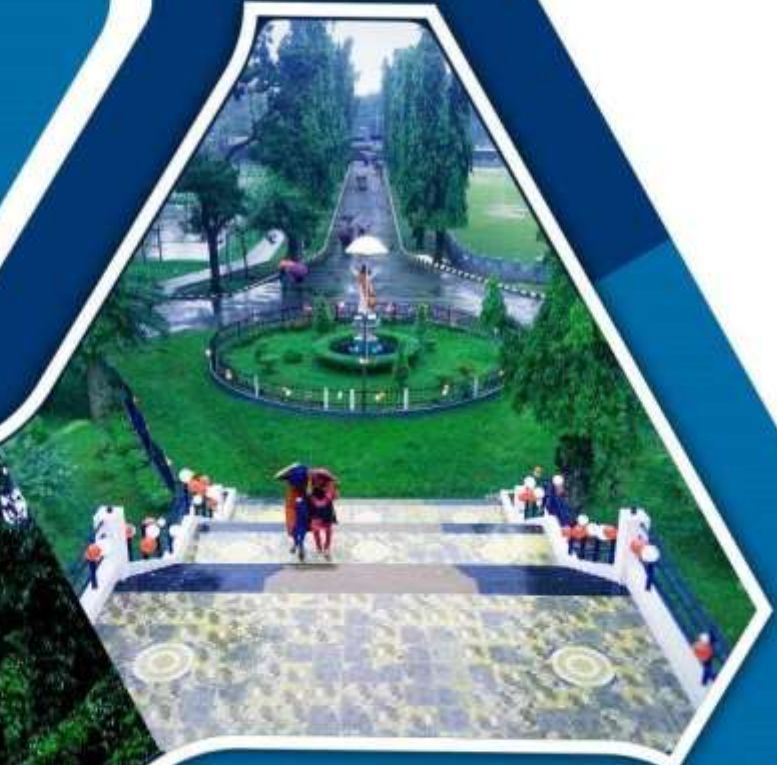


FATIMA MATA NATIONAL COLLEGE

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2.2.1 Student Publication in Journals, Periodicals

IQAC INTERNAL QUALITY ASSURANCE CELL

STUDENT PUBLICATIONS IN JOURNALS, PERIODICALS, AND NEWSPAPERS

Encouraging and supporting advanced learners to publish their research work in reputable journals, periodicals, and newspapers can be a highly beneficial initiative for both the students and the institution. Here are some steps that Fatima Mata National College in Kollam can take to foster a research-friendly environment and assist students in publishing their research:

1. **Establish a Research Support Center:** Create a dedicated research support center within the college where students can access resources, guidance, and assistance related to research and publishing. This center can offer workshops, training sessions, and one-on-one consultations with faculty or experienced researchers to help students navigate the publication process.
2. **Form Research Mentorship Programs:** Pair advanced learners with faculty members who have experience in publishing research in reputable journals. Mentorship programs can provide valuable guidance, feedback, and encouragement to students throughout the research and publication journey.
3. **Conduct Research Seminars and Workshops:** Organize seminars and workshops on research methodologies, academic writing, and publishing strategies. Inviting experienced researchers and journal editors as guest speakers can offer valuable insights and networking opportunities for the students.
4. **Collaborate with Faculty:** Encourage collaboration between students and faculty members on research projects. Jointly authored papers can provide students with the expertise and support needed to publish in reputable journals.
5. **Support Research Conferences and Symposia:** Provide opportunities for students to present their research at college-level conferences or symposia. This can help them gain experience in presenting their work and receive constructive feedback from peers and faculty.
6. **Offer Research Grants and Scholarships:** Establish research grants or scholarships to financially support students engaged in high-quality research projects. This financial assistance can help cover publication fees or conference

travel expenses.

7. **Foster a Research Culture:** Promote a culture of research and scholarly inquiry within the college. Recognize and celebrate students who successfully publish their work in reputable journals, creating incentives for others to pursue similar achievements.
8. **Facilitate Journal Selection:** Assist students in identifying reputable journals that align with their research topics. Help them understand the journal's submission guidelines, peer-review process, and ethical considerations.
9. **Provide Writing and Editing Support:** Offer writing and editing assistance to students, either through peer review or professional editorial services, to improve the quality and language of their research papers.

By implementing these strategies, Fatima Mata National College can encourage and support advanced learners in publishing their research work in reputable journals, periodicals, and newspapers, thereby promoting a strong research culture and enhancing the institution's academic reputation.

UNFOLDING BOTANICAL KNOWLEDGE

A Compilation of Research Papers
in Life Sciences

EDITED BY

Dr. Rogimon P. Thomas

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**Unfolding Botanical Knowledge:
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ESTABLISHMENT OF CALLUS CULTURE OF *TYLOPHORA INDICA* MERRILL AND PHYTOCHEMICAL INVESTIGATION

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Introduction

Tylophora indica (Burm. f.) Merrill, commonly called Antamul or Indian ipecac, is an important medicinal plant belonging to the family Apocynaceae. It is a perennial, woody, climbing shrub and is found on plains, hilly slopes and the outskirts of the forests of eastern and southern India (CSIR1978). About 60 species are found in tropical, sub-tropical Asia, Africa and Australia and about 8 species are reported from India (Kour and Gupta 2015).

In Ayurveda, the plant has been used to cure asthma, dermatitis and rheumatism (CSIR1978; Chopra *et al.* 1986). The leaf and root of this plant are widely used for treating jaundice in Northern Karnataka but there is a paucity of scientific evidence regarding its usage in liver disorder (Kirtikar *et al.* 1991). The presence of tylophorine is responsible for strong anti-inflammatory action (Gopalakrishnan *et al.* 1980) and tylophorinidine is a potential anticancer alkaloid, (Mulchandani *et al.* 1971). Its root also possess stimulant, emetic, cathartic, expectorant, stomachic, diaphoretic, bacteriostatic and antifeedant properties (Varrier *et al.* 1994).

Vegetative propagation is difficult in *T. indica* due to low seed viability and germination rate (Thomas 2005). Stem cuttings failed to produce proper root even after the treatment with different growth regulators. All these, justify the significance of development of an efficient regeneration protocol for rapid multiplication of *T. indica* to meet the demand in the pharmaceutical industry and also to conserve the population by replenishing it in the wild.

Materials and Methods

Plant material

Tylophora indica Merrill, commonly known as Indian Ipecac or Antmool belongs to family Asclepiadaceae. The plant is perennial, small, slender, a twining or climbing herb. Leaves are Ovate to elliptic (6.0-10.5X3.8-6.0 cm), petioles are up to 12mm long. Flowers are minute (1-1.5cm across) and corolla is greenish yellow or greenish purple in color. Fruit is a follicle (Gamble 1984, Sasidharan 2004). Six to eight month old rooted cuttings of *Tylophora indica* grown in the pots in the GreenHouse of KRIBS Bionest were used as the source of explants for initiation of *in vitro* culture.



Fig1: A flowering plant of *T. indica*

Initiation of callus culture

The composition of Murashige and Skoog (MS) media (pH 5.8), used throughout the study. The nutrient media used for callus initiation was MS media supplemented with varied concentrations and combinations of growth regulators. The media for all the cultures contained 3% sucrose and different growth regulators 2, 4-D and Kn at varied concentrations with the pH adjusted to 5.8 before adding 0.6% agar. In all experiments the cultures were incubated at 26°C. Photoperiod provided with white fluorescent tube lights.

The Callus induction frequency was calculated to find out the medium best suitable for the same. Frequency of callus induction was calculated using the following formula:

$$\text{Frequency of callus induction(\%)} = \frac{\text{Number of explants with callus response} \times 100}{\text{Total number of explants inoculated}}$$

Induction and establishment of callus culture

After three weeks of callus initiation, the explants with callus tissues were routinely subcultured to fresh media at 3 week intervals for establishing semifrable callus cultures. The process was continued till sufficient growth and quantity of callus was obtained for further experiments.

Indirect shoot regeneration from callus culture

Callus (about 700 mg) was cultured on MS medium supplemented with different concentration of phytohormones BAP and IAA. At 2 week intervals, the calli were subcultured in their respective fresh medium for shoot induction and further development. The percentage of callus response and the number of shoots per clump of callus was recorded after 15 days of culture third subculture in shoot induction media. The frequency of shoot induction among the callus clumps was also calculated similar to calculation of callus induction frequency.

Shoot proliferation, rooting and hardening

Shoot buds induced on callus clumps were then separated and grown on media containing 2 mg/L BAP and 0.5 mg/L IAA for elongation and shoot proliferation.

Individual shoots were dissected on reaching a height of around 3-4 cm and transferred to half strength MS medium supplemented with 0.2mg/L indole-3-butyric acid (IBA) for rooting. A part of the rooted shoots were taken for further procedures on phytochemical screening. Another batch of the rooted shoots was then acclimatized towards development of a complete micropropagation protocol.

Extraction of Secondary metabolites and Phytochemical analyses

Preparation of plant extracts was done as per Malathi *et al* 2012. Each type of plant material powder was extracted with petroleum ether (60 to 80°C) using Soxhlet apparatus to remove lipids. It was filtered and the filtrate was discarded. The residue was extracted with methanol by soxhlet apparatus at 60°C for 5 hours. The extract was concentrated to a low volume in vacuum evaporator (Cyber Lab) with the final volume of extract being evaporated to dryness at room temperature. The extracts obtained like an amorphous solid mass was stored in refrigerator at 4°C and protected from sunlight until the time for further analysis.

This was later dissolved in 10 ml of methanol to form the crude sample for further phytochemical screening as per Trease (1989), Sofowara (1993) and Harborne (1973).

Results and Discussion

The results of this study establish a successful protocol for micropropagation of *Tylophora indica* through callus mediated regeneration. Best callusing response was obtained on Murashige and Skoog (MS) medium supplemented with 1 mg/L 2,4-D and 0.5 mg/l kinetin. Callusing occurred at either or both of the cut ends after 5-11 days of culturing (Fig. 1A). Callus formation of differentiated tissue begins with a de-differentiation process to produce meristematic and actively

proliferated cell. This process causes the explants tissues to become visibly thick, stiff and swollen. Actively divided cells produce unorganized cell masses on the explants surfaces. In the results the combination of 2,4-D with kinetin induced the cut ends of the internodal segments to produce callus. The morphology of callus derived from various combinations of auxins and cytokinins showed different color and texture after two weeks of initiation. The watery semifriable white translucent callus was more generated in 2,4-D + Kinetin rather than 2,4-D + BAP (Table 1). The hormonal balance proposed by Skoog and Miller (1957) explains the concept of organ formation or unorganized growth of the tissue.

Hormone mg/l			Callus induction (%)	Day for Callus initiation	Qualitative Nature of callus
2,4-D	Kinetin	BAP			
1.5	0.5	-	75.77	9	Watery White semifriable
1	0.5	-	81.35	5	Watery semifriable white translucent
0.5	0.5	-	72.31	7	Pale Yellow semi friable
0.5	1	-	64.09	7	Yellow semi friable
0.5	1.5	-	61.63	11	Yellow semi friable
1.5	-	0.5	47.27	14	Pale yellow sticky
1	-	0.5	56.24	14	Pale yellow sticky
0.5	-	0.5	49.60	18	Yellow sticky
1.5	-	1	53.12	14	Pale yellow compact
1	-	1	49.65	16	Pale yellow compact
0.5	-	-	-	-	-
1	-	-	-	-	-

Table 1. Callusing response in MS media with various combinations of phytohormones.

2,4-dichlorophenoxy-acetic acid (2,4-D) is the most common auxin applied to initiate callus growth since it can revert explant cells to a dedifferentiated state and begin to actively proliferate. A combination of 2,4-D with kinetin is known to promote the cytoplasm of the vacuolated parenchyma cells (derivative cells) of petiole cells in *Arabidopsis thaliana* to become denser (Li *et al.* 2012). In contrast, kinetin alone did not induce the cytoplasm of the derivative parenchyma cells to become denser but remained highly vacuolated.

Faisal and Anis (2005) reported callus induction from stem and petiole segments of *Tylophora indica* on MS medium supplemented with 2, 4, 5-T (10 μ M) and 2, 4-D and TDZ respectively. Thomas and Philip (2005) achieved callus formation from mature leaf pieces on MS supplemented with 2, 4-D (7 μ M) and BAP (1.5 μ M) in 92% explants. Role of auxin alone or in combination with cytokinin for callus proliferation is well documented.

After sufficient callus induction, callus culture was then established through routine subculture into media with 1 mg/L 2,4-D and 0.5 mg/L kinetin, at 3 weeks intervals (Fig. 1B). The subculture processes in callus is important to ensure good growth and development. The failure to do subculture in callus will cause the tissue to gradually die. Narayanaswamy (1994) recommended that callus has to be subcultured at every 4–6 weeks.

Regeneration of shoot is the next step after achieving callus in indirect organogenesis method. Organogenesis can be initiated in callus cultures by the effect of different growth regulators. The necessity of cytokinin for shoot initiation is well established (Evans *et al.* 1984). It

has been established that callus producing roots will never differentiate into shoots because shoot morphogenesis gets terminated with the formation of roots within a callus. Hence, the prospect of plantlet production from callus with roots is not feasible (Narayanaswamy, 1994).

Shoot induction was achieved from the surface of the callus after transferring onto shoot induction medium. The highest rate of shoot regeneration was achieved on MS medium containing 2 mg/L BAP and 0.5 mg/L IAA. The callus when subcultured into this media as small clumps of around (0.7±0.08 g), 73% callus clumps responded with production of shoots from the surface of the inoculated callus (Fig. 1C). This media also produced the maximum number of shoot buds (17.50±1.81), when compared to the other phytohormone combinations.

