each treatment. Culture flasks were then incubated in culture room with controlled conditions of 25±2°C temperature and 16 hours light and 8 hours dark photoperiod. After shoot regeneration, micro-shoots were transferred to rooting medium containing auxins and *in vitro* plantlets obtained were hardened and acclimatized further.

2.4. Plant extract preparation

Roots and leaves of mother and six month old tissue culture raised plants were collected and washed under tap water; moisture was removed using blotting paper. Samples were shade dried for 5 days and then crushed and grinded to form a coarse powder. Dried tissue (1 g) was homogenized in 50 ml of methanol and extractions were carried out in an orbital shaker with slight shaking at 25°C for 48 hours. The resulting suspensions were filtered using the Whatman filter paper no. 42. This extract was used for phytochemical analysis and all measurements were taken on UV-Vis spectrophotometer (Thermo-Fisher Scientific).

2.5. Phytochemical profiling

2.5.1. Qualitative analysis

The presence of secondary metabolites such as alkaloids (Salehi et al. (1992), phenols (Kapoor et al. (1969), phytosterols (Ling and Jones (1955), saponins (Segleman and Farnsworth 1969), tannins (Kapoor et al., 1969), flavonoids (Somolenskin et al., 1972), gum and mucilage (Amelia et al., 2011) was determined by qualitative Phytochemical analysis of *Rauvolofia serpentina extract*.

2.5.2. Quantitative analysis

2.5.2.1. Determination of total phenol content (TPC)

Total phenol content was estimated by Folin-Ciocolteu method (Singleton and Rossi, 1965). Plant extract was prepared as described above and 0.1 ml of plant extract was taken in a test tube from sample solution followed by adding and mixing with 1.8 ml of Folin-Ciocolteu reagent and kept for 6 mins at 25°C. Then, 20% sodium carbonate (1.2%) was added to the resultant mixture and kept for 1 and half hour at room temperature with intermittent shaking. The absorbance of sample was read at 765 nm using UV-Vis spectrophotometer (Thermofischer). Gallic acid was used as reference standard for making calibration curve. The total phenol content was determined as mg of gallic acid equivalent 100 g^{-1} (mg GAE 100 g^{-1}) of tissue. Different concentrations of gallic acid (0.02-0.1 mg ml^{-1}) were used as standard to prepare calibration curve.

2.5.2.2. Determination of total alkaloid content (TAC)

Bromocresol green method was used for measuring total alkaloid content in plant extract (Shamsa et al., 2008). Dry plant extract was formed and dissolved in 10 ml methanol and then filtered. 1 ml of this solution was transferred to a separating funnel and to that mixture 5 ml of Bromocresol green solution along with 5 ml of phosphate buffer was added and mixed properly. The formed mixture was further extracted with chloroform (5 ml) and transferred to 10 ml of volumetric flask and make up the volume with chloroform. The absorbance of sample was read after yellow color development on UV-Vis spectrophotometer at 470 nm. Atropine was used as standard for making calibration curve. The total alkaloid content was determined using linear equation of standard curve prepared with different concentration of atropine (0.5–2.5 mg ml^{-1}). Total alkaloid content in plant extract was expressed as mg atropine equivalent 100 g^{-1} (mg ATE 100 g^{-1}) of tissue.

2.5.2.3. Determination of total flavonoids content (TFC)

Total flavonoids content was measured by using the aluminum chloride colorimetric method (Chang et al., 2002) with some modifications. Firstly, 0.5 ml of plant extract was mixed with 1.5 ml of methanol and then 0.1 ml of aluminum chloride (10%), 0.1 ml of sodium acetate (1 M) and 2.8 ml of distilled water was added. All the reagents were mixed for 5 mins by vortexing and the reaction mixture was incubated for 30 mins at room temperature in dark. Finally the absorbance was measured at 415 nm using UV-Vis spectrophotometer. Quercetin was used as reference standard for making calibration curve. The total flavonoids content was determined

using linear equation of calibration curve prepared with different concentration of quercetin (0.1–0.5 mg ml^{-1}). The content of total flavonoids compounds was expressed as mg quercetin equivalent 1 g^{-1} (mg QE g) of tissue.

2.5.2.4. Determination of total tannin content (TTC)

Folin–Dennis method was used to measure total tannin content (Singelton et al., 1999). Firstly, 0.2 ml of plant extracts was added to 0.5 ml of Folin–Dennis reagent followed by addition of 1 ml of 20% sodium carbonate and 1 ml of autoclaved distilled water. Resultant solution was mixed properly and incubated at room temperature for 30 mins. The absorbance of the reaction was measured at 775 nm. The concentration of the total tannin was determined as mg of tannic acid equivalents (TAE) 100 g^{-1} of tissue using an equation obtained from the tannic acid calibration curve.

