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Enhancing the Wound Healing Efficacy of *Sida acuta* Extract: Development, Characterisation, and *In Vitro* Evaluation of a Novel Phytosomal Delivery System via Scratch Assay

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Abstract

Background: Wound healing is a clinical issue, especially in relation to chronic wounds, and this requires increased therapeutic action by the use of traditional medicinal plants. *Sida acuta* Burm.f. is a commonly used folk medicine containing bioactive compounds that have the potential to be used therapeutically, but it has low bioavailability. **Aim:** The proposed study seeks to develop phytosomes with hydroalcoholic extract of *S. acuta* leaves to improve wound healing due to the ability to deliver drugs. **Methods:** Soxhlet extraction using hydroalcoholic solvent (70% v/v ethanol) yielded 8.02% w/w extract, which was subjected to preliminary phytochemical screening. Phytosomes were prepared by antisolvent precipitation using soy lecithin at a 1:1 extract-to-phospholipid ratio. The formulation was characterised by FTIR spectroscopy, particle size, polydispersity index, and zeta potential. *In vitro* cytocompatibility was evaluated using the MTT assay *in* Vero cells, and wound-healing activity was assessed using the scratch assay. **Results:** The successful formation of phytosomes was established by FTIR, with the presence of characteristic peak displacements, which showed that it forms molecular interactions. Phytosomes had a 2529.0 nm particle size, 0.626 polydispersity index and -44 mV zeta potential. Cytotoxicity experiments produced an outstanding biocompatibility (IC50: 27.9 Mg/mL). The scratch assay revealed that there was a considerable degree of wound healing with 53.33% and 75.75% closure at 24 and 48 hrs respectively, which revealed that cell migration was increased as opposed to the control. **Conclusion:** The evidence indicates that *S. acuta* phytosomes are promising wound healing agents that can be used to combine both traditional and modern phospholipid-based delivery systems to promote better therapeutic effect.

Major Findings: *Sida acuta* phytosomes showed relatively low cytotoxicity within the tested concentration range and demonstrated 53.33% and 75.75% wound closure at 24 and 48 h, respectively, supporting their potential for further *in vitro* and *in vivo* investigation.

Keywords: Bioavailability Enhancement, Phospholipid Complex, Phytosomes, Scratch Assay, *Sida acuta*, Wound Healing

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1. Introduction

The healing of wounds is still a major clinical issue, especially in relation to chronic wounds and diabetes mellitus[1,2]. Although medical research is highly advanced, the creation of potential therapies that can effectively speed up the recovery process without causing excessive complications is a gap that is yet to be filled, worsened by the ageing population and the increasing prevalence of diabetes across the globe[3]. This has heightened the quest to seek innovative and efficient approaches in treatment. Bioactives provide a broad pool of possible

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therapeutic agents and a rich empirical evidence of application in traditional medicine. Nevertheless, their translation into contemporary clinical use is usually limited by the problems of low aqueous solubility, low bioavailability, and chemical instability. *S. acuta* (broomweed), a tropical plant dating back centuries in use in Asia, Africa, and South America to heal wounds and infected skin blemishes, is one such candidate[4]. The synergistic effect of bioactive compounds, such as alkaloids, flavonoids, and phenolic acids, all of which have strong anti-inflammatory, antimicrobial, and pro-healing properties, is believed to be the cause of its pharmacological activity[4–6]. The current literature has already started to prove this ancient application, with results showing that *S. acuta* extracts have the capacity to stimulate collagen production, as well as angiogenesis, critical tissue repair mechanisms[5,6]. Nevertheless, translation of these plant extracts into credible treatment is usually impaired by low bioavailability, unreliability, and intermittent absorption.

Phytosomes are phospholipid-based nanocarrier systems that offer an encouraging approach to these limitations. Phytosomes are complexes of plant extracts and phospholipids and confer high bioavailability and stability of bioactive compounds to help deliver them to the target site[7–9]. This enhanced delivery method has been effectively utilised on a variety of phytoconstituents to allow delivery of much greater efficacy at a lower dose, since the delivery of a stronger regenerative and anti-inflammatory effect can be achieved through improved delivery of bioactive constituents[10–13]. We based our hypothesis on this, by supposing that a phytosomal formulation of *S. acuta* would significantly enhance its wound-healing activity. This paper is the first to develop and *in vitro* test a new *S. acuta* phytosome. We evaluated its wound-healing activity using a standard cell culture model, such as the scratch assay. Our research is an interface between conventional wisdom and new nanotechnology, and we hope to develop one of the time-honoured remedies into a powerful, evidence-based approach to overcome the serious healthcare issue of wound healing impairment.