Conc. of BAP in mg/L	Conc. of IAA in mg/L	Rate of shoot induction (in %)	Average number of shoots per callus clump
0.5	0.5	21.2	6.50±1.32
0.5	1	23.1	8.32±0.76
1	0.5	32.4	11.50±0.89
1	1	52.1	12.50±1.92
2	0.5	73.2	17.50±1.81
2	1	53.8	16.41±0.99
3	0.5	60.8	9.32±1.09
3	1	55.0	11.50±1.17

Table 2. Shoot regeneration response from callus clumps in MS media with various combinations of phytohormones.

The regeneration process was found to vary significantly among the various treatments. The green coloration was noticed as early as 7 days after inoculation in treatments containing 0.5 mg/L of BAP. Majority of green spots later formed shoot primordia, and developed into a young shoot bud. Greening of callus was pronounced in media with 3 mg/L of BAP. But the process of shoot bud regeneration from the greenish required a greater culture period in these media.

Shoot elongation and proliferation occurred well in the media (Fig. 1D&E). The shoots elongated and grew well and developed many leaves. Generally, a wide range of cytokinins like BAP, 2ip and kinetin have been employed in shoot proliferation (Bhojwani and Razdan, 1982). The shoot proliferation effect of BAP observed in the present study is in consonance with other reports (Rathore *et al.* 2008).

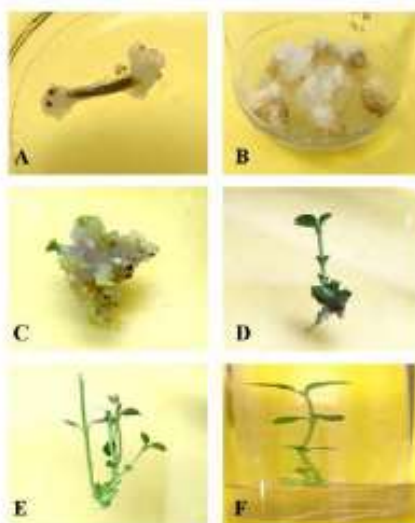


Fig 2. Different stages of indirect regeneration in *Tylophora indica*.

(A) Initiation of callus at cut ends of explants (B) Established Callus Culture (C) Shoot buds developing on callus clumps (D) Shoot elongation and proliferation from callus derived shoot bud (E) Shoots multiplied and proliferated (F) Rooting of individual shoots

The individual shoots (elongated to around 3-4 cm) rooted well when inoculated on to rooting media of half strength MS medium supplemented with 0.2mg/Lindole-3-butyric acid (IBA) (Fig. 1F). These healthy rooted shoots were then transferred to the potting mixture of soil: vermicompost (1:1) and plants with newly formed leaves were shifted to green house for 2 weeks and eventually established in soil with 90% survival rate. Thus, the *in vitro* raised plantlets with well developed shoot and roots were acclimatized successfully and grown in greenhouse, thus establishing a complete micropropagation protocol for *T. indica*.

The methanolic extracts of *T. indica* samples preserved in the refrigerator were utilized for qualitative analysis to determine the presence of chemical constituents of different classes as per the standard protocols. The *in vitro* rooted plant sample showed greater similarity to the phytochemical profile of the field grown plant. Tannins were not observed in the *in vitro* plants which may be due to the difference in tissue strength and type of the *in vitro* plant growing under controlled conditions. The callus culture showed the presence of very few compounds and this may be attributed to the reason that a certain degree of differentiation may be necessary for the biosynthesis of secondary metabolites to occur.

As identified through our study as well, literature extensively suggests that *T. indica* possess various alkaloid and non-alkaloid compounds. The solubility of active constituent is depended on its chemical nature and also a solvent. When methanolic and aqueous extracts of leaf and roots were qualitatively evaluated by Khanna *et al.* (2018) for the presence of different phytochemicals, leaves revealed the presence of alkaloids, carbohydrates, glycosides, saponin and tannins. The root extracts revealed the presence of alkaloids, carbohydrates, glycosides, saponins, tannins, steroids, and amino acids (Khanna *et al.* 2018). Thus the present study reaffirms the presence of various secondary metabolites which are responsible for multiple pharmacological effects of the plant.

Class of Secondary metabolite	Callus culture	<i>In vitro</i> plant	Field grown plant
Alkaloids	+	+	+
Carbohydrates	-	-	-
Cardiac glycosides	-	-	-
Coumarins	-	-	-
Flavanoids	-	+	+
Phenols	-	+	+
Quinones	-	-	-
Saponins	-	+	+
Steroids	+	+	+
Tannins	-	-	+
Terpenoids	-	-	-

Table 3. Preliminary phytochemical screening of methanol extracts of different samples of *Tylophora indica*.

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References

- Bhojwani S.S & Razdan M.K (1982), Plant tissue culture: Theory and practice. *Elsevier Sci Publ. Amsterdam*, pp. 1-520.
- Chopra I.C, Chopra R.N, Nayar S.L (1986), "Glossary of Indian medicinal plants", CSIR, New Delhi, pp. 5-10.
- CSIR (2003), The wealth of India: a dictionary of Indian raw materials and industrial products, vol 10. Council of Scientific and Industrial Research, New Delhi, pp. 398-399
- Evans D. A, Sharp W. R, Bravo J. E (1984), Cell culture methods for crop improvement. In: Sharp WR, Evans DA, Ammirato PV, Yamada Y (Eds.). *Hand Book of Plant Cell Culture. Vol.2. Macmillan Publishing Company*, New York.
- Faisal M, Anis M (2005), *In vitro* regeneration and plant establishment of *Tylophora indica*: Petiole callus culture. *In vitro Cell. Dev. Biol. Plant*, 41: pp. 511-515.
- F. Skoog, and C.O. Miller, (1957), *Symp. Soc. Exp. Biol.* 11, 118-131.
- Gamble, J.S (1984), 'Flora of the Presidency of Madras', vol. 1, 2, 3, *Newman and Adlard Publishers*, London.
- Gopalkrishnan C, Shankamaryanan D, Nazimudeen SK, Kameshwaran L (1980), "Studies of pharmacological effects of extracts and total alkaloids of *Tylophora indica*", *Indian J med Res*, Vol-71; pp. 940-948.
- Harborne, J.B. *Phytochemicals Methods. Chapman and Hall Ltd.*, London (1973), pp.49-188.
- Khanna C, Vyas M, Singh S (2018), Physicochemical, qualitative, and high profile thin-layer chromatography study of *Tylophora indica* (Burm. f) Merr. leaves and roots. *International Journal of Green Pharmacy*, 12 (2), pp.136
- Kirtikar K.R, Basu B.D (1991). "Indian medicinal plants", 2nd Ed. Periodic expert book agency, New Delhi; pp.1-5.
- Kour, N., & Gupta, M. (2015), Utility of *Tylophora Indica* as Antiasthmatic Plant: A Review. *Journal of Plant Development Sciences* Vol. 7(12), 869-873.
- Li F, Cui X, Feng Z, Du X J, Zhu J (2012), The effect of 2,4-D and kinetin on dedifferentiation of petiole cells in *Arabidopsis thaliana*, *Biologia Plantarum*, 56, pp. 121-125.
- Mulchandani, N.B, Iyer, S.S, and Badheka, L.P (1971): *Chem. India*, 19: pp. 505-506.
- Narayanaswamy S (1994), *Plant cell and tissue culture, 9th reprint India Tata McGraw-Hill Education*.
- Sasidharan, N (2004), 'Biodiversity documentation for Kerala', Part 6, *Flowering plants*, KFRI handbook.
- Sofowra, A (1993), Medicinal plants and traditional medicine in Africa. *Spectrum Books Ltd.*, Ibadan, Nigeria, pp.191-289.
- Thomas D, Philip B (2005), Thidiazuron-induced high-frequency shoot organogenesis from leaf-derived callus of a medicinal climber, *Tylophora indica*. *In Vitro Cellular and Dev. Biol. Plant*, 41: pp. 124-128
- Thomas D, Philip B (2005), Thidiazuron-induced high-frequency shoot organogenesis from leaf-derived callus of a medicinal climber, *Tylophora indica*. *In Vitro Cellular and Dev. Biol. Plant*, 41:124-128.
- Trease G.E and Evans W.C (1989), *Pharmacognosy*, 11th edn., *Bailliere Tindall*, London, pp. 45-50.
- Varrier, P.K, Nambiar, V. P. K, and Ramankutty, C (1994), *Tylophora indica* Indian medicinal plants—a compendium of 500 species. New Delhi: Orient Longman 1994;5: pp. 66-68.

TISSUE CULTURE DERIVED AND WILD PLANTS OF *BACOPA MONNIERI* (L.) WETTST.: A PRELIMINARY PHYTOCHEMICAL COMPARISON

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Introduction

Brahmi (*Bacopa monnieri*), a well-known nootropic is an outstanding medicinal plant widely used therapeutically in the orient and becoming increasingly popular ingredient in food products. Brahmi has a high market demand due to its medicinal properties. *Bacopa monnieri* is a plant belonging to the family Scrophulariaceae, has a long history in traditional medicine and is known for its memory-enhancing properties as well as reducing anxiety (Amaravathi *et al.*, 2020). Estimated consumption of this drug in India is about 1000 tons per year. The continued commercial exploitation of this valuable medicinal plant has resulted in reduction of its population in the natural habitat. The natural regeneration of this endangered plant is hampered by death of the plant at two leaf stage and specific habitat (marshy areas) requirement. Moreover, with heavy demand and limited supply, it is the most adulterated species in Ayurvedic formulations. More than 90 percent of medicinal plant species used by the industry are however collected from the wild source of which 70 percent involve unorganized harvesting.

These factors contribute towards posing a serious threat to the genetic stock and the biodiversity of medicinal plants (Tripathi *et al.*, 2012). The National Medicinal Plant Board (NMPB), Government of India and Technology Information Forecasting and Assessment Council (TIFAC) recommended immediate attention to seven medicinal plants, among which *Bacopa monnieri* took a prominently place and was included in the category of highly endangered medicinal plants in India (Tripathi *et al.*, 2012)

So, it is important to conserve this medicinal plant through application of robust technologies that can help augment the production of Brahmi and also serve as an alternate source of production of its medicinal bioactives. In the present scenario, Plant tissue culture emerges as one of the finest technologies, with its varied and vast applications. It is an attractive alternative approach to regenerate as well as to conserve natural populations of Brahmi for future needs (Rao and Ravishankar 2002). Hence, the present study was undertaken to develop an efficient protocol that can be economically up-scaled for faster *in vitro* propagation.

Materials and Methods

The important aspects of the study include Collection and identification, Aseptic operations including surface sterilization, preparation of nutrient media, providing appropriate culture conditions for growth of plant tissues, preliminary phytochemical comparison of *in vitro* rooted shoots with field grown plants, observations and data collection for understanding the result etc.

Plant material

Bacopa monnieri (L.) Pennel (Family: Scrophulariaceae, Common name: Brahmi), was selected for the present investigation (Fig.1). The plant is a glabrous somewhat succulent, creeping herb, rooting at the nodes, with numerous prostrate branches, each 10-30 cm long; Leaves oblong to spatulate, sessile, decussate, rather fleshy, entire, punctuate, obtuse; Flowers axillary, solitary, peduncles often much longer and deflexed in fruiting stage; Corolla about one cm long, pale, lobes 5, oblong, obtuse, subequal, tips purple; Capsule ovoid-acuminate or slightly beaked at the apex; Seeds oblong, truncate, longitudinally ribbed with transverse striations in between the ribs (Gamble 1984, Sasidharan 2004).



Fig. 1: A flowering plant of *B. monnieri*

Aseptic operations, nutrient media and culture conditions:

Culture initiation

Nodal explants trimmed as above were then inoculated in erect position into the test tubes with into media with various concentrations of cytokinin BAP. At least 15 replicates were used for each treatment in each of the following experiments. Observations made through a period of 15 days of culture and the rate of contamination, number of days taken for bud initiation in each of the media etc. were noted.

Shoot Multiplication and establishment of multiple shoots

After 3 weeks of culture, initiation shoot buds emanated from the nodal meristem were dissected out and subcultured to medium found best as per above experiment for establishment of multiple shoot culture. The shoots were routinely subcultured into the same media at 2 week intervals to establish sufficient culture stock for further experiments. After 2-3 subcultures, the initiated plants were taken out the test tube under the laminar flow hood, medium adhered to the plants was removed, and were placed into the culture bottles containing autoclaved semi-solid media having the various combinations of phytohormones that promote rooting.

Rooting and hardening

Shoots of 3.5-4cm length were separated individually and transferred to MS basal and medium supplemented with varying concentrations of auxins for finding the media suitable for best rooting response.

Rooted shoots obtained in 3 weeks of culture were weaned away from bottles and washed well in running tap water to remove remnants of agar. Some of these plants were kept away for further procedures on extraction of secondary metabolites. The other deflasked plantlets were potted in small plastic cups filled with fine sand and farmyard manure (3:1) mixture and kept under 25% shade provided with irrigation. The rate of establishment was calculated.

