International Journal of Environmental Sciences
ISSN: 2229-7359
Vol. 11 No. 15s,2025
https://theaspd.com/index.php

Propagation Of Rauwolfia Serpentina (L.) Benth.Ex Kurz (Sarpagandha) Through In Vivo Seed Germination

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Abstract:
Rauwolfia serpentina known as Sarpagandha is an indigenous medicinal plant of India, documented in medicinal literature of ancient India, 3000 years back. As it has many medicinal properties, the demand for crude drug has been increasing continuously leading to its exploitation due to lack of cultivation. Because of this reason this plant has been listed in endangered category. Though it is commercially propagated by seeds, its irregular and low percentage of germination is varied from 10-60%. Hence to facilitate better germination and to produce higher quality and quantity of planting material, an experiment has been carried out with different soil media, different types of seed treatments for germination and growth of the seedlings of Sarpagandha.
Key words: *Crude drug, Medicinal properties, Exploitation, Growing Media, Seedlings.*

INTRODUCTION:

Rauwolfia serpentina (L.) Benth.ex Kurz, commonly known as Sarpagandha, belong to the Apocynaceae family is a commercial source of plant alkaloids. *Rauwolfia serpentina* is a perennial woody herb with tall plants and irregular tubular roots. The bark is green when young and brown when mature. The leaves are grouped in whorls, nearly elliptical in shape, tapering gradually at the apex. The plant is a tiny, erect, hardy shrub that grows 40 to 60 cm in height. The leaves range in colour from light to deep green and are glossy and silky. Petiole is short; pedicels, calyx, and bottom portion of the corolla tube are all pink, and the colour of the pedicels is persistent. Flowers are small and slightly inflated above the middle; the inflorescence has numerous flowered cymes with a long peduncle; the flower bud is white when very young and pinkish white when mature. The fruit is drupe with one or two seeds, the fruit progressively changes colour as it ages, going from green to brown to reddish brown to purple to pinkish black. Often called a snake root plant, the thick, sturdy roots snap quickly under pressure and have a sinuous, snake-like appearance. Under pressure, the fresh root bark can be readily separated from the root wood (1&2). The root wood has a light-yellow hue. Both the wood and the bark have a rather bitter flavour.

Classification:
Division: Magnoliophyta
Class: Magnoliopsida
Sub Class: Asteridae
Order: Gentianales
Family: Apocynaceae
Genus: *Rauwolfia*
Species: *serpentina*
Botanical name: *Rauwolfia serpentina* (L.) Benth.ex Kurz
Other common names: Indian snakeroot, Devil pepper, Serpentine wood

Fig.1: Habitat of *Rauwolfia serpentina* (L.) **Fig.2**: Seeds of *Rauwolfia serpentina* (L.)

The Sarpagandha holds great significance within the medical system. However, despite tales of its therapeutic benefits, overexploitation by indigenous and tribal people has resulted in a decline in Sarpagandha's natural reserves. As a result of this, the International Union for Conservation of Nature and Natural Resources (IUCN) has listed this species as "Endangered" (3). Since 1969, the Indian government has made it illegal to harvest and export plants that are growing wild in forests. This plant must be grown commercially and scientifically in order to meet current and future demand. The roots of plants are employed in Ayurvedic, Homeopathic, Siddha, and Unani treatments to treat asthma, high blood pressure, heart disease, and sleeplessness. Juice from leaves is used to treat corneal opacities in the eye (4).

In addition to its use in conventional medicine, sarpagandha is becoming more and more significant in the global pharmaceutical sector. The root of sarpagandha is used to make the "Serpasil" tablet for high blood pressure. Reserpine, ajmaline, rescinnamine, deserpidine, ajmalicine, and rauwolfinine are among the approximately 30 distinct alkaloids known to exist in plants (5&6).

More than fifty indole alkaloids have been identified from the plant, making them the most significant alkaloids. A class of nitrogenous chemicals known as indole alkaloids is produced from the amino acid tryptophan. According to Pandey *et al.*, (2010) they share a heterocyclic ring structure with five and six carbons and a single nitrogen molecule (7). Although indole alkaloids are present in many plant parts, including the stem and leaves, the root bark has the largest concentration. According to Bhuyar *et al.*, (2000), Ponkumar *et al.*, (2008) and Bhupendra K. Dorkar, (2021) reserpine is one of the main alkaloids derived from plants (7,8&9). Reserpine content has been shown to be higher in the base and lower in the stems and leaves. Rahul *et al.*, (2018) stated that various assays have cast doubt on the scientists' belief that it is the foremost plants paramount indole alkaloids (10).