2.6. Thin layer chromatography (TLC) analysis

2.6.1. Plant extract preparation

Leaves and roots were collected from mother plants and *in vitro* regenerated plants. Afterwards, samples were shade dried at room temperature for 5 days and grinded to coarse powder for extraction. Dry powder of mother plant as well as *in vitro* raised plants was collected in different flasks containing 1.25 ml of methanol. The conical flask was kept at 25˚C for 12 hrs in orbital shaker. After 12 hrs, the suspension was filtered through Whatman's filter paper no. 42 and collected in flasks. These were allowed to dry completely in water bath set at 40±0.2˚C for 30 mins. Dried extracts were scraped out by using scalpels and was collected in vials separately. Extracted powders were made available to use as per requirements by redissolving in methanol every time.

2.6.2. Thin layer chromatography analysis

The qualitative estimation of alkaloids from leaves and roots of mother plants as well as *in vitro* raised plants of sarpagandha was done by TLC technique on preparative silica gel plates. Retardation factor (Rf) values were compared with reserpine standard (Himedia). Mobile phase or solvent system used for alkaloid estimation was chloroform and methanol in 97:3 ratios. Samples (roots and leaves from mother plant and root and leaves of *in vitro* raised plant) were spotted on TLC preparative silica gel plates along with reserpine standard dissolved in methanol. Spots were observed on plate under UV- transilluminator. Fluorescent green bands showed the presence of alkaloids on preparative silica gel plate.

3. Results and Discussion

3.1. In vitro regeneration studies

In vitro culture establishment was done using shoot tips and leaf explants of *R. serpentina*. After surface sterilization explants was cultured on MS medium containing varied concentrations of TDZ in combination with auxins. It was observed that MS medium containing 1.0 mg l^{-1} TDZ and 0.2 mg l^{-1} NAA showed highest shoot regeneration i.e. 75.3% using shoot tips as explants and 85.0% using leaf explants (Table 1). Healthy shoots were multiplied further on same medium composition. *In vitro* raised shoot were transferred further on half strength MS medium containing different concentrations of auxin for *in vitro* rooting and maximum root regeneration percentage (89.00%) was achieved on 0.5 mg l^{-1} NAA. Healthy plantlets were acclimatized further on coco-peat first and then on mixture of 1:1 of sand: FYM. Previous studies have also reported the efficacy of TDZ hormone for inducing shoot regeneration in *Rauvolfia serpentina* L. Mukherjee et al. (2020) induced indirect shoot regeneration from leaf explants of *R. serpentina* using 1 mg l^{-1} TDZ in combination with 0.2 mg l^{-1} NAA. They reported that TDZ in combination of NAA significantly outperformed the other cytokinins (BA or KIN). Similarly in an another study nutrient media amended with

Table 1: Influence of different concentrations of TDZ in combinations with auxins on *in vitro* shoot regeneration of *Rauwolfia serpentina* L. using shoot tips and leaf explants

Sl. No.	Medium code	Medium composition MS medium basal+GR (mg l^{-1})			Percent regeneration (%)		Average no. of shoot explants^{-1}	
		TDZ	NAA	IBA	Shoot Tips	Leaves	Leaves	Shoot tips
1.	MS-1	0.25	0.2		59.3	59.0	1.00	1.66
2.	MS-2	0.5	0.2	-	71.3	76.6	2.69	3.00
3.	MS-3	1.0	0.2	-	75.3	85.0	3.33	3.33
4.	MS-4	1.5	0.2	-	60.0	65.0	1.66	1.53
5.	MS-5	2.0	0.2	-	53.0	56.3	0.97	1.51
6.	MS-6	0.25	-	-	46.0	65.6	1.66	0.66
7.	MS-7	0.5	-	0.2	61.6	69.0	2.00	1.66
8.	MS-8	1.0	-	0.2	70.6	72.6	2.36	2.33
9.	MS-9	1.5	-	0.2	70.0	58.0	0.99	2.00
10.	MS-10	2.0	-	0.2	43.6	52.3	0.89	0.33

BAP in range of 1.0–3.0 mg l^{-1} and TDZ in range of 0.1–1.0 mg l^{-1} were responded well, more than 80% and 92%, respectively (Tripathi et al., 2022) (Figure 1).