Extraction of secondary metabolites and phytochemical analyses

Whole plant of *B. monnieri* uprooted from greenhouse and a known quantity of *in vitro* rooted plants were dried under shade at room temperature and then powdered using a grinder. The shade dried plant powder was soaked with methanol (1:10 ratio). The flask was covered with aluminum foil to avoid evaporation and then kept for 48 hrs in 50 rpm shaker incubator at room temperature. After 48 hrs the solution was filtered by using Whatman filter paper No.1 and the filtrate was collected in a beaker. Then, the filtrate was kept in incubator at 37 °C to evaporate the solvent. The prepared extract was then stored at 4 °C for further use and subjected to phytochemical screening as per Trease and Evans (1989), Sofowara (1993), Kokate (1997) and Harborne (1973).

Results and Discussion

As stated by Jain *et al.*, (2013), the rapidity of multiplication of true-to-type plants and efficient transplantation of *B. monnieri* can be advantageous in the conservation of the endangered species and also be helpful in propagation of elite plants for commercial exploitation. Thus a suitable *in vitro* regeneration protocol that is rapid and cost-efficient is very much necessary towards mass multiplication. Thus, the significance of the present study can be established on the basis of the conservational and commercial utilization aspects of *B. monnieri*.

Surface sterilization is known to be the most important step before inoculation of any explants. Shrivastava and Rajani (1999) have described sterilization treatment of *Bacopa*, which includes use of 0.1% Mercuric chloride (w/v) for 2 mins followed by rinsing thoroughly with sterile distilled water. Mathur and Kumar (1998) in which leaves and stem explants were shaken for 10 minutes in Tween 20 (Ranbaxy) and Savlon (Johnson & Johnson) in water for 10 minutes followed by treating with 0.1% Mercuric chloride for 3-4 minutes and several washes with sterile distilled water. Latest reports are available of much elaborate processes of surface sterilization using 20% Savlon, followed by 0.1% of Tween-20 for 2 min and washed with water thoroughly. Following this, sterilization was then done by 70% alcohol for 30 sec and washing with three times with distilled water. After transferring the explants in autoclaved flask, final surface sterilization was done with 0.1% HgCl₂ solution for 2 min 30 sec with agitation, inside the laminar flow cabinet (Rahe *et al.*, 2020). Our results showed 86.17% of contamination free explants with all of them responding with bud formation, which indicates that the sterilization procedure has not been too rough on the plant tissues or in any way harmfully

affected the meristematic activity of the nodal explants. Of the contamination free explants obtained, the first response was obtained by day 9 of inoculation and all explants showed bud break by day 17.

Hormone concentration mg/l BAP	Average number of shoots per explant	Length of shoots (cm)
0.1	3.21±0.87	4.09±1.02
0.2	4.35±0.52	4.24±1.23
0.5	7.52±1.09	6.23±1.11
0.75	5.21±0.87	5.19±1.12
1	4.29±0.73	3.99±0.54
1.5	4.61±1.98	3.22± 0.71
2	4.22±0.87	2.12±0.87
2.5	3.20±0.25	2.09±9.35

Table 1. Initiation response of *B. monnieri* in various phytohormone combinations of MS media

Axillary bud break was achieved in all media supplemented with (0.1–2.5 mg/l) BAP. The number of shoots proliferating from each explant ranged from a minimum of 2 to maximum of 11, over a period of 3 weeks. Shoots that emerged towards the later end of incubation were allowed to elongate for a period of 7 days more for taking the observations on shoot length and number of shoots. The regenerative response of explants towards axillary bud multiplication was greatly influenced by the concentration of the growth regulator (BAP) in the medium. Increase in concentration of BAP reduced the percentage of bud break and the number of shoots that proliferated. At a concentration of 0.5 mg/L BAP, distinct average of 7.52±1.09 shoots were formed that had an average length of 6.23±1.11 cm.

Cluster formation initiated from the basal node; progressively increases in size from each subculture and number of shoots initiated from the nodal portion vary from medium to medium. It was observed that during each passage, the number of leaves/shoots has increased substantially along with the height of shoots. Further multiplication was continued in the same medium (0.5 mg/L BAP) and after a period of 4 weeks, a total number of 122.28±4.50 shoots were obtained. Shrivastava and Rajani (1999), reported that out of two cytokinins used BAP was found to be suitable than Kn, as BAP resulted in quicker and better response. Tiwari *et al.*, (2001) also reported that addition of BAP resulted in the increase in number of shoots. Both these reports clearly supported our observations as well.

After two cycles of multiplication subculture, elongated shoots of 5-6 cm in length were excised and cultured on MS basal medium having different combinations of MS media with auxins for rooting. Initiation of rooting took place after 5-6 days of inoculation. Single and multiple roots were formed from the base and the nodal portions and the length of the roots were 2-3 cm within 25-30 days.

To get preliminary idea about the active constituents present in *in vitro* and field grown plants of *B. monnieri* phytochemical screening was performed and this showed the presence of several important phytochemical constituents like saponin, flavanoids, alkaloids, steroids, phenols etc. These compounds have been identified in the preliminary studies conducted by Basak *et al.*, (2016). The preliminary phytochemical studies received pronounced importance, because the crude drugs possess varied composition of secondary metabolites.

IBA (mg/l)	Minimum Days required for root initiation	% of responsive explants	Average no. of roots/ plant	Average length(cm) of roots/plant
0.1	10	95	7.5 ± 0.18	2.5 ± 0.22
0.2	10	100	10.6 ± 0.32	3.1 ± 0.15
0.5	5	100	14.0 ± 0.23	4.5 ± 0.21
1.0	7	90	10.3 ± 0.17	2.3 ± 0.51

Table 2. Effect of media supplemented with different concentrations of IBA on root induction from the *in vitro* regenerated shoots of *B. monnieri*.

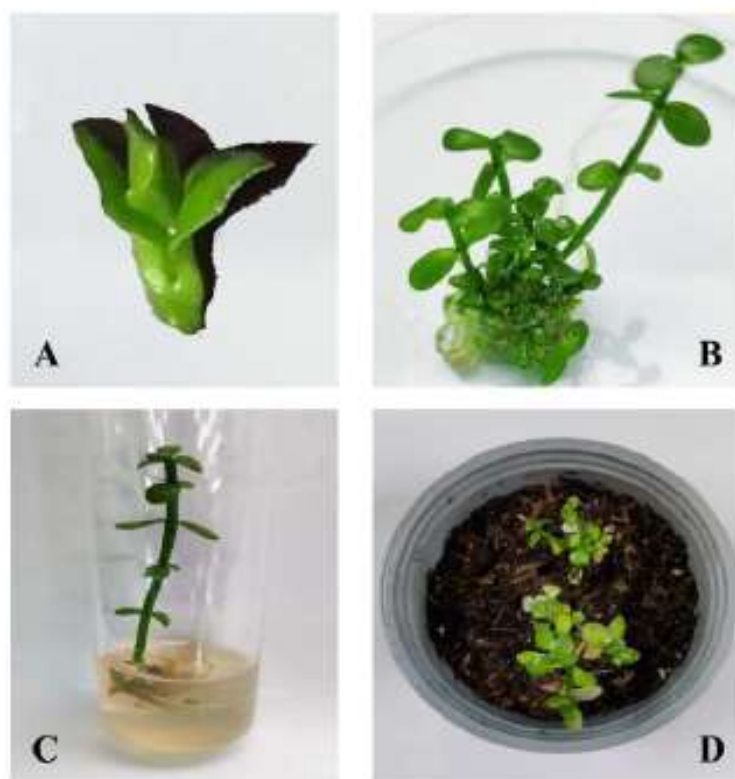


Fig 2. Establishment of micropropagation protocol of *B. monnieri* through direct Regeneration. (A) Initiation of axillary bud from nodal explants (B) Multiplication and formation of shoot cluster (C) rooting of individual shoot (D) Hardening of shoots.

In a similar study, the phytochemical screening of aqueous and hydro alcoholic extracts of *B. monnieri* has revealed the presence of flavanoids and phenols which are considered to be responsible for antioxidant activity reported prominently in *Bacopa* (Shah *et al.*, 2012). But in the ethanolic and aqueous extracts of leaf callus of *B. monnieri*, bioactive chemical constituents identified included amines, Saponins, Terpenoids, Steroids, Phytosterol, Anthraquinone, Glycosides Flavonoids etc.

Growing commercial interest in the use of *B. monnieri* for the development of pharmaceutical agent, posed the interest to investigate the *in vitro* production of 'bacosides'. Plant cell and organ cultures have been exploited as an alternative for sustained production of bacosides.

Sl No.	Phytochemical (Test)	Methanol extract of <i>in vitro</i> plants	Methanol extract of field grown plants
1.	Tannins	-	-
2.	Saponins	+	+
3.	Terpenoids	-	-
4.	Glycosides	-	-
5.	Anthroquinone	-	-
6.	Flavanoids	+	+
7.	Alkaloid	+	+
8.	Steroids	+	+
9.	Phytosterol	-	-
10.	Phenolic	+	+

Table 3. Phytochemical comparison of *in vitro* derived and field grown plants of *B. monnieri*

The similarity between preliminary phytochemical profiles of *in vitro* and field grown plants being evident, comprehensive studies in future can help identify if the *in vitro* derived plants can be used as an alternate source of bioactives of *Bacopa*.

References

- Amaravathi T, P.S Geetha, M Murugan, S Selvam and S Kanchana (2020), Traditional value added products from Indian penny wort (*Centella asiatica*) and water hyssop (*Bacopa monnieri*) to alleviate ADHD, *The Pharma Innovation Journal*. 9(7): pp.432-441.
- Basak A, Hossain M.L, Parvin N.M (2016), *Int J Sci Rep.*, 2(10): pp. 242-247.
- Gamble J.S(1984), 'Flora of the Presidency of Madras', vol. 1, 2, 3, *Newman and Adlard Publishers*, London.
- Harborne, J.B. *Phytochemicals Methods*. *Chapman and Hall Ltd.*, London (1973), pp.49-188.
- Jain M, Rajput R and Mishra A (2013), Enhancement of secondary metabolite biosynthesis in *Bacopa monnieri*: An *in vitro* study. *Res. J. Rec. Sci.* 2(1): pp. 13-16.
- Mathur Shalini and Kumar Sushil (1998), Phytohormone self-sufficiency for regeneration in the leaf and stem explants of *Bacopa monnieri*. *Journal of Medicinal and Aromatic Plant Sciences*. 20(4): pp. 1056-1059.
- Rahe M.A, Mollika S.R , Khan M.S, Bamu T.A, Al-Amin G.M, Habib M.A, Akter S, Islam M and Sharmin R (2020), *In vitro* Micropropagation of *Bacopa monnieri* (L.) Penn. - An Important Medicinal Plant. *Plant Tissue Cult. & Biotech.* 30(1): pp.57-63.
- Rao R.S and Ravishankar G.A (2002), Plant cell cultures: Chemical factories of secondary metabolites. *Biotechnol. Adv.* 20: pp.101-153.
- Sasidharan, N (2004), 'Biodiversity documentation for Kerala', Part 6, Flowering plants, *KFRI handbook*.
- Shah M, Behara Y.R and Jagadeesh B (2012), Phytochemical screening and *in vitro* antioxidant activity of aqueous and hydroalcoholic extract of *Bacopa monnieri* Linn. *IJPSR*, 2012; Vol. 3(9): pp. 3418-3424
- Shrivastava N and Rajani M (1999), Multiple shoot regeneration and tissue culture studies on *Bacopa monnieri* (L.) Pennell. *Plant Cell Reports*. 18 (11) : pp. 919-923.
- Sofowara, A (1993), *Medicinal plants and traditional medicine in Africa*. *Spectrum Books Ltd.*, Ibadan, Nigeria, pp.191-289.
- Tripathi N, Chouhan D.S, Saini N and Tiwari S (2012), Assessment of genetic variations among highly endangered medicinal plant *Bacopa monnieri* (L.) from Central India using RAPD and ISSR analysis. *Biotech.* 2: pp. 327-336.

QUALITATIVE ANALYSIS AND *IN VITRO* STUDIES IN *PLUMBAGO ROSEA* L. FOR PLUMBAGIN PRODUCTION

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Introduction

Plumbago rosea L. (Plumbaginaceae) is a perennial shrub distributed throughout India, and grown in home and public gardens both as an ornamental and as a medicinal plant. Tuberos roots of the plant are medicinally important and used for many treatments in indigenous Ayurvedic system of medicine (Nadkarni, 1976). The tuberos roots of the plant are used as an important indigenous drug for treating a variety of symptoms including oedema, piles, intestinal worms, skin disease, common wart, rheumatism, secondary syphilis and leprosy (Agarwal, 1985). The medicinal properties of the roots are attributed to the presence of plumbagin, 5-hydroxy 2-methyl 1, 4-naphthoquinone (Modi, 1961). Plumbagin is a yellow needle compound with the melting point 72-79°C, slightly soluble in hot water; soluble in alcohol, acetone, chloroform, benzene and acetic acid.

As with many medicinal plants, in *Plumbago rosea* L. also, quality planting material is becoming inaccessible and it becomes necessary to develop tissue culture protocols that help preservation of the germplasm as supply of planting materials for cultivation in new areas. As proposed through the present work, the development of efficient *in vitro* protocol for production of secondary metabolites to prevent destructive harvest of plants from their natural habitat, holds great socio-economic relevance.

Materials and Methods

Plant material

Plumbago rosea L. (Family: Plumbaginaceae, Syn.: *Plumbago indica*, Common name: Scarlet leadwort, Rose-colored Leadwort), was selected for the present investigation (Fig. 1). The plant commonly cultivated in gardens throughout India. This is an erect or spreading, more or less branched, herbaceous or half-woody plant 1.5 meters or less in height. The leaves are ovate to oblong-ovate, 8 to 13 centimeters long, slightly drooping, and smooth, with entire, undulate or wavy margins, pointed or blunt tip, and pointed base. The spikes are 15 to 30 centimeters long. The calyx is tubular, 8 to 10 millimeters long, and covered with stalked, sticky glands. The corolla is bright red, the tube is slender and about 2.5 centimeters long, and the limb, which spreads, is about 3 cm in diameter. (Gamble 1984, Sasidharan 2004).