From Moiadabad to Sikkim, the plants are dispersed throughout the plains close to the base of the hills and the tropical Himalayas. It can be found in the Sub-Himalayan plains that rise to a height of 4,000 feet, as well as in Assam, Dehra Dun, and the Siwalik range (11&2).

Commercial Sarpagandha cultivation requires the availability of high-quality planting material. Sarpagandha is grown commercially using seeds. The primary challenge with Sarpagandha seed multiplication is irregular and low germination rates. According to Farooqui and Sreeramu (2001), the percentage of seeds that germinate varies greatly, ranging from 10 to 60 percent (12). The negative impact of the stony endocarp is partially one of the reasons for poor germination and non-viability of seeds (about 50%) is another significant aspect. Another significant obstacle to Sarpagandha seed propagation is irregular germination combined with a lengthy germination period.

Due to the remarkable and valuable importance of Sarpagandha the current work has been designed to carry out an experiment to investigate the impact of growing media and seed treatments on Sarpagandha seed germination and seedling growth in order to overcome the hard stony endocarp's inhibitory effect on dormancy, promote better germination and yield a greater quantity of high-quality planting materials, which will be helpful in conserving the plant in natural habitat.

MATERIALS AND METHODS

Propagation and Plant material

Rauwolfia serpentina was conventionally propagated through seeds.

Experimental study area

The experiment was carried out during the months of February to May- 2025 in shade net house of medicinal plant garden, Department of Biosciences and Sericulture, Sri Padmavati Mahila Visvavidyalayam, Tirupati, Andhra Pradesh.

Source and collection of seeds

Seeds were collected from Mudupula Vemula Village, Pileru Mandal, Chittoor district, Andhra Pradesh, India. These were stored at room temperature for the experimental study.

Experimental details

Preparation of growing media

For the germination of *R. serpentina* seeds, media composition was prepared by the combination of Soil: Sand: Mixed Manure in the ratio of 3: 1: 1. The mixed manure was prepared by mixing the individual components FYM: Vermicompost: Cocopeat @ 1: 1: 1 on volume basis as per the requirement. The mixture of growing media was filled in pro trays and kept inside the naturally ventilated shade net house.

Seed preparation

The seeds were washed with water for 2-3 times and the cleaned seeds were thoroughly dried. Further seeds were allowed for floating test to assess the viability. The sunken seeds were used to carry out the present study.

To break the dormancy and to increase the efficiency of seed germination seeds were soaked in different treatments by using hormone Gibberellic acid (GA3), an adsorbent Activated charcoal (Ac) in alone and in combination for about 24 hr respectively. Seeds without treatment is used as control.

Soaking treatments

200 seeds were soaked for each treatment to carry out the germination. The experiment was set out with 16 treatments alone, in combinations and without treatment. The treatments which were followed are T1: GA3 1000 ppm + Ac 50 mg; T2: GA3 500 ppm + Ac 75 mg; T3: GA3 400 ppm + Ac 100 mg; T4: GA3 300 ppm + Ac 125 mg; T5: GA3 200 ppm + Ac 150mg; T6: GA3 1000; T7: GA3 500 ppm; T8: GA3 400 ppm; T9: GA3 300 ppm; T10: GA3 200 ppm; T11: Ac 50 mg; T12: Ac 75 mg; T13: Ac 100 mg; T14: Ac 125 mg; T15: Ac 150 mg; T16: Control (without-soaking).

After treatment the soaked seeds were sown in pro trays filled with soil: sand: soil mixture and maintained as per the above-mentioned treatment schedule. All the three replications of pro trays for each treatment were maintained in the shade net house.

Pattern of sowing and maintenance of seedling in pro trays

One pro tray has been used in each treatment accommodating 98 seeds. The prophylactic plant protection measures were taken during the course of investigation. Regular watering and weeding were done as per the requirement. The observation on germination and seedling growth was recorded from the date of sowing and continued up to 45 days. Germinated seeds were counted until no further germination was recorded for five consecutive days. The data recorded on seed germination, survivability, seedling shoot and root growth and development.