Figure 1: TDZ induced organogenesis in Rauvolfia serpentina on MS medium containing 1.0 mg l^{-1} TDZ+0.2 mg l^{-1} a) Shoot regenerated after 25–30 days b) Shoots mutiplied c) Root regenerated on half strenght MS medium containing 0.5 mg l^{-1} NAA d) Rooted plantlets of *R. serpentina* L. with well developed root system e) 2 week hardened in vitro raised plants in cocopeat f) Hardened plants of *R. serpentina* in soil:FYM

3.2. Phytochemical profiling

3.2.1. Qualitative analysis

Preliminary qualitative phytochemical profiling of *Rauvolfia serpentina* extract was done by the phytochemical profiling test (Table 2). It was observed that alkaloids, flavonoids, phytosterols, saponins, tannin and phenolic compounds were present in leaves and roots of mother plant as well as *in vitro* raised plants whereas gum and mucilage was seen in mother plant leaves and roots but absent in *in vitro* raised plants root and leaf samples. In a similar study, Vaishnav and Sahoo (2020) reported the presence of different phytochemicals viz., alkaloids, flavonoids, steroids, tannins, glycosides, terpenoids and phenols in acetone root extract of *Rauwolfia serpentine* L. However in other study methanolic root extract of *Rauwolfia serpentina* showed presence of alkaloids, saponins, flavonoids, phenol, terpenoids and absence of tannins and glycosides (Ratnam, 2021).

Table 2: Qualitative analysis of phytochemicals present in the leaves and roots of donor and regenerated plant

S l . No.	Metabolites	Tests	Mother plant	Donor plant
1.	Alkaloid	Salehi et al. (1992)	+	+
2.	Flavanoids	Somolenskin et al. (1972)	+	+
3.	Phytosterols	Ling and Jones (1955)	+	+
4.	Saponin	Segleman and Farnsworth (1969)	+	+
5.	Tannin	Kapoor et al. (1969),	+	+
6.	Phenol	Kapoor et al. (1969)	+	+
7.	Gum and Mucilage	Amelia et al. (2011)	+	-

3.2.2. Quantitative analysis

Comparative evaluation of different phytoconstituents such as phenols, flavanoids and alkaloids of leaf and root extracts for both the regenerated and mother plants was carried out by spectrophotometric estimation also. Calibration curve of gallic acid, atropine and quercetin was prepared and measurements were taken at wavelengths of 765 nm, 700 nm and 420 nm, respectively. Total phenol content was expressed as milligrams of gallic acid equivalent (GAE) 100 g^{-1}, total alkaloid content was expressed as atropine equivalent $gram^{-1}$ of extract, total tannin content was expressed as mg of tannic acid equivalents (TAE) 100 g^{-1} of tissue and total flavanoid content was expressed as Quercetin equivalent 100 g^{-1} of extract. It was observed that total phenol, flavanoid, alkaloid and tannin content in leaf methanolic extract of mother plant was 1.05 mg 100 g^{-1}, 1.94 mg 100 g^{-1}, 0.995 mg 100 g^{-1} and 0.612 mg 100 g^{-1}, respectively which was slightly less than in the leaf extract of *in vitro* raised plant that showed 1.55 mg 100 g^{-1}, 2.45 mg 100 g^{-1}, 1.23 mg 100 g^{-1} and 0.788 mg 100 g^{-1}, respectively (Table 3).

Root extracts of regenerated and mother plant was also studied for quantitative analysis and phenol, tannin, flavonoids and alkaloid content was measured accordingly. Root extract of mother plants contain 1.25 mg 100 g^{-1} Phenol content, 1.066 mg 100 g^{-1} tannin content, 1.55 mg 100 g^{-1} Alkaloid content and 0.512 mg 100 g^{-1} flavonoids content whereas 1.31 mg 100 g^{-1} phenol content, 1.550 mg 100 g^{-1} tannin content, 1.66 mg 100 g^{-1} alkaloid content and 0.678 mg 100 g^{-1} flavonoids content was observed in root extract of regenerated plants. Results shown here are in agreement to earlier findings where elevation of phytochemicals was observed in hardened tissue culture raised plants of medicinal plants (Mukherjee et al., 2020; Qahtan et al., 2023; Khan et al., 2021)

3.3. TLC analysis

Crude plant extract was also qualitatively analyzed by separating the components in Thin Layer Chromatography (TLC). Samples were spotted on silica gel plates and separated using solvent system. Reserpine was used as standard and it was observed that Rf value of reserpine was 0.95. Fluorescent bands of reserpine were observed under UV light. It was observed that reserpine bands were present in mother plant methanolic root extract and *in vitro* raised (six month) old plant root extract also. Similarly, TLC analysis of root extracts of *Rauvolfia serpentina* was reported in other studies also and Rf value of reserpine was found to be in the range of 0.56 to 0.95 (Rohela et al., 2021, Irshad and Khatoon 2021, Ghate et al., 2022).