Two to three month old rooted cuttings of *Plumbago rosea* L. grown in the pots in the GreenHouse of KRIBS Bionest, Kalamassery were used as the source of explants for initiation of *in vitro* shoot culture. The media for all the cultures contained 3% sucrose and different growth regulators at varied concentrations with the pH adjusted to 5.8 before adding 0.6% agar. The composition of Murashige and Skoog (MS) media (pH 5.8), used throughout the study is provided in Appendix I. Media was prepared with various plant growth regulators as per the requirement of the experiment.

After 3 weeks of culture initiation shoot buds emanated from the nodal meristem were dissected out and subcultured to MS agar medium containing 3mg/l BAP for multiplication of shoots. After 4 weeks of incubation, shoots of 3-3.5 cm length were separated individually and transferred to MS basal and medium supplemented with 0.1 mg/l IBA for rooting. All The cultures were kept under 12 h photoperiod (50-60mol⁻²s⁻¹) at 26±2°C.

Rooted shoots obtained in 15 days of culture were weaned away from bottles and washed well in running tap water to remove remnants of agar. Some of these plants were kept away for further procedures on extraction of secondary metabolites. The other deflasked plantlets were potted in small plastic cups filled with fine sand and farmyard manure (3:1) mixture and kept under 25% shade provided with irrigation.

Extraction of secondary metabolites and Phytochemical analyses

Maceration extraction method using methanol as solvent was done to analyse the phytochemical composition of the *in vitro* plant samples as well as tuberous roots of field grown plants. For the extraction of plumbagin, the maceration was carried out following the protocol of Balasubramanian *et al.*, (2018). It was also subjected to phytochemical screening as per Trease and Evans (1989), Sofowara (1993), Kokate (1997) and Harborne (1973, 1998). The concentrated extracts appropriately diluted in methanol were taken for quantitative measurements at 420 nm using a Xp-Explorer 3000 UV-visible Spectrophotometer, by comparison with a known concentration of pure plumbagin (Sigma) taken as the standard solution.

Results and Discussion

Plumbago rosea, is a non-seed setting plant being propagated vegetatively either using root suckers or stem cuttings. Tuberous root production in vegetatively propagated plants was found less attractive compared to the tissue culture-derived regenerants that produced rich root biomass with higher concentration of plumbagin (Satheeshkumar & Seeni 2003).



Fig 1. A) Mature plants of *Plumbago rosea* growing in pots in Greenhouse conditions B) Flowering twigs of *P. rosea*

Fig 2. A) Emergence of bud during shoot initiation from nodal explants B) More number of buds emerging from nodal explant during initiation

It was observed that 68% of the nodal explants obtained as contamination free. Explants cultured in the medium containing cytokinins (BAP) invariably responded positively and shoot bud development was observed in 5 days of culture (Fig 1). There was no shoot bud induction observed on explants cultured in media devoid of hormones during this period. Each nodal explant had produced shoot buds after a period of 2 weeks and maximum number of shoots (2.41 ± 0.49) was obtained in medium containing 3 mg l^{-1} BAP (Fig 2, Table 1).

Initial bud break in the axillary meristem of nodal explants in a very short period of incubation in MS solid medium supplemented with cytokinin (BAP) at different concentrations is due to the influence of BAP on the resident meristem of nodal explants as reported in several taxa. The better response was on BAP containing media is consistent with the observations on *Holarhena antidysenterica* and *Cereis yumanensis* (Cheong & Pooler 2003). Supplementation of varied concentration of BAP induced additional shoot bud initiation and obtained maximum shoot proliferation in medium with BAP at higher concentration (3.0 mg/l). The results confirmed the specific requirement of BAP for inducing shoot multiplication which is in consonance with the earlier

results of Binoy *et al* (2006). Higher concentrations of BAP ($>3 \text{ mg l}^{-1}$) induced increased callusing and also the shoots produced were vitrified in nature.

Concentration of BAP (mg/l)	Number of buds	Number of days for bud initiation	Average Length of shoot buds (cm) after 7 days of bud initiation
0	1.02 ± 0.13	14	2.05 ± 0.21
0.5	1.25 ± 0.25	11	1.33 ± 0.51
1.0	1.66 ± 0.33	7	1.62 ± 0.25
1.5	1.78 ± 0.22	7	1.41 ± 0.11
2.0	2.00 ± 0.58	8	1.37 ± 0.26
2.5	2.22 ± 0.31	7	1.13 ± 0.31
3.0	2.41 ± 0.49	5	0.94 ± 0.13
3.5	2.11 ± 0.14	5	0.97 ± 0.21
4.0	2.06 ± 0.66	5	1.03 ± 0.42
5.0	2.04 ± 0.48	5	0.87 ± 0.11

Individual shoots obtained through initiation were then subcultured into media with varying concentration of BAP for production of multiple shoots and the results showed that the media with 1.5 mg l^{-1} BAP produced good rate of multiplication of shoots (5.50 ± 0.40) (Table 2). So, further proliferation of shoots was obtained in the same medium through repeated subcultures at 4 week intervals to establish multiple shoot culture (Fig 3).

Hormone conc. BAP	Number of shoots	Average length of multiple shoots (cm)
0.5	1.02 ± 0.32	1.16 ± 0.09
1.0	2.05 ± 0.51	1.58 ± 0.08
1.5	5.50 ± 0.40	1.76 ± 0.09
2	2.37 ± 0.28	0.38 ± 0.02
2.5	2.26 ± 0.54	0.36 ± 0.03
3	2.29 ± 0.37	0.28 ± 0.02

*Observations were made after 4 weeks of culture.

Table 2. Multiplication of shoots derived from nodal explants, in media supplemented with different concentrations of BAP *

Once the shoots elongated to 3.0- 4.0cm length in the elongation medium (MS basal medium devoid of any growth hormones), they were separated individually and transferred to media with various combinations of growth regulators, for rooting. Other smaller shoots were again divided and subcultured to the multiplication medium for further shoot proliferation. All the shoots of 3.0-4.0 cm length transferred to basal agar media as well as media containing auxins readily formed roots with or

without accompanying callus formation in 2 weeks. Earliest root formation was noted in the base of shoots by 5 days of culture in Full strength MS medium containing 0.5 mg/l IBA. Root formation was prolonged for two weeks in all media after observance of root initiation on the shoots, before making all observations given in Table 3.



Fig 3. Multiple shoot culture established through subculture of shoot buds initiated from nodal explants.

Among the auxins tested, callus free production of maximum number of roots (5.32 ± 1.21) was observed in presence of 0.5 mg l^{-1} IBA. Around 4.5 ± 2.5 roots were observed per shoot together with substantial callusing, on media containing 0.5 mg l^{-1} IAA. Combinations of IBA and IAA did not enhance rhizogenesis but induced more callusing than rooting, as also observed slightly in the shoots in multiplication media containing BAP.

The formation of upto 2.9 ± 0.87 roots/shoot at 100% efficiency even in the basal medium further suggested that there was a residual effect of hormones supplied during shoot multiplication stages and this was also sufficient to induce rhizogenesis in all the shoots. The exogenous addition of IBA,

IAA in rooting media caused only quantitative and qualitative enhancement in the rooting of shoots. Precisely, half strength MS medium supplemented with 0.5 mg/L IBA was the best for root induction. In individual shoots, average of 5.32 ± 1.21 roots were produced with well developed lateral branching enabling easy hardening of the *in vitro* derived shoots (Fig 4).

Media strength	Hormone conc.		Number of roots
	IBA	IAA	
Full strength MS	-	-	2.90 ± 0.87
	0.1	-	3.39 ± 0.71
	0.5	-	4.21 ± 1.06
	1	-	4.08 ± 0.59
	-	0.1	3.54 ± 0.82
	-	0.5	3.98 ± 1.07
	-	1	4.27 ± 0.54
	0.1	0.5	-
	0.5	0.1	-
	0.5	0.5	-
Half strength MS	0.1	-	2.57 ± 1.04
	0.5	-	5.32 ± 1.21
	1	-	3.98 ± 0.65
	-	0.1	3.54 ± 0.82
	-	0.5	4.50 ± 1.52
	-	1	4.28 ± 2.07
	0.1	0.5	-
	0.5	0.1	-
	0.5	0.5	-

Table 3. Rooting of shoots in media with different concentrations and combination of auxins



Fig 4. *In vitro* developed roots in *P. rosea*

Fig5. Hardening of *in vitro* derived *P. rosea* plantlets

After 2 weeks, the rooted shoots (plantlets) were deflasked and potted in plastic cups filled with fine sand farmyard manure (3:1) mixture and kept under 25% shade house. It was noticed that all the plants got established and produced new leaves in the 6 weeks (Fig 5). The overall result obtained in the present investigation reveals that *P. rosea* is found to be highly amenable to tissue culture. Several reports on Tissue culture propagation and field establishment of *P. rosea* have already been published (Satheesh kumar & Seeni 2003). However, this study has identified the ideal media conditions for a faster and more cost-effective micropropagation of *P. rosea*, through use of lesser quantity of plant growth hormones and media chemicals (by using half strength media) for multiplication as well as rooting, when compared to the other latest studies (Mangala N and M Saradha, 2020).

Phytochemical screening and Quantitative analysis

Our results indicated the presence of various secondary metabolites in the methanol extracts *in vitro* and greenhouse grown whole plant. It was found that the *in vitro* rooted shoots exhibit a phytochemical profile more similar to the whole plants. Therefore it was analysed that *in vitro* shoots culture may be a prospective alternative to replenish the declining natural population of *P. rosea* in the wild as well as for the production of secondary metabolites present in *P. rosea*. Methanol extracts of *in vitro* shoots and whole plant showed the presence of alkaloids, quinones, steroids and flavanoids. (Table 4). However, further detailed phytochemical procedures are necessary to form a comprehensive comparison of the two systems. In a similar study, screening of phytochemicals from the root extract of *Plumbago zeylanica* prepared in different solvents revealed the presence of biochemicals of the group alkaloids, carbohydrates, saponins, tannins, steroids and flavonoids (Subhash *et al.*, 2013).

The quantitative analysis of the methanol extracts using UV –Visible spectrophotometer, the presence of plumbagin was ascertained and preliminary quantification done in comparison to the standard Pure Plumbagin (Sigma). Plumbagin production was found to be 0.88 ± 0.13 % DW in the *in vitro* derived whole plants and 1.21 ± 0.98 % DW in the tuberous root from field grown plants (Fig 6).

Class of Secondary metabolite	<i>In vitro</i> plant	Field grown plant
Alkaloids	+	+
Carbohydrates	-	-
Cardiac glycosides	-	-
Coumarins	-	-
Flavanoids	+	+
Phenols	-	-
Quinones	+	+
Saponins	-	-
Steroids	+	+
Tannins	-	-
Terpenoids	-	-

Table 4. Phytochemical screening of secondary metabolites from the total methanol extract of plant samples.

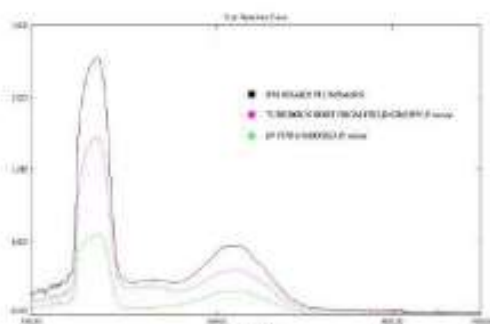


Fig. 5: Quantitative estimation of plumbagin in methanol extracts using UV Visible spectrophotometry

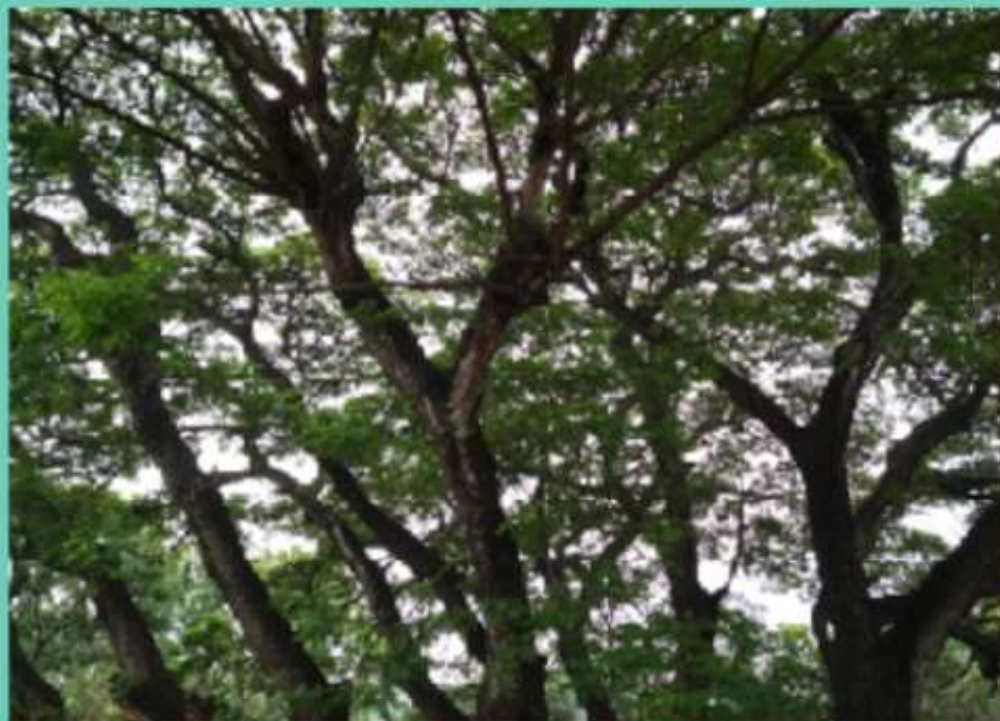
There is a recent publication that has compared the different parts of *in vitro* and field grown plants of *P. rosea* for plumbagin content. The leaves, stem and roots of the *in vitro* grown plant yielded higher content of plumbagin when compared to the respectively field grown parts (Malaiyandi *et al.*, 2020). In a similar research on *P.*

auriculata, the authors have stated that the spectrophotometric analysis showed that plumbagin content from *in vitro* callus is less than that of the roots but more than stem and leaves of the wild grown plant (Deshpande *et al.*, 2014). Lenora *et al.* also reported higher plumbagin content in roots of *in vivo* plants than in *in vitro* callus. All these reports substantiate the findings of our study that *in vitro* systems can also serve as alternate sources of plumbagin, apart from supporting conservation of germplasm.