2. Materials and Methods

2.1 Collection and Authentication

The plant specimen for the proposed study, *S. acuta* leaves, was collected from Madurai, Tamil Nadu. The plant was authenticated by Mr G. Ganasekar, Senior Assistant Professor, Department of Botany, Madras Christian College, Tambaram, Chennai.

2.2 Extraction

The dried leaves of *S. acuta* were crushed to obtain a homogeneous powder. The powder sample was extracted with hydroalcoholic solvent (70% v/v ethanol) using a Soxhlet extraction method, followed by filtration and rotary evaporation at 50°C. The extract was stored in the refrigerator for further use.

2.3 Preliminary Phytochemical Screening of Extract

The hydroalcoholic extract was subjected to preliminary phytochemical investigation to validate the presence of secondary metabolites, viz., alkaloids, flavonoids, glycosides, saponins, and steroids, as per standard procedure[14].

2.4 Formulation of Phytosomes

Phytosomes were prepared using the anti-solvent precipitation method. The concentrated *S. acuta* extract was mixed with soy lecithin (phosphatidylcholine) in a 1:1 weight ratio. The mixture was dissolved in dichloromethane as the organic solvent and subjected to reflux at 50–60°C for 4 hours under continuous stirring to ensure interaction between the extract constituents and the phospholipid.

The anti-solvent precipitation method was selected because it facilitates the controlled precipitation of the extract–phospholipid complex upon gradual addition of the organic phase into an aqueous anti-solvent under continuous stirring. This approach has been reported for the preparation of phytosomal formulations and was therefore employed in the present study[15]. Soy lecithin was selected as the phospholipid because it contains phosphatidylcholine, a commonly used phospholipid in the preparation of phytosomal formulations. The 1:1 extract-to-phospholipid weight ratio was selected based on the formulation procedure reported in the supporting literature[15].

After refluxing, the resulting mixture was gradually added dropwise into distilled water (anti-solvent) under continuous magnetic stirring at room temperature. The addition was performed slowly to allow proper formation of the phytosomal dispersion. Stirring was

continued for an additional 30 minutes to facilitate dispersion and removal of the organic solvent. The final phytosomal dispersion was collected, filtered through a 0.45 µm membrane filter, and stored in sterile, airtight containers at 4°C for further characterisation and biological evaluation[15].

2.5 Characterisation of Phytosomes

2.5.1 Fourier Transform Infrared Spectroscopy (FTIR)

Fourier Transform Infrared (FTIR) spectroscopy was used to study interactions between the *S. acuta* leaf extract and phospholipids in the phytosomal suspension. The extract, soy lecithin, and the final formulation were prepared as samples and analysed using the KBr pellet method[16].

2.5.2 Particle Size Analysis and Polydispersity Index (PDI)

Dynamic Light Scattering (DLS) was used to determine the mean particle size of the phytosomes and the Polydispersity Index (PDI). The suspension was diluted with distilled water before the measurement, and the analyses were conducted in triplicate at 25°C[17].

2.5.3 Zeta Potential

We assessed the surface charge of the phytosomes by electrophoretic light scattering. Samples were diluted with distilled water and placed into a specialised cuvette for measurement. Zeta potential values were recorded in triplicate at 25°C[18].

2.6 *In Vitro* Biological Evaluation

2.6.1 Cell Culture and Cytotoxicity Assay

Vero cells were cultured in DMEM supplemented with 10% fetal bovine serum (FBS) and 1% penicillin–streptomycin (100 IU/mL and 100 µg/mL, respectively) at 37°C in a 5% CO_2 atmosphere. For the cytotoxicity assay, cells were seeded at a density of 1×10^4 cells/well in 96-well plates and exposed to a concentration range (25–400 µg/mL) of the phytosomal formulation for 48 hours. Cell viability was then quantified using an MTT assay, whereby the reduction of MTT to formazan was measured spectrophotometrically at 540 nm after dissolution in DMSO. Cell viability was expressed relative to the untreated control, and the half-maximal inhibitory concentration (IC_{50}) was calculated from the concentration–response data[19].

