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Bovine Serum Albumin Nanoparticle For Homeopathic Medicine Thuja Mother Tincture Enhanced Bioavailability: Characterization, Anticancer And Antioxidant Studies

Chandana Yesudas[1], Uma Maheshwari[2], Illakkiam Devaraj[1*]

[1]Department of Biotechnology, Mother Teresa Women's University, Kodaikanal, Tamil Nadu, India
[2]Department of Physics, Mother Teresa women's University, Kodaikanal, Tamil Nadu, India

***Corresponding Author:** Dr. Illakkiam Devaraj
*Assistant professor, Department of Biotechnology, Mother Teresa Women's University, Kodaikanal, Tamil Nadu, India, Phone No: +91 9865375795, Email: illakiam@gmail.com

Abstract
The study explores the development and evaluation of BSA nanoparticles encapsulated with homeopathic medicine Thuja Mother Tincture (TMT) for enhanced therapeutic applications. BSA nanoparticles were synthesized and loaded with TMT using desolvation technique, achieving high entrapment efficiency and a controlled release profile. The BSA-TMT nanoparticles demonstrated improved stability compared to free TMT, with a notable sustained release of bioactive compounds over 72 hours. Anticancer and antioxidant activities were significantly enhanced in the encapsulated formulation, surpassing the effects of free TMT. DLS and SEM-EDX analyses confirmed the nanoparticle size, shape, and elemental composition, revealing a uniform and stable structure. This study underscores the efficacy of BSA nanoparticles in improving the delivery and therapeutic potential of traditional remedies, offering a promising approach for developing advanced drug delivery systems.

Keywords: Nanomedicine, Antioxidant activity, Bioavailability, Albumin, Anticancer activity, Controlled release.

Highlights.
- Utilized a desolvation method to encapsulate Thuja Mother Tincture in BSA nanoparticles, enhancing the stability and delivery of therapeutic compounds.
- Demonstrated superior anticancer and antioxidant activities of BSA-encapsulated Thuja nanoparticles compared to free Thuja tincture and non-drug-loaded BSA nanoparticles, revealing improved therapeutic potential.
- Achieved a controlled and sustained release of Thuja Mother Tincture over 72 hours, providing insights into prolonged therapeutic effects and potential for oral drug delivery.
- Conducted stability assessments of BSA-TMT nanoparticles in both physiological and acidic environments, highlighting their robust performance and potential for practical applications in drug delivery systems.

INTRODUCTION

The field of drug delivery systems using nanoparticles as carriers for medicinal compounds is rapidly increasing. Proteins are particularly useful for generating nanoparticles for drug administration due to their natural abundance, biocompatibility, biodegradability, ease of production, and cost-effectiveness[1]. Additionally, proteins can potentially evade opsonization by the reticuloendothelial system, allowing them to remain in the bloodstream for extended periods[2]. Using protein nanoparticles for drug delivery applications could provide a better option for improving the pharmacokinetic and pharmacodynamic aspects of diverse therapeutic compounds.

In this context, bovine serum albumin (BSA) nanoparticles have gained popularity due to their biocompatibility, biodegradability, as well as its capability to improve the solubility and stability of encapsulated agents. Its natural abundance and low cost make it an attractive material for the development of nanoparticles. The protein's ability to form stable nanoparticles through simple methods such as desolvation further enhances its utility in biomedical applications[3]. Encapsulating bioactive chemicals within BSA nanoparticles protects them against degradation, increases their solubility, and allows for better control over their release profile. This can lead to better therapeutic outcomes because bioactive substances are transported more efficiently to their target areas in the body[4]. Furthermore, BSA nanoparticles can be modified with targeting ligands to promote site-specific drug delivery, reducing off-target effects and increasing therapeutic efficacy[5].

Thuja occidentalis, commonly called as arborvitae, is a widely utilized homeopathic remedy known for its diverse medicinal properties[6]. Thuja exhibits anticancer, antiviral, antifungal, antioxidant, and immunomodulatory effects[7]. The mother tincture of Thuja, a concentrated plant extract, is used in homeopathic treatments. However, the clinical application of Thuja is often limited by its poor bioavailability and stability[8]. Encapsulation within BSA nanoparticles offers a potential solution to these challenges, potentially enhancing the delivery and efficacy of Thuja.

The study aims to characterise BSA-encapsulated Thuja mother tincture (BSA-TMT) nanoparticles, with special focus on their stability, release profiles, and physicochemical characteristics. Furthermore, the study aims to assess the BSA-TMT antioxidant and anticancer properties in vitro and contrast its effectiveness with free Thuja tincture. Through encapsulating Thuja in nanoparticles, this study aims to increase its bioavailability and provide a more potent formulation for medicinal uses.