References

- Agarwal V.S & Barin Gosh (1985), *Drug plants of India: Root drugs*, New Delhi. Kalyani Pub. pp. 204.
- Balasubramanian M, Anbumegala M, Surendran R, Arun M, Girija S (2018). Elite hairy roots of *Raphanus sativus* (L.) as a source of antioxidants and flavonoids. *3biotech*, 8(2): pp. 128.
- Binoy Jose, Satheeshkumar, K and Seeni S (2007), A protocol for high frequency regeneration through nodal explant cultures and *ex vitro* rooting of *Plumbago rosea* L. *Pakistan Journal of Biological Sciences*, 10(2): pp. 349-355.
- Cheong E & Pooler M.R (2003), Micropropagation of Chinese red bud (*Cereis yunnanensis*) through axillary bud breaking and induction of adventitious shoots from leaf pieces, *In Vitro Cell Dev Biol Plant*, 39. pp. 455-458.
- Gamble J.S (1984), *Flora of the Presidency of Madras*, vol. 1, 2, 3, Newman and Adlard Publishers, London.
- Mangala N and M Saradha (2020) "Effective protocol for conservation of plumbago rosea - a plant of novel and potent anticancer compound Plumbagin" *Journal of Global Biosciences*. Vol 9, Number 4, pp. 6943-6952.
- Modi J (1961), *Text book of medicinal jurisprudence and toxicology*, Pripati Pvt.Ltd, Bombay, India, 1, pp. 595-596.

- Nadkarni A.K (1976), *Indian Materia Medica*. Vol I, pp. 990-993.
- Sasidharan, N (2004), *Biodiversity documentation for Kerala*, Part 6, Flowering plants, KFRI handbook.
- Satheeshkumar K and Bhavanandan K.V(1988). Micropropagation of *Plumbago rosea*L. *Plant Cell Tissue and Org Cult.* 15: pp. 275-278.
- Satheeshkumar, K and Seeni S (2003) *In vitro* mass multiplication and production of roots in *Plumbago rosea* L. *Planta Medica* , 69: pp 83-86.
- Subhash K, A. S. Wabale and M. N. Kharde (2013), Phytochemical Screening and Antimicrobial Studies on *Plumbago zeylanica* L. *Advances in Bioresearch*, Vol 4 (3), pp. 115-117.



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22. Imprints of mycorrhizal association on the Cadmium-induced structural and functional modifications in leaves of *O. sativa* and *Z. mays*
E. Janeeshma and Jos I. Puthur
23. Estimation on the bioactivity of secondary metabolites and antioxidant analysis of *Syzygium cumini* (L.) skeels
C. Catherine and S. Sofia
24. A study on germination percentage of *Syzygium caryophyllatum* (L.) Alston seeds stored at different conditions
S.G. Chithra, Neethu S Kumar and R. Santhoshkumar
25. Morphological and anatomical alterations in *Artemisia nilagirica* (Clarke) Pamp. Upon copper toxicity
P. Fathimath Zuhra and K. Hussain

26. Phytochemical screening and anti-inflammatory properties of *Thottea siliquosa* rhizome extract
M. Athira, P.N. Shaiju and S. R. Suja
27. Total antioxidant assay of *Gnidia glauca* (Fresen.) Gilg leaf and bark extracts using Phosphomolybdate method
S.S. Gayathri and P.B. Raveendran
28. Effect of divalent cations (Zn^{2+} , Pb^{2+} , Co^{2+} , Ca^{2+}) on the enzyme activity of Acid Phosphatase in *Murraya koenigii*
Haripriya Santosh, Laxmi Chaudhary, Manukriti Chauhan and Taruna Arora
29. Preliminary phytochemical studies on the leaves and roots of *Canthium angustifolium* Roxb.
Anjali Rajan and J. Nirmala Jeyarani
30. Copper oxide nanoparticles mediated photocatalytic degradation of Thiabendazole. antimycotic potential of CuONP-TBZ complex and its *in silico* docking analysis
M.V. Hridhya and Vimala Jose
31. Effect of seaweed extracts on *in vitro* rhizogenesis and growth of *Stevia rebaudiana* (Bert.) Bertoni
S. Aiswarya and A. S. Rubin Jose
32. Toxicological impacts of synthetic dye on plants and phytoremediation potential
Jyotshana Sharma and Vineet Soni
33. A Study on stomatal ontogeny and stomatal clustering of *Dendrophthoe falcata* (L.f.) Ettingsh. (Loranthaceae) from Eastern Ghats, Tamil Nadu, India
K. Murugesan, M. Thennarasu, B. Sathish Kumar, V. Dhaarani and Mathew Dan
34. Bioactive macromolecules coated silver nanoparticles synthesized from *Curcuma zanthorrhiza* Roxb., its antifungal activity supported with *In silico* docking studies
K. S. Aiswarya
35. Elite screening of superior germplasm of *Garcinia gummi-gutta* (L.) Roxb. across southern Kerala
L. V. Aswanilal, B. Vishnu, Remya Ramachandran, A. Ganagaprasad and E. A. Siril
36. Evaluation of bioactive compounds and mosquitocidal activity in *Cnidioscolus aconitifolius* (Mill.) I.M.Johnst.
Kavya K. Sasikumar and C.T. Anitha
37. Effect of phosphate solubilising bacteria on vegetative growth, yield and metabolism of butter beans (*Phaseolus lunatus* L.) cultivated in Kodai Hills
U. Muthuvel and K. Suresh

38. Physiological impact of CO₂ enrichment and associated high temperature on photosynthetic efficiency of tomato (*Solanum lycopersicum* L.) and their improvement through certain mitigation strategies.
Lakshmi G. Ajay, R.V. Manju and K.K. Manu Govind
39. Association between water and electrolyte leakage in the recalcitrant seeds of *Humboldtia vahliana* Wight.
Lima S. Lawrance and G. Sunil Kesava Deth
40. Effect of elevated blood pressure and circulating iodine levels in blood on thyroid functioning
Manjri malhotra, Shefali Mehra, Bhupender Kumar and Taruna Arora
41. Phytochemical profiling of *Cocos nucifera* L. haustorium
Manju Manoharan, Bindu R. Nair and Laija S. Nair
42. Early-stage waterlogging in pea induced reduction in grain filling capacity and Chlorophyll fluorescence at maturity stage
Shubhangani Sharma and Vineet Soni
43. The inflorescence variations of the genus *Echinochloa* P. Beauv. (poaceae, panicoideae, paniceae) in Kerala
M.J. Mithraja, K.R. Kavitha and R.V. Sushama Raj
44. Phytochemical screening, GC/MS and pharmacological analysis of *Rivina humilis* L.
J. J. Monusha, P. A. Mary Helen and S. Sujatha
45. Changes in endogenous ABA levels during embryogenesis and the recalcitrant behaviour of *Syzygium zeylanicum* (L.) DC seeds
K.P. Sharanya and K. G. Ajith Kumar
46. A comparative study on the Biochemical and Nutritional values of selected leafy vegetables
Lekshmi R. Nair and S. Sofia
47. Expression and phylogenetic analysis of PISTILLATA (PI) gene in *Coccinia grandis* (L) Voigt (Cucurbitaceae)
N. R. Raseena and S. Suhara Beevy
48. Standardization of light condition for the artificial culture of *Azolla pinnata*
Rejo Kurien and Benoj Mathew
49. GC-MS analysis of leaves essential oil in *Eugenia sphaerocarpa* Vadhyar, Sujana, J.H.F.Benj & Murthy
S.S. Neethu and A.R. Sivu
50. Phytochemical screening and Biochemical quantification of a medicinal mangrove fern *Acrostichum aureum* L.
K. Neethu Simon and Neethu S. Kumar

51. Antibacterial study on green synthesized Manganese dioxide nanoparticles.
Nithya S. George, Riya Mary Cherian, Arun Aravind and R. Dinesh Raj
52. Conservation of *Madhuca neriifolia* (Moon) H.J.Lam through plant tissue culture
Princy Raju and G. Sunil Kesava Deth
53. An appraisal on the biochemical characteristics and antioxidant status of *Rhynchospora Corymbosa* (L.) Britton in Aruvikkara Reservoir, South India
S.S. Navami and D.S. Jaya
54. Identification, characterization and comparison of seasonal Calcium oxalate crystal load in *Amaranthus dubius*
Renu Rajan and Justin R. Nayagam
55. Preliminary phytochemical and pharmacognostical studies in *Jasminum cordifolium*, Wall.ex G.Don
Priyanka Mohan, J. Nirmala Jeyarani, V. Devi Priya and Regy Yohannan
56. Effect of type 2 Diabetes on cardiovascular disease
Riya Roy, Himanshi, Bhupender Kumar and Taruna Arora
57. Phytochemical, histochemical and anti-microbial screening of *Murraya koenigii* L.
M. Rizwana, V. Rajani and S.R. Dhanya
58. Study on the diversity of parmelioid lichens in Thiruvananthapuram hills of Kerala, India
S.M.Arsha, Stephen Sequeira, A. Aswathi and Arun Christy
59. *Lasiodiplodia theobromae* infection on *Myristica malabarica* seeds: Threat in artificial regeneration
Saira George and Justin R. Nayagam
60. Evaluation of bioactive compounds in speciality land races of rice for nutraceutical potential through *in silico* analysis
M.S. Mikhina, P.S. Abida, A. Jayadeep, Deepu Mathew, V. Ravisankar, M.S. Parvathy and G.K. Krishna
61. Effect of various auxins and cytokinins on shoot multiplication of *Aristolochia bracteata* Retz.
A. Ramsiya and A. S. Rubin Jose
62. Study of different nutrient application for sustainable and production in radish (*Raphanus Sativus*) with different soil moisture.
G.A. Srikanth, Rohith Bopparthi, A. Rohith Bali, H.K. Preetham and N. Abhishek
63. Data generation and analysis for biodiversity screening of endophytic bacterial communities from medicinal plant *Emilia sonchifolia* (Linn.) DC. Metagenomics through illumine Mi Seq
Sithara K. Urumbil and M. Anilkumar

64. Moisture content dynamics and FT-IR spectral analysis of seeds of *Spondias pinnata* (L. f.) Kurz
Sneha John and K. G. Ajith Kumar
65. A study on factors influencing seed viability in *Artocarpus hirsutus*. Lam
S. Sreedevi and K. G. Ajith Kumar
66. Diversity analysis of endophytic fungi in the roots of *Eclipta prostrata* (L.) L using metagenomic approach
Sreelakshmi Rajesh and M. Anilkumar
67. Anti-oxidant activity studies of leaf and stem bark of *Syzygium palodense*
S.P. Smitha Rani, P. Nusaifa Beevi and Sreeja Thankappan
68. A comparative study on the heavy metal phytoremediation potential of some common ornamental plants
C. Akshaya Prakash and Delse P. Sebastian
69. SEM-EDX study to evaluate the absorption and translocation of copper in *Plectranthus amboinicus* (LOUR.) SPRENG.
P.K. Sudheeshna and K. Hussain
70. Effect of heavy metal stress (Cadmium) on morphological physiological activity and anatomy of cow pea plant. (*Vigna uncuiculata*)
S. Swathy Lekshmi and Ayona Jayadev
71. Preliminary physiological studies on improved seed shelf life of *Machilus macrantha* Nees.
P.R. Vidya and P. A. Jose
72. Influence of stress signals on vegetative growth, yield and secondary metabolite production in turmeric
E.S. Sindhu and Delse P. Sebastian
73. Study of biochemical and biophysical adjustments during the transition from desiccation-to-fully-hydrated states in *Riccia gangetica* and *Semibarbula orientalis*
Upma Bhatt and Vineet Soni
74. Activity of extracts from Sargassum, Gracillaria and Padina as growth promoters of *Withania somnifera* (L.) Dunal
Renju Ammu Joseph and A. S. Rubin Jose
75. The effect of music on plants growth
Akshata Mandloi and Shweta Kulshreshtha
76. Molecular regulation of the oxidative damages during drought stress in *Momordica charantia* L.
M.S. Jayaraj and S. Suhara Beevy

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77. Antibacterial activity of green synthesized nanoparticles
Reshma Rajan, Silvy Mathew and Vimala Jose
78. Study on nutritional composition in five varieties of cassava (*Manihot esculenta* Crantz) tubers
M.C. Haritha and Ayona Jayadev

Report of
**International Conference on Materials -
Properties, Measurements, and Applications
(ICMPMA 2022)**

09 – 13 May 2022



Organized by
**Research & Postgraduate Department of Physics
Fatima Mata National College (Autonomous)
Kollam, Kerala, India**

About the Conference

ICMPMA primarily aims to bring together the ignited and inquisitive minds of students, researchers, academicians, and scientists, thereby creating a platform where they can exhibit and observe the myriad research works so that a forum is built to share innovative developments in material science. In due course, we intend to be an acknowledged global forum for researchers working in material science by organizing a triennial conference through which we expect to attract a large number of participants.