2.6.2 Scratch Wound Healing Assay

Confluent Vero cells (2×10^5 cells/well) were grown in 24-well plates and scratched with sterile pipette tips to simulate a wound. Following PBS washing, the cells were treated with the phytosomal formulation in serum-free DMEM[20]. The wound areas were monitored at 0, 24, and 48 hours following treatment. The percentage of wound closure was determined from the reduction in the wound area over time and was used to evaluate the *in vitro* wound-healing activity of the phytosomal formulation.

3. Results and Discussion

The present study was intended to formulate phytosomes incorporating hydroalcoholic extract of *S. acuta* and evaluate its wound-healing activity.

3.1 Phytochemical Screening

The percentage yield of hydro alcoholic extract of *S. acuta* was about 8.02%w/w and showed the presence of the secondary metabolites, viz., alkaloids, steroids, flavonoids, glycosides, terpenoids, tannins, lignans, and phenols.

3.2 FT-IR Analysis

Molecular interactions between the *S. acuta* hydroalcoholic extract and soy lecithin in the phytosome were investigated using FT-IR spectroscopy (Figure 1). The extract spectrum displayed characteristic peaks: O–H stretching (~3400 cm^{-1}), C–H stretching (~2920 cm^{-1}), C=O stretching (~1730 cm^{-1}), aromatic C=C (~1600 cm^{-1}), and C–O–C vibrations (1050–1250 cm^{-1}), confirming the presence of phenolics, flavonoids, and glycosidic compounds. Soy lecithin exhibited peaks for O–H (~3400 cm^{-1}), aliphatic C–H (~2920, 2850 cm^{-1}), ester C=O (~1740 cm^{-1}), and phosphate-related P=O and P–O–C stretching (1230–1050 cm^{-1}). In the phytosomal spectrum, notable shifts and broadening were observed in the O–H and C=O regions, along with overlapping of C–O–C and P–O–C bands. These spectral modifications indicate hydrogen bonding and non-covalent interactions between phytoconstituents and phospholipids, confirming successful complexation.

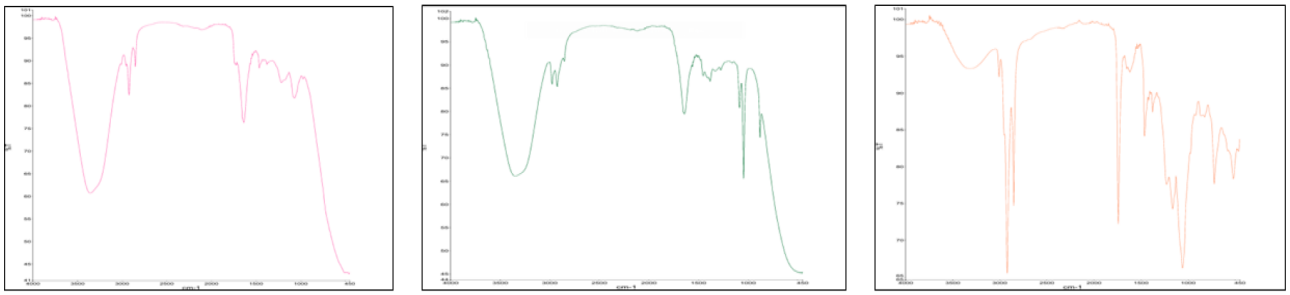


Figure 1. FT-IR spectra of **(a)**. *Sida acuta* extract, **(b)**. soy lecithin, and **(c)**. phytosomal formulation showing characteristic peak shifts indicative of complexation.

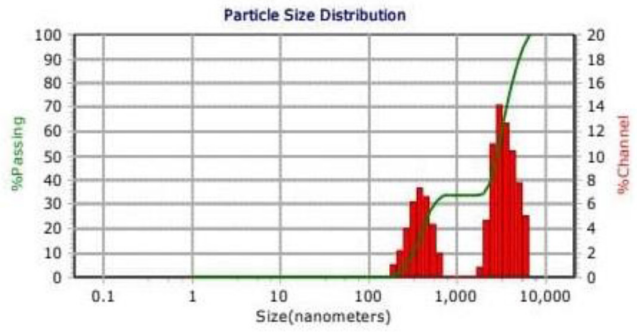


Figure 2. Particle size distribution and zeta potential of *Sida acuta* phytosomes.