Transplanting and maintenance of seedling growth and development in grow bags

20 seedlings of uniform growth were transplanted in the grow bags of size 6"x4" filled with the afore-mentioned growing media after 45 days of sowing under each treatment in order to study the growth performance. The survivability percentage data was recorded after 4 weeks of transplantation in growing media.

RESULTS

The data recorded for *in vivo* germination of *Rauwolfia serpentina* seed is presented in the Table 1 and 2.

Seed Germination (%)

Variation in seed germination was observed from 65-100%. Maximum germination percentage (100%) was observed in T1: GA3 @1000 ppm + Ac @ 50 mg/l followed by T2: GA3 500 ppm+ Ac 75 mg (96%)

and the lowest (65%) in T5: GA3 @ 200 ppm + Ac @ 150mg/l. Germination was not observed in T15: Ac @ 150mg/L and in T16: Control (without pe-soaking).

Seed Geramination period for initiation and completion (Days)

Seed germination period was observed to be lowest (10-13 days) in T1: GA3@1000 ppm + Ac@ 50 mg followed by T2: GA3 500 ppm+ Ac 75 mg (12-17 days). The activated charcoal (alone) treatments (T11: Ac @ 50 mg/l, T12: 75 mg/l, T13: 100 mg/l and T14: 125mg/l) showed long duration for seed germination (17-24).

Survivability after 45 days in pro trays (%)

The survivability percentage at 45 days has also showed variation ranges from 84 to 94. Highest (94%) survivability was observed in T1: GA3@1000 ppm +Ac @50mg/L and the lowest (84%) in T5: GA3@ 200 ppm + Ac @ 150mg/l.

Shoot Length of the Seedling after 45 Days (cm)

Wide variation in shoot length was observed from 4.2 ± 0.10 to 9.2 ± 0.027 cm. Maximum shoot length (9.2 ± 0.027cm) was observed in T1: GA3@1000 ppm + Ac @ 50mg/L followed by T2: GA3 500 ppm+ Ac 75 mg (8.9±0.027 cm) and the lowest (4.2 ± 0.10 cm) in T8: GA3@ 400 ppm/l.

Root Length of the Seedling after 45 Days (Cm)

Variation in root length was observed from 5.2±0.02 cm to 8.3±0.02 cm. Maximum root length (8.3±0.02) was observed in T1: GA3@ 1000 ppm +Ac @ 50 mg/l followed by T2: GA3 500 ppm+ Ac 75 mg (8±0.05) and the lowest (5.2 ± 0.02cm) in T14: Ac @ 125 mg/l.

Survivability after 4 weeks in grow bags (%)

The survivability at 75 days has also showed variation ranging from 80 to 94. Highest (94%) survivability percentage was observed in T1: GA3@ 1000 ppm +Ac @50mg/l and the lowest (80%) in T5: GA3@ 200 ppm + AC @ 150mg/l.

Table 1: Effect of different soaking treatments on seed germination (%), Initiation and completion of germination

S.No	Soaking Treatments for Seed Germination	Seed germination (in No.)	Seed germination (%)	Initiation of Gemination (No. of Days)	Completion of Gemination (No. of Days)
1.	T1: GA3 1000 ppm +Ac 50mg/l	200	100	10	13
2.	T2: GA3 500 ppm+ Ac 75 mg/l	192	96	12	17
3.	T3: GA3 400 ppm + Ac 100 mg/l	187	87.5	14	18
4.	T4: GA3 300 ppm + AC 125mg/l	170	85	14	18
5.	T5: GA3 200 ppm + Ac 150 mg/l	130	65	15	19
6.	T6: GA3 1000 ppm	160	80	15	19
7.	T7: GA3 500 ppm	150	75	16	19
8.	T8: GA3 400 ppm	150	75	16	19
9.	T9: GA3 300 ppm	148	74	16	20

10.	**T10**: GA3 200 ppm	150	75	16	20
11.	**T11**: Ac 50mg/l	168	84	17	24
12.	**T12**: Ac 75 mg/l	142	71	17	24
13.	**T13**: Ac 100 mg/l	142	71	17	24
14.	**T14**: Ac 125mg/l	140	70	17	24
15.	**T15**: Ac 150mg/l	0	0	0	0
16.	**T16**: Control (without Treatment)	0	0	0	0