The first edition of the ICMPMA series was organized by Research & Postgraduate Department of Physics, Fatima Mata National College (Autonomous), Kollam, Kerala, India from May 09 to May 13, 2022. The assemblage of ardent scientific minds from all over the world has made this conference series an outstanding scientific program. The leading experts in the domain of materials research engaged us in deliberations that enlightened and enriched our knowledge, and also bestowed on us new perspectives to approach the various allied disciplines of material science. This scientific program was keen on emphasizing the recent developments in material science at an international level and has created an opportunity for the participants to build intellectual associations with researchers across the world. ICMPMA 2022 has been well represented globally as international researchers have held diverse roles in the course of the conference as Editors and Reviewers of the Proceedings, Conference Chairs, Speakers, and Scientific Advisors.

ICMPMA 2022 comprised 6 plenary talks, 21 invited talks, 8 technical sessions, 195 oral presentations, 160 poster presentations, 14 oral presentation sessions, 6 poster sessions, and 6 conference chairs. The active involvement of 856 participants from 33 countries is one of the key highlights of the first edition of the conference. Photonic Materials and Devices, Energy Materials, Electronic Materials, Biomaterials, and Magnetic Materials were the thrust areas of discussion across the course of the conference series.

The abstract book of ICMPMA 2022 is published with ISBN: 978-81-950724-2-2, and the conference proceedings is published in IOP Conference Series: Material Science and Engineering (<https://iopscience.iop.org/issue/1757-899X/1263/1>)

List of Conference Organizers, Conference Chairs, Speakers, Advisory Committee, Best Oral Presentations, Best Poster Presentations, Reviewers and Editors of the Proceedings are available in this pdf.

Conference Organizers

Dr. Sunil Thomas* and **Dr. Sachin P C**

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Conference Convener

Dr. Sheena Mary Y

Fatima Mata National College, Kollam, Kerala, India

Local Organizing Committee

**Mr. Ratheesh Kumar R, Mr. Sunil A, Dr. Ben Byju, Dr. Bhagya Uthaman,
Dr. Manu Raj R, Ms. Arya Anilkumar, Dr. Soniya S R, Ms. Syama Nath H,
Ms. Mary Lizy P, and Mr. Albert R**

Fatima Mata National College, Kollam, Kerala, India

The sessions have been conducted as per the following schedule

DAY 1: 9 May 2022, Monday		
OPENING CEREMONY		
Meeting Link: https://us06web.zoom.us/j/84089730570?pwd=dkJnRThsaVRVMW5ZRTd0UVRRTUxtdz09 (Meeting ID: 840 8973 0570; Pass code: ICMPMA)		
9:15 am – 10:00 am (IST)	Welcome	Dr. Sheena Mary Y (Convener)
	Conference Introduction	Prof. Gin Jose, University of Leeds, UK (Conference Chair)
	Inaugural speech	Fr (Dr.) Abhilash Gregory (Manager)
	Felicitation	Prof. Jojo P J (Principal)
	Vote of Thanks	Dr. Sunil Thomas (Organizer)

DAY 1: 9 May 2022, Monday		
SESSION 1: Photonic Materials and Devices		
Meeting Link: https://us06web.zoom.us/j/84089730570?pwd=dkJnRTlsaVRVMW5ZRTd0UVRRTUxtdz09 (Meeting ID: 840 8973 0570; Pass code: ICMPMA)		
Session CHAIR: Prof. P R Biju (Mahatma Gandhi University, India)		
10:00 am – 10:40 am (IST)	Plenary Talk-1	Prof. C Vijayan (Indian Institute of Technology Madras, India) <i>Title: Can You have Your Laser and Eat it Too?</i>
10:40 am – 11:10 am (IST)	Invited Talk-1	Prof. Reji Philip (Raman Research Institute, India) <i>Title: Nonlinear Optics: Fundamentals and Applications</i>
11:10 am – 11:40 am (IST)	Invited Talk-2	Prof. Sajan D George (Manipal Academy of Higher Education, India) <i>Title: Nanoscale Surface Engineering for Photonics Applications</i>
11:40 am – 12:10 pm (IST)	Invited Talk-3	Dr. Kavita Devi (Indian Institute of Technology, Dharwad) <i>Title: Tunable Coherent Devices Based on Nonlinear Optical Materials</i>
12:10 pm – 2:00 pm (IST)	Poster Session-1	Photonic Materials and Devices (Visit the conference website: https://icmpma.fmnc.ac.in/)
SESSION 2: Photonic Materials and Devices		
Meeting Link: https://us06web.zoom.us/j/84089730570?pwd=dkJnRTlsaVRVMW5ZRTd0UVRRTUxtdz09 (Meeting ID: 840 8973 0570; Pass code: ICMPMA)		
Session CHAIR: Prof. Riju C Issac (Cochin University of Science and Technology, India)		
2:00 pm – 2:40 pm (IST)	Plenary Talk-2	Prof. Laetitia Petit (Tampere University, Finland) <i>Title: Novel glass-based materials for (bio)photonics applications</i>
2:40 pm – 3:10 pm (IST)	Invited Talk-4	Prof. M L Chithambo (Rhodes University, South Africa) <i>Title: Procedures and analysis of phototransferred thermoluminescence</i>
3:10 pm – 3:40 pm (IST)	Invited Talk-5	Prof. Radhakrishnan Prabhu (Robert Gordon University, Scotland) <i>Title: Lanthanide based micro/nano-photonic devices and their emerging smart applications</i>
3:40 pm – 4:10 pm (IST)	Invited Talk-6	Prof. Gin Jose (University of Leeds, UK) <i>Title: Femtosecond Laser Induced Plasma Manufacturing of Functional Materials</i>

DAY 2: 10 May 2022, Tuesday**SESSION 3: Energy Materials**

Meeting Link: <https://us06web.zoom.us/j/84089730570?pwd=dkJnRThsaVRVMW5ZRTd0UVRRTUxtdz09>
(Meeting ID: 840 8973 0570; Pass code: ICMPTMA)

Session CHAIR: Prof. Pradyumnan P P (University of Calicut, India)

9:30 am – 10:10 am (IST)	Plenary Talk-3	Prof. Aldrin Antony (Cochin University of Science and Technology, India) <i>Title: Transition Metal Oxides and 2D Materials for Selective Charge Extraction in Silicon Solar Cells</i>
10:10 am – 10:40 am (IST)	Invited Talk-7	Prof. R Prasanth (Pondicherry University, India) <i>Title: Titania Nanotubes: A sustainable Green Energy Material</i>
10:40 am – 11:10 am (IST)	Invited Talk-8	Prof. S Assa Aravindh (University of Oulu, Finland) <i>Title: Applications of density functional theory (DFT) in materials science</i>
11:10 am – 11:40 am (IST)	Invited Talk-9	Dr. Libu Manjakkal (University of Glasgow, Scotland) <i>Title: Textile based Wearable Energy Storage for Autonomous Systems</i>
11:40 am – 12:10 pm (IST)	Invited Talk-10	Prof. Biju V (University of Kerala, India) <i>Title: Storing Energy at Reduced Graphite Oxide- Water Interface</i>
12:10 pm – 2:00 pm (IST)	Poster Session-2	Energy Materials (Visit the conference website: https://icmpma.fimnc.ac.in/)

SESSION 4: Energy Materials

Meeting Link: <https://us06web.zoom.us/j/84089730570?pwd=dkJnRThsaVRVMW5ZRTd0UVRRTUxtdz09>
(Meeting ID: 840 8973 0570; Pass code: ICMPTMA)

Session CHAIR: Prof. Cyriac Joseph (Mahatma Gandhi University, India)

2:00 pm – 2:30 pm (IST)	Invited Talk-11	Dr. Rakhi R B (CSIR - National Institute for Interdisciplinary Science and Technology, India) <i>Title: Direct growth of MoS₂ nanowires over Ni foam substrates for supercapacitor application</i>
2:30 pm – 4:30 pm (IST)	Oral Presentations (Three parallel sessions)	Photonic Materials and Devices–A (Chair: Dr. Arun Kumar K V, CMS College, Kerala, India) Meeting Link: https://us06web.zoom.us/j/89874647755?pwd=bldTQk1vdDVIUEMyTmQwYmlMeGdjUT09 (Meeting ID: 898 7464 7755; Pass code: ICMPTMA2)
		Biomaterials–A (Chair: Dr. Ann Mary K A, St. Thomas College, Thrissur, India) Meeting Link: https://us06web.zoom.us/j/88405362479?pwd=M3g4NkZlYzN2aFo4WVJlZEw5cWRLUT09 (Meeting ID: 884 0536 2479; Pass code: ICMPTMA3)
		Other Materials–A (Chair: Prof. Cyriac Joseph, Mahatma Gandhi University, Kerala, India) Meeting Link: https://us06web.zoom.us/j/84089730570?pwd=dkJnRThsaVRVMW5ZRTd0UVRRTUxtdz09 (Meeting ID: 840 8973 0570; Pass code: ICMPTMA)

DAY 3: 11 May 2022, Wednesday		
9:30 am – 12:30 pm (IST)	Oral Presentations <i>(Three parallel sessions)</i>	Photonic Materials and Devices–B (Chair: Dr. Rithesh Raj D, SASTRA Deemed University, Tamil Nadu, India) Meeting Link: https://us06web.zoom.us/j/898746477552?pwd=bldTQk1vdDVIUEMyTmQwYmI0eGdjUT09 (Meeting ID: 898 7464 7755; Pass code: ICMPMA2)
		Energy Materials-A (Chair: Dr. Suneesh C. V., University of Kerala, India) Meeting Link: https://us06web.zoom.us/j/884053624792?pwd=M3g4NkZJYzN2aFo4WVJlZEw5cWRLUT09 (Meeting ID: 884 0536 2479; Pass code: ICMPMA3)
		Other Materials–B (Chair: Dr. Jayakrishnan R, University of Kerala, India) Meeting Link: https://us06web.zoom.us/j/84089730570?pwd=dkJnRThsaVRVMW5ZRTd0UVRRTUxtdz09 (Meeting ID: 840 8973 0570; Pass code: ICMPMA)
12:30 pm – 2:00 pm (IST)	Poster Session–3	Biomaterials (Visit the conference website: https://icmpma.fnncc.ac.in/)
SESSION 5: Biomaterials		
Meeting Link: https://us06web.zoom.us/j/84089730570?pwd=dkJnRThsaVRVMW5ZRTd0UVRRTUxtdz09 (Meeting ID: 840 8973 0570; Pass code: ICMPMA)		
Session CHAIR: Prof. Jayachandran K (Mahatma Gandhi University, India)		
2:00 pm - 2:30 pm (IST)	Invited Talk-12	Prof. Harikrishna Varma P R (Sree Chitra Tirunal Institute for Medical Sciences and Technology, India) <i>Title: Search for ideal biomaterials</i>
2:30 pm - 3:00 pm (IST)	Invited Talk-13	Prof. Virginia Pensabene (University of Leeds, UK) <i>Title: Material toxicity and manufacturing challenges in microfluidics</i>
3:00 pm - 3:30 pm (IST)	Invited Talk-14	Prof. C. Sudarsanakumar (Mahatma Gandhi University, India) <i>Title: Probing the interaction of natural drugs and their functionalized nanoparticles with macromolecules: Spectroscopic, calorimetric, and computational approaches</i>
3:30 pm - 4:10 pm (IST)	Plenary Talk-4	Prof. Saji George (McGill University, Canada) <i>Title: Taming the 'Nano' Giant for Food Safety and Security</i>

DAY 4: 12 May 2022, Thursday**SESSION 6: Magnetic Materials**

Meeting Link: <https://us06web.zoom.us/j/84089730570?pwd=dkJnRThsaVRVMW5ZRTd0UVRRTUxtdz09>
(Meeting ID: 840 8973 0570; Pass code: ICPMA)

Session CHAIR: Prof. Nirmala R (Indian Institute of Technology Madras, India)