3.3 Particle Size and Zeta Potential Analysis

As shown in Figure 2, the phytosomal formulation had a mean particle size of 2529.0 nm with a Polydispersity Index (PDI) of 0.626, which indicates a wide size distribution and a high degree of heterogeneity. The two bimodal peaks of 374.0 nm (33.6% volume) and 3380 nm (66.4% volume) of the sample indicate the presence of smaller vesicles in the mix with relatively larger aggregates, which is associated with low uniformity and inherent instability. The zeta potential was -44 mV, which is a strong repulsive force on the vesicles, which is usually caused by a strong electrostatic repulsive force. A formula whose absolute zeta potential is above ±30 mV is said to be colloidally stable because repulsive forces prevent the coalescence of particles. Although the PDI is relatively high, the fact that having none of the precipitation or phase separation when the formulation is stored at 4, demonstrates that there is sufficient physical stability of the formulation. The particle size distribution and size data, in conjunction with the zeta potential data, reveal that the phytosomes are heterogeneous, with a positive contribution of the strong negative surface charge to the stability of the phytosomes in terms of colloidal stability, which is a major characteristic of dispersion integrity in drug delivery.

Table 1. *In vitro* cytotoxicity effect of phytosomes against Vero cell line

Concentration (µg/mL)	Cell Viability (%)
0.0 (Control)	100.00
25.0	95.50
50.0	93.25
100.0	90.30
200.0	84.11
400.0	72.08

3.4 *In Vitro* Study of Wound Healing Activity

3.4.1 *Cytotoxicity Assay*

The Phytosomal Complex (PC) was tested in terms of cytotoxicity on Vero cells through the MTT assay. The formulation was found to be of low toxicity at 400 µg/mL, and cell viability was observed to be over 70% (Table 1). The maximum cytotoxicity of 27.92% was recorded at the highest concentration (400 µg/mL). The IC_{50} was estimated to be 727.9 µg/mL from the concentration–response curve, which was above the highest concentration tested (400 µg/mL), indicating relatively low cytotoxicity of the formulation within the tested concentration range. It is important to note that no morphological evidence of cell disintegration was observed after treatment of the cells with phytosomes for 48 h, further supporting the low cytotoxic potential of the phytosomes (Figures 3 and 4).

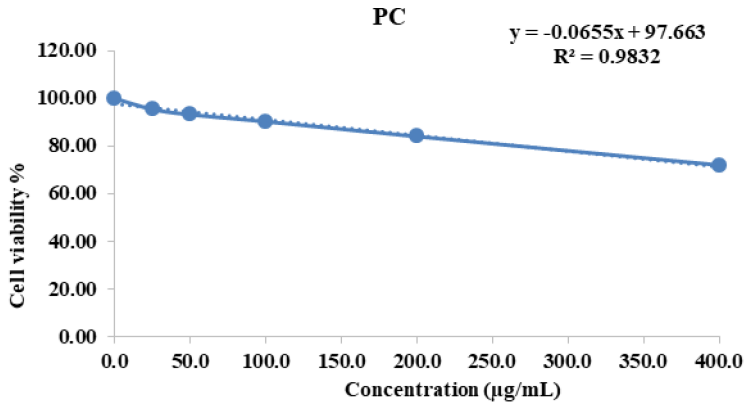


Figure 3. Dose–response cytotoxicity curve of phytosomal complex.