Table 2: Effect of different soaking treatments on survivability in pro trays, shoot length, root length, and survivability in grow bags

S.No	Treatment for Seed Germination	Survivability After 45days in pro trays (%)	Shoot Length (Cm) After 45 Days	Root Length (Cm) After 45 Days	Survivability After 4 weeks in grow bags (%)
1.	**T1**: GA3 1000 ppm +Ac 50mg/l	94	9.2±0.027	8.3±0.02	94
2.	**T2**: GA3 500 ppm+ Ac 75 mg/l	93	8.9±0.027	8.0±0.05	90
3.	**T3**: GA3 400 ppm + Ac 100 mg/l	92	6.5±0.027	6.8±0.051	90
4.	**T4**: GA3 300 ppm + AC 125mg/l	91	5.2±0.05	6.4±0.09	90
5.	**T5**: GA3 200 ppm + Ac 150 mg/l	84	5.4±0.10	6.0±0.051	80
6.	**T6**: GA3 1000 ppm	87	6.2±0.09	6.1±0.075	85
7.	**T7**: GA3 500 ppm	87	4.6±0.07	6.1±0.08	85
8.	**T8**: GA3 400 ppm	86	4.2±0.10	6.2±0.08	85
9.	**T9**: GA3 300 ppm	86	4.7±0.27	6.1±0.04	85
10.	**T10**: GA3 200 ppm	86	4.5±0.027	5.8±0.046	85
11.	**T11**: Ac 50mg/l	86	5.2±0.08	6.1±0.04	85
12.	**T12**: Ac 75 mg/l	86	4.6±0.16	6.2±0.04	85
13.	**T13**: Ac 100 mg/l	86	5.0±0.04	5.8±0.02	85
14.	**T14**: Ac 125mg/l	86	4.7±0.027	5.2±0.02	85
15.	**T15**: Ac 150mg/l	0	0	0	0

16.	**T16**: Control (without Treatment)	0	0	0	0

Fig 3: Seed germination in *Rauwvolfia serpentina* (L.) at **GA3 1000ppm+ Ac 50mg/lit**