9:30 am – 10:10 am (IST)	Plenary Talk-5	Prof. S M Yusuf (Bhabha Atomic Research Centre, India) <i>Title: Physics of Magnetization Reversal Phenomenon</i>
10:10 am – 10:40 am (IST)	Invited Talk-15	Prof. Santhosh P N (Indian Institute of Technology Madras, India) <i>Title: Competing magnetic interactions and exchange bias behaviour in complex oxides materials</i>
10:40 am – 11:10 pm (IST)	Invited Talk-16	Prof. K G Suresh (Indian Institute of Technology Bombay, India) <i>Title: Recent studies on magnetic Heusler alloys</i>
11:10 am – 11:40 am (IST)	Invited Talk-17	Prof. P S Anil Kumar (Indian Institute of Science, Bangalore, India) <i>Title: Current-induced magnetization reversal</i>
11:40 am – 12:10 pm (IST)	Invited Talk-18	Dr. Senoy Thomas (Cochin University of Science and Technology, India) <i>Title: Non-collinear spin textures in soft ferromagnets</i>
12:10 pm – 2:00 pm (IST)	Poster Session-4	Magnetic Materials (Visit the conference website: https://icmpma.fimnc.ac.in/)
2:00 pm – 4:00 pm (IST)	Oral Presentations <i>(Three parallel sessions)</i>	Energy Materials-B (Chair: Dr. Sibi K. S., University of Kerala, India) Meeting Link: https://us06web.zoom.us/j/89874647755?pwd=blJlTQk1vdDVIUEMyTmQwYmlMeGdjUT09 (Meeting ID: 898 7464 7755; Pass code: ICPMA2)
		Biomaterials-B (Chair: Dr. Derry Holaday M. G., University of Calicut, India) Meeting Link: https://us06web.zoom.us/j/88405362479?pwd=M3g4NkZlYzN2aFo4WVJlZEw5cWRLUT09 (Meeting ID: 884 0536 2479; Pass code: ICPMA3)
		Other Materials-C (Chair: Dr. Saritha A C, Mahatma Gandhi University, India) Meeting Link: https://us06web.zoom.us/j/84089730570?pwd=dkJnRThsaVRVMW5ZRTd0UVRRTUxtdz09 (Meeting ID: 840 8973 0570; Pass code: ICPMA)
4:00 pm – 6:00 pm (IST)	Poster Session-5	Other Materials (Visit the conference website: https://icmpma.fimnc.ac.in/)

DAY 5: 13 May 2022, Friday		
SESSION 7: Electronic Materials		
Meeting Link: https://us06web.zoom.us/j/84089730570?pwd=dkJnRThsaVRVMW5ZRTd0UVRRTUxtdz09 (Meeting ID: 840 8973 0570; Pass code: ICPMA)		
Session CHAIR: Dr. S N Potty (Centre for Materials for Electronics Technology, India)		
9:30 am – 10:10 am (IST)	Plenary Talk-6	Dr. K P Surendran (CSIR - NIIST, India) <i>Title: Magnetic Control of Electricity in Multiferroic Composites: New Frontiers</i>
10:10 am – 10:40 am (IST)	Invited Talk-19	Dr. Subodh G (University of Kerala, India) <i>Title: Electromagnetic Interference Shielding: Current Status and Future Challenges</i>
10:40 am – 12:40 pm (IST)	Oral Presentations (Three parallel sessions)	Magnetic Materials (Chair: Dr. Savitha Pillai S., University of Kerala, India) Meeting Link: https://us06web.zoom.us/j/89874647755?pwd=blDTQk1vdDVIUEMyTmQwYmlMeGdjUT09 (Meeting ID: 898 7464 7755; Pass code: ICPMA2)
		Electronic Materials (Chair: Dr. Pranay Ranjan, Indian Institute of Technology Jodhpur) Meeting Link: https://us06web.zoom.us/j/88405362479?pwd=M3g4NkZlYzN2aFo4WVJlZEw5cWRLUT09 (Meeting ID: 884 0536 2479; Pass code: ICPMA3)
		Other Materials–D (Chair: Dr. K P Zuhail, University of Calicut, Kerala, India) Meeting Link: https://us06web.zoom.us/j/84089730570?pwd=dkJnRThsaVRVMW5ZRTd0UVRRTUxtdz09 (Meeting ID: 840 8973 0570; Pass code: ICPMA)
12:40 pm – 2:00 pm (IST)	Poster Session-6	Electronic Materials (Visit the conference website: https://icpma.fmnc.ac.in/)
SESSION 8: Electronic Materials		
Meeting Link: https://us06web.zoom.us/j/84089730570?pwd=dkJnRThsaVRVMW5ZRTd0UVRRTUxtdz09 (Meeting ID: 840 8973 0570; Pass code: ICPMA)		
Session CHAIR: Dr. S N Potty (Centre for Materials for Electronics Technology, India)		
2:00 pm – 2:30 pm (IST)	Invited Talk-20	Qassim Abdullahi (Heriot-Watt University, UK) <i>Title: Thermally modified TRMC for characterisation of VO₂ Conductivity</i>
2:30 pm – 3:00 pm (IST)	Invited Talk-21	Dr. Sheetal Kaushik Bhardwaj (University of Amsterdam, Netherlands) <i>Title: Hybrid Pixel detectors</i>
3:00 pm – 4:00 pm (IST)	Oral Presentations (Two parallel sessions)	Photonic Materials and Devices-C (Chair: Dr. Jitumani Kalita, Cotton University, Assam, India) Meeting Link: https://us06web.zoom.us/j/89874647755?pwd=blDTQk1vdDVIUEMyTmQwYmlMeGdjUT09 (Meeting ID: 898 7464 7755; Pass code: ICPMA2)
		Energy Materials-C (Chair: Dr. I. Hubert Joe, University of Kerala, India) Meeting Link: https://us06web.zoom.us/j/88405362479?pwd=M3g4NkZlYzN2aFo4WVJlZEw5cWRLUT09 (Meeting ID: 884 0536 2479; Pass code: ICPMA3)

DAY 5: 13 May 2022, Friday

CLOSING CEREMONY

Meeting Link: <https://us06web.zoom.us/j/84089730570?pwd=dkJnRTlsaVRVMW5ZRTd0UVRRTUxtdz09>
(Meeting ID: 840 8973 0570; Pass code: ICMPMA)

4:00 pm – 4:30 pm (IST)	Welcome	Dr. Bhagya Uthaman
	Feedback	Participants
	Announcement of best presentations	Fr (Dr.) Abhilash Gregory (Manager)
	Concluding Remarks	Prof. Jojo P J (Principal)
	Vote of Thanks	Dr. Sachin P C (Organizer)



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CMS College Kottayam (Autonomous)

Prof. Filcy T. Baby Memorial

National Web-Seminar on Dissertation based paper presentation
(Unfolding Botanical Knowledge - UBK-2021)

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Structural analysis of $\text{La}_{0.7}\text{Sr}_{0.3}\text{MnO}_3$ manganite synthesized by sol-gel and solid-state route

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Abstract. In this study, the structural properties of $\text{La}_{0.7}\text{Sr}_{0.3}\text{MnO}_3$ (LSMO) manganite synthesized in bulk and nano form have been analyzed in detail. The bulk LSMO was synthesized by the solid-state method whereas the LSMO nanoparticles were prepared using the sol-gel route. The structural properties of the prepared samples were examined using X-Ray diffraction (XRD). Rietveld refinement confirmed that all the samples crystallize in $R\bar{3}c$ space group with a rhombohedral structure. Williamson-Hall (WH) Analysis using Uniform deformation model and Size strain plots (SSP) were employed to determine the average crystallite size and microstrain in the synthesized samples. SEM and TEM measurements have also been taken to analyse their size distribution.

1. Introduction

Manganites are an important class of compounds with the formula $\text{RE}_{1-x}\text{B}_x\text{MnO}_3$ where RE is a rare earth and B denotes divalent alkaline earth metal cation. Among the manganites, Lanthanum Strontium manganites are of scientific and technological importance with a wide variety of exotic properties such as magnetocaloric effect (MCE), colossal magnetoresistance (CMR), as catalysts, sensors, etc [1-4]. Manganites exhibit a strong interplay between the charge, orbital and spin degrees of freedom [4]. Double exchange mechanism in manganites is responsible for the ferromagnetism and metallicity exhibited by them. They also exhibit strong electron-phonon coupling which is attributed to Jahn-Teller effect [5-7].

$\text{La}_{0.7}\text{Sr}_{0.3}\text{MnO}_3$ manganites are of special interest owing to their high value of Curie temperature (TC), half-metallic ferromagnetic ground state which leads to 100% spin polarization, easy tunability of TC by changing doping species, concentration and pressure [5-7]. The discovery of CMR in Lanthanum Strontium manganites has made them potential candidates as magnetic field sensors [8-10] and magnetic recording applications [9, 11]. Hence, in this study, we have analysed the structural behavior of $\text{La}_{0.7}\text{Sr}_{0.3}\text{MnO}_3$ synthesized by sol-gel and solid-state route.

2. Experimental Details

LSMO nanoparticles were synthesized using sol-gel route. Stoichiometric amounts of Lanthanum nitrate ($\text{La}(\text{NO}_3)_3 \cdot 6\text{H}_2\text{O}$), Strontium nitrate ($\text{Sr}(\text{NO}_3)_2$) and Manganese nitrate ($\text{Mn}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$) were dissolved separately in double-distilled water and ultrasonicated for 30 minutes. Citric acid ($\text{C}_6\text{H}_8\text{O}_7$) and ethylene glycol ($\text{C}_2\text{H}_6\text{O}_2$) in the ratio 1.5:2.25 were added drop wise with stirring. The mixture was then stirred continuously on a hot plate at 80°C . The pH of the resulting solution was kept at 9 by adding small amounts of ammonia. Subsequently, the temperature of the solution was increased slowly to 100°C and then increased to 300°C to form the sol. The sol then ignited to form the precursor. The precursor was then finally ground and pre-calcined at 400°C for 5 hours. The final product LSMO nano (designated as NANO) was obtained by calcining at 750°C for 5 hours which was then ground and pressed into pellets of diameter 10 mm and thickness 2–3 mm and sintered at 850°C for 5 hours. The bulk sample (designated as BULK) was synthesized by solid-state reaction using the corresponding



La_2O_3 , SrCO_3 , and MnO_2 with ethanol as the medium. The final bulk LSMO was obtained by a series of calcinations followed by sintering at 1300°C for 12 hrs.

Phase purity of the samples was analysed by X-ray powder diffraction using PANalytical X'pert Pro diffractometer with $\text{Cu-K}\alpha$ radiation of wavelength 1.5406 \AA and operated at 30 mA, 40 kV. The powder samples were scanned with a step size of 0.017° in the 2θ range 20° to 80° . General Structure Analysis System (GSAS)-EXPGUI software was used to carry out the Rietveld refinement of the X-ray diffraction patterns. The crystallite size was estimated using Scherrer equation. The β_{hd} values were corrected for instrumental broadening by using standard silicon sample. The microstructural features were examined using Carl Zeiss Evo 18 scanning electron microscope (SEM) in secondary electron (SE) imaging mode. High-Resolution Transmission electron microscopy (HRTEM) measurements were done on NANO and BULK samples using a JEOL-2010 (Japan) electron microscope.

3. Results and Discussion

3.1. Structural Analysis

The Rietveld refined powder X-ray diffraction patterns at room temperature of the LSMO samples labeled as NANO and BULK is shown in figure 1 (a) and 1 (b) respectively. Both the samples were found to be in a single phase with rhombohedral structure and R-3c space group. It has been observed that certain peaks present in BULK are absent in NANO samples. In bulk samples, there are infinite number of planes and hence the XRD peaks are due to diffraction from these planes. But in nano samples, there are less number of atoms and hence less number of planes hence diffraction is not complete. Hence, the peak in nano samples gets broadened. Due to incomplete constructive interference, the diffraction from certain peaks may not be evident in the nano sample. However, in bulk samples, due to the presence of large number of crystallites oriented in all possible directions, all possible diffraction peaks should be observed.

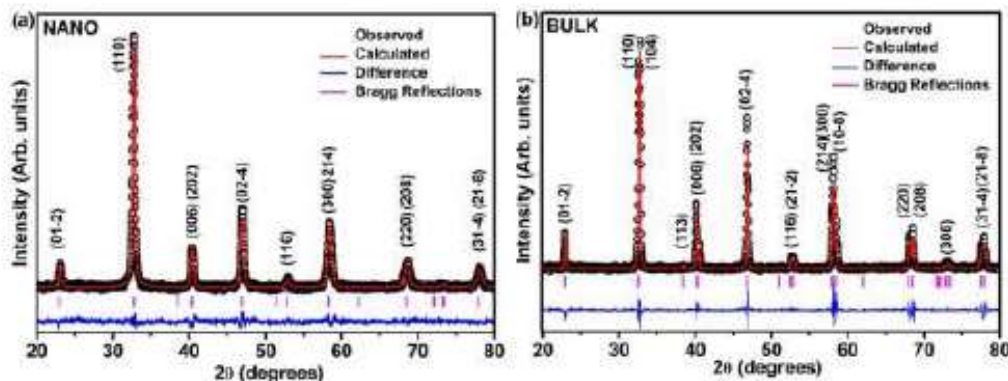


Figure 1. Refined XRD patterns of (a) NANO and (b) BULK samples at 300 K.

The lattice parameters obtained from the Rietveld analysis of the diffraction patterns were found to be $a = 5.4875(3) \text{ \AA}$, $b = 5.4875(3) \text{ \AA}$ and $c = 13.38(1) \text{ \AA}$ for the NANO sample and $a = 5.5074(2) \text{ \AA}$, $b = 5.5074(2) \text{ \AA}$, and $c = 13.3644(5) \text{ \AA}$ for the BULK sample. It is observed that, there is a slight increase in the unit cell parameters along a and b axis as the sample changes from NANO to BULK, whereas along the c axes, it is found to decrease. The estimated cell volumes of NANO and BULK are $348.95(5)$ and $351.06(3) \text{ \AA}^3$, respectively. The increase in cell volume is attributed to the increase in particle size from nano to bulk. In the present work, we have used sol-gel method for the synthesis of NANO sample. In this method, the growth of the grain is restricted due to the presence of surfactants and capping agents. Further, we have used a low temperature of 850°C for the synthesis of nano samples. In case of bulk sample, high sintering temperatures of 1300°C have been used for the complete phase formation which

results in the growth of crystallites. Hence the crystallite size of the nano sample would be much less compared to the bulk. Lattice parameters, cell volume, atomic positions, bonding distances (Mn-O-Mn) and tilt bond angles obtained from Rietveld refinement are presented in Table 1. The flattening of the Mn-O-Mn tilt angles from 175.262° in NANO to 166.337° in BULK is attributed to the increase in the double exchange (DE) mechanism in BULK sample [5].