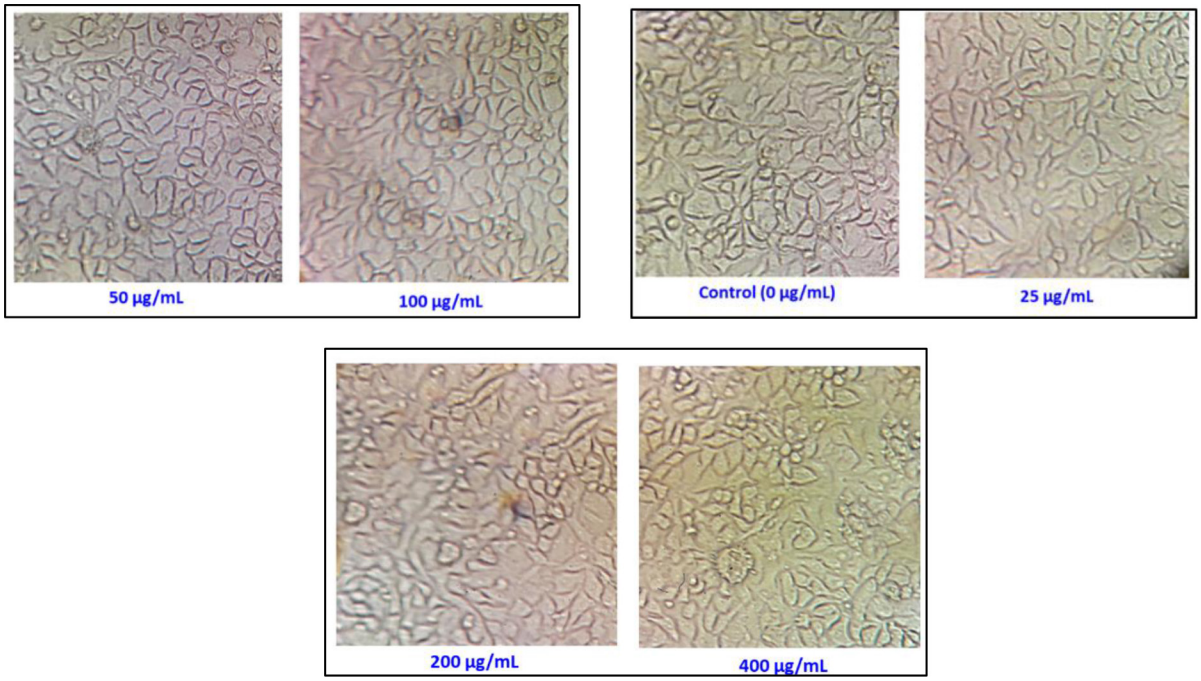


Figure 4. Cytotoxicity effect of phytosomes against Vero cell lines.

3.4.2 Wound Healing Assay

Scratch assay was used to determine the potential of the Phytosomal Complex (PC) for *in vitro* wound closure in Vero cell monolayers. The assay with the minimal active concentration selected from the cytotoxicity studies was used for this evaluation. Follow-up of cell migration after 24 h and 48 h exposure showed a considerable increase in cell migration, which implies that the artificially induced wound gap was progressively closed. Quantitative analysis showed that PC led to 53.33% and 75.75% wound closure at 24 and 48 h, respectively (Figure 6), indicating that PC could enhance cell migration and wound closure. Representative images of the *in vitro* scratch wound-healing assay are shown in Figure 5. The results of the study reveal the preliminary *in vitro* wound-healing potential of the phytosomal formulation and support its further investigation for tissue healing and regeneration. However, the scratch assay is a preliminary *in vitro* screening model and cannot fully reproduce the complex processes of wound healing *in vivo*. Therefore, the present findings require further validation using appropriate *in vivo* wound-healing models.