Table 3: Total phenol, flavonoids, alkaloids and tannins content in aqueous methanol extract from leaves and roots of donor and regenerated plant

Sl. No.	Planting material	Sample	Total phenols (mg GAE 100 g^{-1})	Total flavonoids (mg Q 100 g^{-1})	Total alkaloid (mg 100 g^{-1})	Total tannins (mg 100 g^{-1})
1.	Donor plant	Leaves	1.05	1.94	0.995	0.612
		Roots	1.25	0.512	1.55	1.066
2.	Regenerated plant	Leaves	1.55	2.45	1.230	0.788
		Roots	1.31	0.678	1.66	1.550

4. Conclusion

TDZ hormone efficiently regenerated shoot i.e. 75.3% using shoot tips and 85.0% using leaf explants. Qualitative and quantitative characterization of phytochemicals showed that total phenol, tannins, flavonoids and alkaloid content of *in vitro* raised plants was comparable higher than *in vitro* raised plant.

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6. References

Amelia, M., Rakesh, A., Dash Shilpa, R., Shrotriya, N., 2011. Recent investigations of plant based natural gums, mucilages and resins in novel drug delivery systems. Indian Journal of Pharmaceutical Education Research 45, 86–99.

Chang, C., Yang, M., Wen, H., Chern, J., 2002. Estimation of total flavonoids contents in Propolis by two complementary colorimetric methods. Journal of Food and Drug Analysis 10(3), 178–182.

Cruz-Cruz, C.A., González-Arnao, M.T., Engelmann, F., 2013. Biotechnology and conservation of plant biodiversity. Resources 2(2), 73–95.

Dey, A., De, J.N., 2010. *Rauwolfia serpentina* (L.) Benth. ex. Kurz. A Review. Asian Journal of Plant Sciences 9, 285–298.

Gantait, S.S., Dutta, K., Majumdar, J., 2017. *In vitro* regeneration and conservation of endangered medicinal plant sarpagandha (*Rauvolfia serpentina*). Journal of Horticulture Sciences 12, 71–77. DOI: https://doi.org/10.24154/jhs.v12i1.74.

Ghate, V.U., Namdeo, A.G., Patil, S.H., Gajendragadkar, M.P., 2022. Phytochemical screening of reserpine in wild and cultivated *Rauvolfia Serpentina* mother tincture. Jundishapur Journal of Microbiology 15(1), 5031–5042.

Hasnain, A., Naqvi, S.A.H., Ayesha, S.I., Khalid, F., Ellahi, M., Iqbal, S., Hassan, M.Z., Abbas, A., Adamski, R., Markowska, D., Baazeem, A., Mustafa, G., Moustafa, M., Hasan M.E., Abdelhamid, M.M.A., 2022. Plants *in vitro* propagation with its applications in food, pharmaceuticals and cosmetic industries; current scenario and future approaches. Frontiers in Plant Science 13, 3894.

Ilahi, I., Rahim, F., Jabeen, M., 2007. Enhanced clonal propagation and alkaloid biosynthesis in cultures of *Rauwolfia*. Pakistan Journal of Plant Science 13(1), 45–56.

Irshad, S., Khatoon, S., 2021. A validated HPTLC method for the simultaneous determination of seasonal alterations of two antihypertensive monoterpenoid indole alkaloids in *Rauvolfia* species from northern India. South African Journal of Botany 142, 193–200. https://doi.org/10.1016/j.sajb.2021.06.027.

Kapoor, L.D., Singh, A., Kapoor, L., Srinivastava, S.N., 1969. Survey of Indian plants forsaponins, alkaloids and flavonoids. Lloydia 32, 297–304.

Khan, A., Shah, A.H., Ali, N., 2021. *In vitro* propagation and phytochemical profiling of a highly medicinal and endemic plant species of the Himalayan region (*Saussurea costus*). Scientific Reports 11, 23575. https://doi.org/10.1038/s41598-021-03032-1.