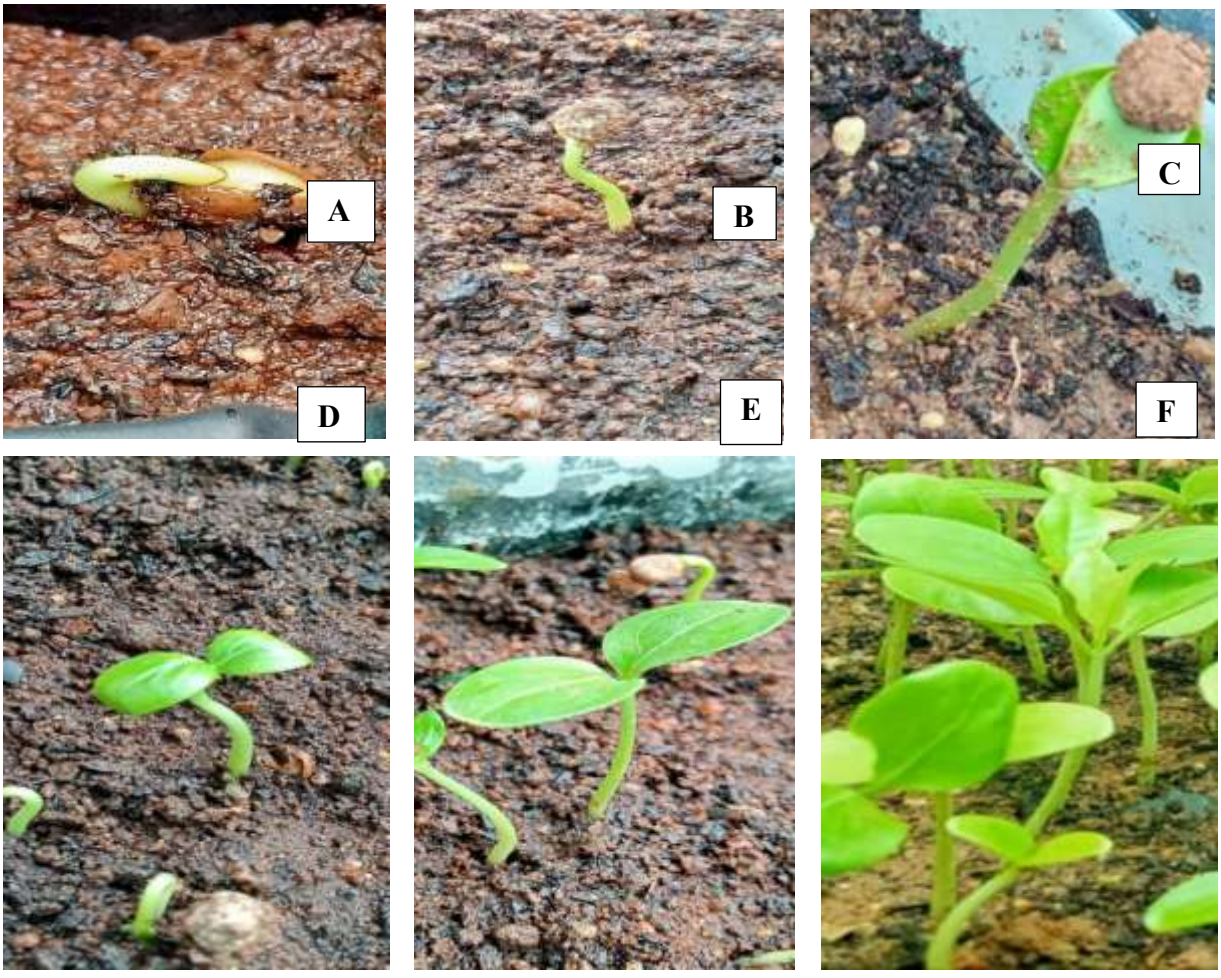

Fig 4: Seed germination in *Rauwvolfia serpentina* (L.) in different stages
A. After 10th day of sowing, **B.** 14th day **C.** 20th day **D.** 24th day **E.** 27th day **F.** 30th D

G **H**

Fig 5: Seed germination of *Rauwvolfia serpentina* (L.)
G. After 45 Day of sowing in pro trays **H**. After 4weeks of transplantation in grow bags

DISCUSSION

Farmers commercially follow seed propagation but, the two main challenges in propagating Sarpagandha seeds are seed dormancy and the absence of viable embryos. An endogenously regulated yet externally enforced temporary halt in growth that is unaffected by external factors is known as dormancy. In Sarpagandha, a stiff seed coat and a high ABA content may cause seed dormancy. Exogenous applications of various substances and growth regulators have been used in a number of cases to overcome these barriers. Different treatments were tried in order to increase seed germination.

According to the results obtained, the treatment with GA3 @1000 ppm + Ac 50 mg/l has the highest percentage of germination. This could be because early induction of α-amylase activity allowed the seeds to efficiently use the limited food supply. The results reported by Hussain and Jha (2014), Bhuyar *et al.* (2000), Ponkumar *et al.* (2008), Anonymous (2017), and Phatak *et al.* (2017) in Sarpagandha are in support with the current findings (13,8,9&14). But according to Paul *et al.* (2008), none of the acidic or chemical seed treatments considerably increased the germination rate in Sarpagandha (4).

Sarpagandha seedlings treated with GA3 @1000 ppm + Ac 50 mg/l were sown in a soil media (soil, sand, mixed manure @3:1:1) showed improved germination (Mixed Manure is made up of FYM, vermicompost and cocopeat @1:1:1). This mixed manure may have offered the favorable physical circumstances required to initiate enzymatic and biochemical activities. Along with soaking treatments, may be the growing media composition in particular with mixed manure ratio composition may have suitable and favourable factors like nutritional status. Physical environment may also show favourable effect which facilitates better growth and development of seedlings.

The endogenous GA3 in the embryo may be in low concentration, so exogenous GA3 treatment through seed soaking combined with a 3:1:1 ratio of soil, sand, and mixed manure may have accelerated the germination process and increased germination by eliminating inhibitory compounds, promoting embryo growth, and lowering the inherent ABA/GA3 ratio.