The average crystallite size (t) of both the samples have been determined from Scherrer's formula:

$$t = \frac{0.9\lambda}{\beta_{hkl} \cos \theta_{hkl}} \quad (1)$$

where β_{hkl} is the full width at half maximum (FWHM), λ is the wavelength of the X-rays and θ is the Bragg angle. The FWHM has been determined by curve fitting the XRD peaks using Pseudo Voigt 1 function. The curve fitting of (110) peak for NANO and BULK sample has been shown in figure 2 as a representative of the series. Table 2 gives the parameters obtained from the curve fitting for determining the crystallite size (t) of NANO and BULK samples. The average crystallite size is found to be approximately 19 and 63 nm for the NANO and BULK samples sintered at 850°C and 1300°C , respectively. The crystallite size is found to increase with increase in sintering temperature.

Table 1. Refinement parameters obtained for LSMO NANO and BULK samples.

Sample	NANO	BULK
Lattice parameters		
a (Å)	5.4875(3)	5.5074(2)
c (Å)	13.38(1)	13.3644(5)
Volume (Å ³)	348.95(5)	351.06(3)
Atomic Positions		
La/Sr (0.7-0.3) x	0	0
La/Sr (0.7-0.3) y	0	0
La/Sr (0.7-0.3) z	0.25	0.25
O x	0.5146(0)	0.5422(0)
O y	0	0
O z	0.25	0.25
Mn x	0	0
Mn y	0	0
Mn z	0	0
Bond distance Mn-O (Å)	1.9389(10)	1.9550(5)
Bond angle (°) Mn-O-Mn	175.262	166.337
R _{wp} (%)	3.22	5.17
χ^2	1.107	2.730

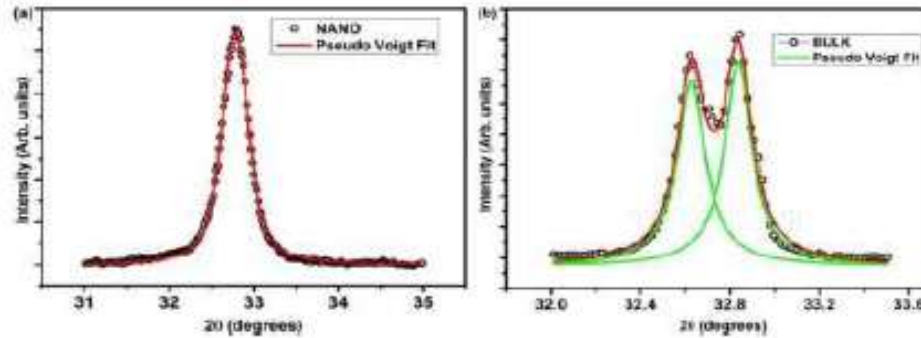


Figure 2. Peak fitting of (110) peak of (a) NANO and (b) BULK samples.

Table 2. Parameters obtained from peak fitting for determining crystallite size

NANO				BULK			
Peak	2θ (degrees)	β _{hkl} (degrees)	t (nm)	Peak	2θ (degrees)	β _{hkl} (degrees)	t (nm)
110	32.7827	0.3722	22.2581	110	32.62834	0.15423	53.6953
006	40.4393	0.4711	17.9802	104	32.83652	0.14782	56.0536
02-4	47.0061	0.4105	21.1097	006	40.24655	0.16183	52.3050
300	58.43049	0.61355	14.8406	202	40.61693	0.15977	53.0424
				122	46.9954	0.11285	76.7943
				300	58.2104	0.13216	68.8235
				214	58.6319	0.1233	73.9207

The microstrain (ϵ) and crystallite size has been estimated only for the NANO sample using WH analysis and SSP. WH analysis using Uniform deformation model (UDM) has been used to determine the size and strain contribution to line broadening. In UDM, the anisotropic character of the crystal is not considered and ϵ is presumed to be homogeneous along all the crystallographic axes. WH equation is,

$$\beta_{hkl} \cos \theta = \frac{K\lambda}{t} + 4 \langle \epsilon \rangle \sin \theta \quad (2)$$

where β_{hkl} is the observed width, K is the shape factor, θ is the Bragg angle, t is the average crystallite size and ϵ is the root mean square (RMS) value of microstrain. By plotting $\beta_{hkl} \cos \theta$ with respect to $4 \sin \theta$, the crystallite size is found from the y-intercept and ϵ is estimated from the linear slope of the line. The UDM plot for the NANO sample is shown in Figure 3 (a). The estimated crystallite size and microstrain is 43 nm and 0.00293 for the NANO sample from the UDM model. UDM model being less accurate for the calculation of t and ϵ , we have estimated them using the size strain plots (SSP). In this approximation, the crystallite size broadening is described using the Lorentzian function while gaussian function is used to estimate the strain broadening [12,13]. Hence we have used the equation,

$$\left(\frac{d_{hkl} \beta_{hkl} \cos \theta}{\lambda} \right)^2 = \frac{K\lambda}{t} \left(\frac{d_{hkl}^2 \beta_{hkl} \cos \theta}{\lambda^2} \right) + \left(\frac{\epsilon}{2} \right)^2 \quad (3)$$

where K is a constant, d_{hkl} is the interplanar distance and $\Sigma = (2(2\pi))^{1/2} \langle \epsilon \rangle$ [12]. Here, the term $\left(\frac{d_{hkl} \beta_{hkl} \cos \theta}{\lambda} \right)^2$ is plotted a function of $\left(\frac{d_{hkl}^2 \beta_{hkl} \cos \theta}{\lambda^2} \right)$ for the all orientation of peaks. In SSP, the particle size is determined from the slope of equation (3) and the square root of the y-intercept gives the value of ϵ [14]. Figure 3 (b) show the SSP plot for the NANO sample. The estimated crystallite size and ϵ

from SSP is 27 nm and 0.00255 for the NANO sample. The variation of average crystallite size and ϵ obtained from different methods is presented in Table 3. It could be seen that the average crystallite size obtained from SSP agrees well with that obtained from the Scherrer equation.

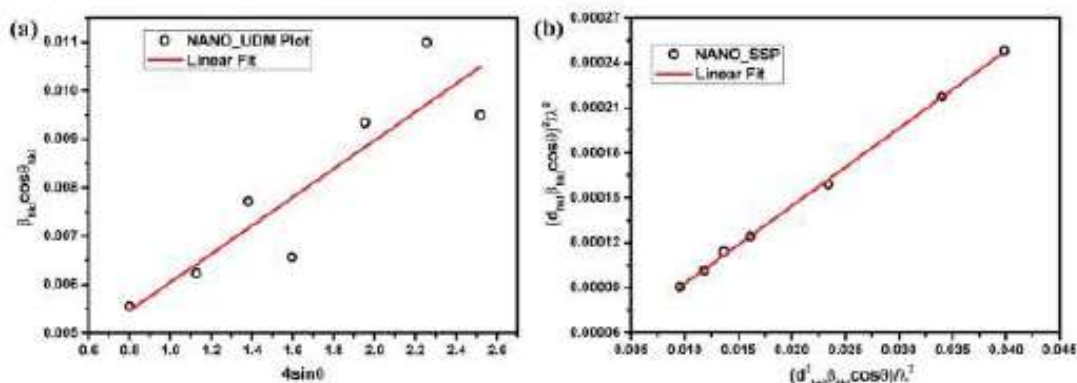


Figure 3. (a) UDM plot and (b) SSP plot for NANO sample.

Table 3. Average Crystallite size and microstrain obtained by different methods for NANO sample.

	Average Crystallite Size	Microstrain
Scherrer Equation	19.05	-
WH Analysis	44.44	0.00293
SSP	26.76	0.00255

3.2. Morphological Analysis:

In order to understand the surface morphology and microstructure of $\text{La}_{0.7}\text{Sr}_{0.3}\text{MnO}_3$ nanocrystals, SEM images of sintered, polished and thermally etched pellets of NANO and BULK samples have been taken and are shown in figure 4(a) and 4(b) respectively. SEM micrographs indicate that the surface of the samples are homogeneous with well-defined grain boundaries. The NANO and BULK samples has been further analyzed using HRTEM. Figure 5(a and b) represents the HRTEM images of the NANO and BULK samples and their corresponding SAED patterns are shown in figure 5(c and d) respectively. It could be seen that the particles are agglomerated both in NANO and BULK which could be attributed to the strong magnetic moment between the particles. The average grain sizes is found to be around 28 and 78 nm respectively for the NANO and BULK samples. It is evident that the crystallite size increases with increase in sintering temperature and the results obtained agree well with that obtained from Scherrer equation and SSP.

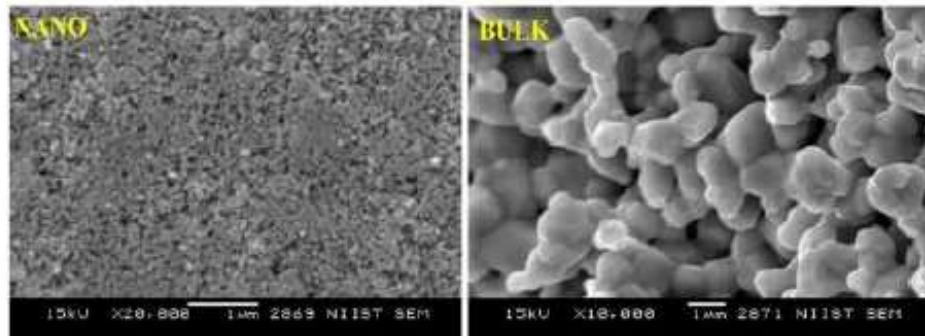


Figure 4. SEM micrographs of (a) NANO and (b) BULK sample.

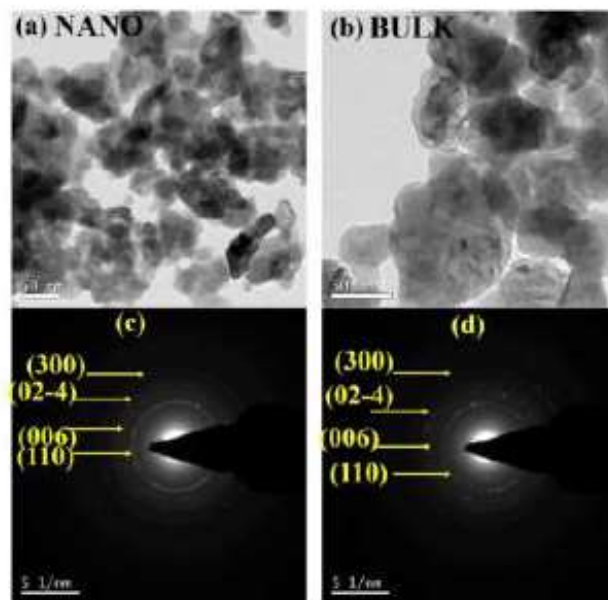


Figure 5. HRTEM micrographs of (a) NANO and (b) BULK sample; SAED patterns of (c) NANO and (d) BULK samples.

4. Conclusion

$\text{La}_{0.7}\text{Sr}_{0.3}\text{MnO}_3$ perovskite manganites have been synthesized by solid state and sol-gel technique. Powder X-ray diffraction technique was performed to examine the crystal structure and all the compounds assumed rhombohedral structure with space group $R-3c$. The crystallite size has been estimated from the Scherrer equation, WH analysis and SSP. The crystallite size determined from the SSP was found to be more accurate than that obtained from the WH analysis and agrees well with the results obtained by Scherrer Equation. Homogeneity of the compound was confirmed from TEM & SEM analysis.

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Reference

- [1] Arakawa T, Yoshida A and Shiokawa J 1980 *Mater. Res. Bull.* **15** 269
- [2] Phan M H and Yu S C 2007 *J. Magn. Magn. Mater* **308** 325
- [3] Xia W, Pei Z, Leng K and Zhu X 2020 *Nanoscale Res. Lett.* **15** 1
- [4] Zener C 1951 *Phys. Rev.* **82** 403
- [5] Liao Z *Appl. Sci.* 2019 **9**(1) 144
- [6] Tiwari A and Rajeev K P 1999 *Solid State Commun.* **111** 33
- [7] Goodenough J B 1998 *Annu. Rev. Mater. Sci.* **28** 1
- [8] Sadhu A and Bhattacharyya S 2014 *Chem. Mater.* **26** 1702
- [9] Lau L N, Lim K P, Ishak A N, Kechik M M A, Chen S K, Ibrahim N B, Miryala M, Murakami M and Shaari A H 2021 *Coatings* **11**(3) 361
- [10] Navin K and Kurchania R 2018 *Ceram. Int.* **44** (5) 4973
- [11] Zhang C *et al.* 2021 *ACS Appl. Mater. Interfaces* **13** (24) 2844
- [12] Maniammal K, Madhu G and Biju V 2017 *Physica E* **85** 214
- [13] Biju V, Sugathan N, Vrinda V and Salini S L 2008 *J. Mater. Sci.* **43** 1175
- [14] Warren B E and Averbach B I 1950 *J. Appl. Phys.* **21** 595