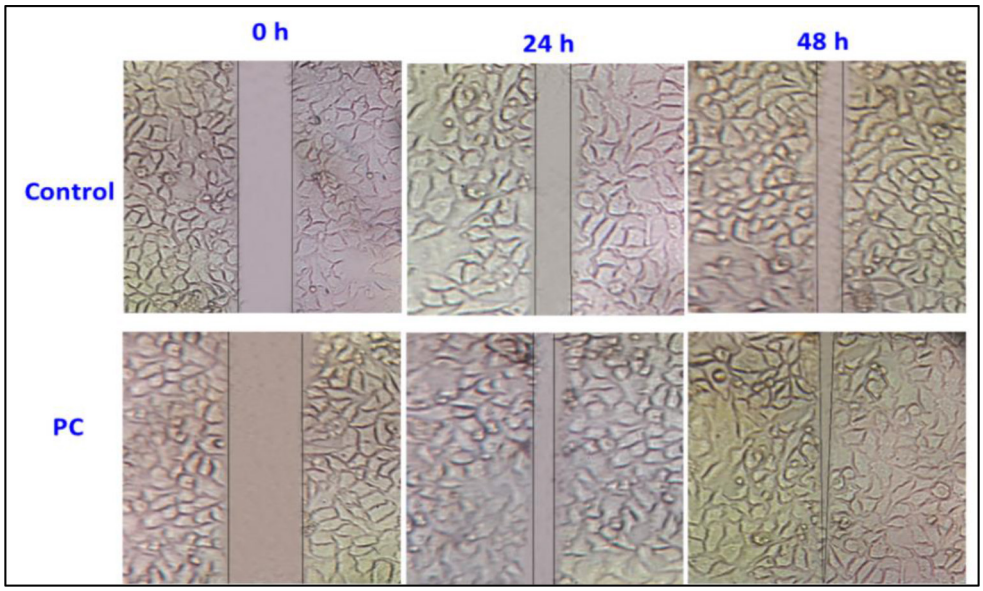


Figure 5. *In vitro* wound healing (scratch) assay of phytosomal complex in Vero cell monolayers.

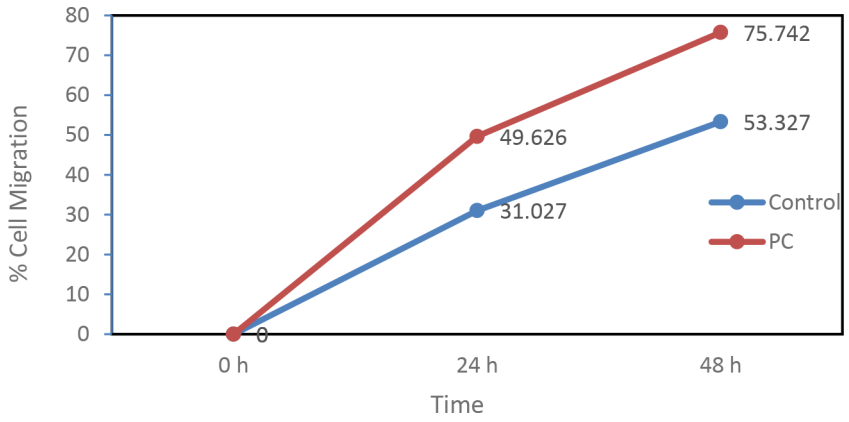


Figure 6. Graphical representation of the effect of phytosomes on wound healing in Vero cell lines.

4. Conclusion

This research describes the achievement of developing and characterising *S. acuta* phytosomes as a new wound healing system. The phytochemical screening of the plant hydroalcoholic extract revealed the presence of alkaloids, flavonoids, glycosides, tannins, and terpenoids, which supports the therapeutic relevance of the plant. FTIR spectroscopy was used to support the formation of phytosomes due to the characteristic changes in the O–H, C=O, and P–O–C bands, which reflected the presence of hydrogen bonding and non-covalent interactions between phytoconstituents and phospholipids. The phytosomes had an average particle size of 2529.0 nm, PDI of 0.626 and zeta potential of -44 mV, which indicates heterogeneity in particle size distribution and a relatively strong negative surface charge, respectively. The cytotoxicity examinations showed relatively low cytotoxicity within the tested concentration range (IC_{50} = 727.9 µg/mL), whereas the scratch test showed wound closure of 53.33 per cent after 24 hours and 75.75 per cent after 48 hours, indicating that cell migration and wound closure were enhanced. On the whole, the *S. acuta* phytosomes represent an encouraging nanocarrier approach that would address the solubility and bioavailability barriers of crude extracts and maintain their therapeutic effects. To determine the clinical utility of the therapeutic particles as wound healing therapeutics, further optimisation of size distribution and *in vivo* studies are justified.

5. Acknowledgement

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Author Contribution

Conceptualisation, Vijayalakshmi A; methodology and formal analysis, Prabhakar V and Vaishali J; software and data curation, Sudarshen Yadav B; validation and resources, Vijayalakshmi A and Arivalagan C; investigation, Vaishali J and Sudarshen Yadav B; writing—original draft, Prabhakar V; writing—review and editing, Vijayalakshmi A and Jayakumari S; visualisation, Vaishali J; supervision and project administration, Vijayalakshmi A. All authors have read and agreed to the published version of the manuscript.