Seedling growth and Survival percentage

Plant development was significantly influenced by GA3 at 1000 ppm and activated charcoal at 50 mg/l. Higher concentrations of GA3 applied externally may have stimulated growth by promoting cell expansion and multiplication, which in turn led to increased plant growth. It's also possible that the early and quick germination gave the plants more time to grow vegetatively. It's possible that the seed that germinated early would have grown vigorously later. Soaking treatment of GA3 results in homogeneous germination, an intensified hydrolytic process, and improved nutrient and moisture uptake, all of which

contribute to the increase in shoot and root length. Ponkumar *et al.* (2008) observed that GA3 has a positive effect on the vegetative growth of seedlings in Sarpagandha(9).

The various seed treatments had a substantial impact on the Sarpagandha seedlings' survival percentage. Maximum (94%) survivability percentage was observed in GA3 @ 1000 ppm +Ac 50mg/l and the minimum (80%) in GA3 @ 200 ppm + Ac 150mg/l. The results also showed that maximum shoot length (9.2 ± 0.027cm) was observed in GA3 @1000 ppm + Ac 50mg/l followed by GA3 @ 500 ppm + Ac 75 mg/l and the minimum (4.2 ± 0.10 cm) in GA3@ 400 ppm/l. Maximum root length (8.3±0.02) was observed in GA3@ 1000 ppm +Ac 50mg/l followed by GA3 500 ppm + Ac 75 mg/l and minimum (5.2 ± 0.02cm) in Ac 125 mg/l. This may be because early and quick germination allowed for more time for vegetative growth, which improved plant establishment.

Malik and Swain (2018) conducted the different growing media composition and sowing seed treatments on seed germination (16). The results of initiation of germination recorded minimum days (15) which has minimal variation more or less in comparison to all treatments in the present study (10-17) whereas completion of germination (62) exhibited contrary results with wide variation. In all the variables, results indicated that growing media garden soil+FYM+sand exhibited better performance than coco peat+vermiculite+perlite in the ratio of 2: 1: 1. The results in soaking treatments found better in GA3 @150ppm but germination percentage (38) was showed contrary results in the present germination percentage (100). In contrast to this, present study results confined to GA3 @1000 ppm in combination with Ac 50 mg/l. Phatak *et al* (2017) studied seed germination with different soaking treatments but results yielded in GA3 @1000 ppm (15). With respect to germination and seedling growth Rahul *et al* (2018) also yielded results in overnight soaking treatment in GA3 @ 1000 ppm. In both the studies, results obtained by using alone coco peat filled in pro trays (10). Bhupendra K Dorkar (2021) studied germination activity and prepared nursery bed with partial shade by using specific farm yard manure which comprises 1/3rd FYM and leaf mould and 2/3rd medium of silt-loam soil (2). Initiation of germination noticed in 19th day, peak was recorded in 28th day and germination percentage (65) were recorded as per 10 replicates.

Role of activated charcoal has less evidence and it has not been reported earlier in any *in vivo* germination studies of *R. serpentina*. This may influence indirectly for the early initiation or germination which may be due to mechanism of adsorption of toxic substances or growth inhibitors and removes unwanted substances from the environment. Of many soaking treatments for the germination of *R. serpentina,* none of them have tried activated charcoal alone or in combination. Among all the treatments Activated charcoal (Ac) in combination with GA3 promoted high performance. Alone activated charcoal (Ac) treatments were expressed minimal performance whereas Alone GA3 @1000 ppm has showed little better results comparatively with other alone GA3 treatments.

CONCLUSION:

Techniques for mass production and multiplication in agricultural settings must be developed in order to close the gap between supply and demand for *R. serpentina* roots. After the research findings, it is observed that the application of Gibberellic acid @ 1000 ppm in combination with Activated charcoal @ 50 mg/l has shown best results among all the treatments in seed germination percentage, survivability % and shoot and root growth. Hence, to overcome the lower germination % of Sarpagandha, application of Gibberellic acid @ 1000 ppm in combination with Activated charcoal @ 50 mg/l can be recommended for maximum seed germination, survivability and growth. In view of the germination percentage, according to literature reviewed, none of the experimental studies showed above 90 per cent seed germination in *R. serpentina*.

Acknowledgements:

The authors gratefully acknowledge that the funding for this publication was provided by the Pradhan Mantri Uchchatar Shiksha Abhiyan (PM-USHA), under the Multi-Disciplinary Education and Research Universities (MERU) Grant sanctioned to Sri Padmavati Mahila Visvavidyalayam, Tirupati.

Conflict of interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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