Klyushnichenko, V.E., Yakimov, S.A., Tuzova, T.P., Syagailo, Y.V., Kuzovkina, I.N., Wulfson, A.N., Miroshnikov, A.I., 1995. Determination of indole alkaloids from *R. serpentina* and *R. vomitoria* by high-performance liquid chromatography and high-performance thin-layer chromatography. Journal of Chromatography 704, 357–362.

Ling, W.H., Jones, P.J.H., 1955. Dietary phytosterols: a review of metabolism, benefits and side effects. Life Science 57, 195–206.

Mukherjee, E., Sarkar, S., Bhattacharyya, S., Gantait. S., 2020. Ameliorated reserpine production via *in vitro* direct and indirect regeneration system in *Rauvolfia serpentina* (L.) Benth. ex Kurz. 3 Biotech 10, 294. Doi: 10.1007/s13205-020-02285-3.

Murashige, T., Skoog, F., 1962. A revised medium for rapid growth and bio assays with tobacco tissue cultures. Plant Physiology 15, 473–497.

Panwar, G.S., Attitala, I.H., Guru, S.K., 2011. An efficient *in vitro* clonal propagation and estimation of reserpine content in different plant parts of *Rauwolfia serpentina* L.

American-Eurasian Journal of Scientific Research 6, 217–222.

Qahtan, A.A., Alatar, A.A., Faisal, M., 2023. *In vitro* regeneration, phytochemical profiling and antioxidant activity in *Ruta chalepensis* plants established from alginate encapsulated synthetic seeds. South African Journal of Botany 161, 575–585. https://doi.org/10.1016/j.sajb.2023.08.065.

Ratnam, D.P.S.K., 2021. Studies on phytochemical, antimicrobial activity and micro propagation of medicinal plants from Eastern Ghats of Andhra Pradesh. Asian Journal of Pharmacy and Technology 11(2), 111–115. doi: 10.52711/2231-5713.2021.00018.

Rohela, G.K., Bylla, P., Pendli, S., Korra, R., Gandu, R., Reuben, C., 2021. High performance liquid chromatography based quantification of reserpine in *Rauwolfia tetraphylla* L. and enhanced production through precursor feeding. Acta Chromatographica 34(2), 120–129.

Salehi, S.M.H., Aynehchi, Y., Amin, G.H., Mahmoodi, Z., 1992. Survey of Iranian plants for saponins, alkaloids, flavonoids and tannins IV. Daru 2, 281–291.

Segleman, A.B., Farnsworth, N.R., 1969. Biological and photochemical screening of plants IV. A new rapid procedure for the simultaneous determination of saponins and tannins. Lloydia 21, 53–56.

Shamsa, F., Monsef, H., Ghamooshi, R., Verdian-rizi, M., 2008. Spectrophotometric determination of total alkaloids in some Iranian medicinal plants. Journal of Applied Horticulture 32(1), 17–20. DOI:10.56808/3027-7922.2196.

Sharma, S., Devi, B., Sharma, V., 2021. Estimation of phytochemicals from mother plants and *in vitro* raised plants of *Gloriosa superba* L. International Journal of Economic Plants 8(3), 127–130. DOI. ORG/10.23910/2/2021.0430.

Singelton, V.L., Orthofer, R., Laumela-Raventos, R.M., 1999. Analysis of total phenols and other oxidation substrates and other antioxidants by means of Folin-Ciocolteu reagent. Methods in Enzymology 299, 152–178.

Singleton, V.L., Rossi, J.A., 1965. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. American Journal of Enology and Viticulture 16, 144–158.

Somolenskin, S.J., Slilins, H., Farnsworth, N.R., 1972. Alkaloid screening. Llyodia 35, 1–34.

Swain, H., Gantait, S., Mandal, N., 2023. Developments in biotechnological tools and techniques for production of reserpine. Applied Microbiology and Biotechnology 107, 4153–4164. Doi: 10.1007/s00253-023-12570-9.

Tripathi, M.K., Tiwari, G., Tiwari, S., Tripathi, N., Mishra, N., Tomar, Y.S., Uikey, S., Tiwari, S., 2022. Plant regeneration in *Rauvolfia serpentina* (L.) Benth via organogenic mode. In: Hanif, A. (Ed.), Research aspects in biological science. B P International, West Bengal, India, 124–155. DOI: 10.9734/bpi/rabs/v7/3678A.

Vaishnav, Sahoo, D., 2020. Comparative phytochemical analysis of stem and root extracts from *Rauwolfia serpentina* Virendra. International Journal of Advanced Scientific Research and Management 5(1), 5–7.