MATERIALS AND METHODS

Chemicals, Cell line and Reagents.

In this investigation, most of the chemicals used are analytical grade. Bovine Serum Albumin (BSA) and Glutaraldehyde were purchased from Sigma-Aldrich. The Dulbecco's Modified Eagle medium/nutrient mixture F-12 Ham, enhanced with 10% foetal bovine serum, was used to cultivate the ATCC human gastric cancer cell line AGS (No: CRL-1739). The culture media is added with antibiotics such as 100 µg/ml streptomycin and 100 U/ml penicillin, as well as 1.5% sodium bicarbonate, 2 mM glutamine were used. Thuja mother tincture was purchased from SBL Pvt Ltd in Madurai.

Preparation of Thuja Mother tincture-loaded BSA nanoparticles

Thuja Mother tincture-loaded BSA nanoparticles was prepared by the desolvation method[9]. The 100 mg of BSA was dissolved in 1 ml of 10 mM NaCl solution. Then, it is added with 8.0 ml of ethanol dropwise into the BSA solution under constant stirring at normal room temperature. Later, the prepared BSA nanoparticles were cross-linked with 100 µl of 8% glutaraldehyde solution. Then, to produce BSA-encapsulated Thuja mother tincture, the solution was added with different ratios of Mother Tincture to BSA (1:2, 1:3, and 1:4) and kept for 24 hours. The nanoparticles that formed were centrifuged and cleaned with distilled water. The particles were resuspended and freeze-dried for 24 hours in 2% mannitol.

Entrapment Efficiency and Loading Efficiency of Thuja Mother Tincture in BSA nanoparticle

An absorbance spectrum was first conducted using a UV-Vis spectrophotometer to identify the ideal wavelength (λmax) at which the absorbance is maximum to understand the entrapment and loading efficiency of Thuja mother tincture in BSA nanoparticles. 2 mg of BSA nanoparticles encapsulating Thuja mother tincture (BSA-TMT) were dissolved in methanol(1ml). It was gently agitated for 24 hrs at 37°C to ensure the complete extraction of the Thuja mother tincture into the methanol. The liquids were then centrifuged for 10 minutes at 12,000 rpm. After that, the supernatant was collected, and at the established λmax, the optical density was measured with a UV-Vis spectrophotometer. The experiments were performed three times in triplicates[10]. The amount of Thuja mother tincture entrapped and loaded in the BSA nanoparticles was expressed as entrapment efficiency and loading efficiency and calculated using the following equation (1) and equation (2) respectively:

Entrapment Efficiency (EE%) = (Amount of Thuja in nanoparticles / Total amount of Thuja used) × 100. (1)

Loading Efficiency (LE%) = (Amount of Thuja in nanoparticles / Total weight of nanoparticles) × 100. (2)

Stability of Thuja Mother Tincture in BSA nanoparticle

For stability testing, 1 mg/ml of BSA-TMT nanoparticles is dissolved in phosphate buffer (0.01M) of pH 7.4 for 24 hours at 37^0C. At designated time points (0.5, 1, 2, 3, 4, 5, 6, 12, 18, 24 hours), the mixture was sampled and centrifuged at 200 rpm and the OD of the supernatant was measured using a UV-Vis spectrophotometer at predetermined λmax[10]. The stability of the BSA-TMT nanoparticles was calculated using the following equation (3):

$$\text{Stability}=(C_0/C_t) \times 100. \quad (3)$$

C_0 is the initial absorbance and C_t is the absorbance of the sample at different time point.

In Vitro Release of Thuja Mother Tincture- BSA nanoparticle

1 mg/ml of BSA-TMT nanoparticles was suspended in 10 ml of the phosphate buffer solution. The suspension was agitated at 200 rpm and 37°C. 1 ml of samples was were taken out of the release medium at predefined time intervals (0, 0.5, 1, 2, 3, 4, 5, 6, 12, 24, 48, and 72 hours). After every sampling, the release medium was readded with an equivalent volume of new phosphate buffer solution to maintain sink conditions. The collected samples were then filtered through a 0.22 µm Millipore membrane. The filtrate was appropriately diluted, and the optical density (OD) was measured using a UV-Vis spectrophotometer at the predetermined λmax[11].

In Vitro Release of Thuja Mother Tincture- BSA nanoparticle in modified gastric environment

Thuja mother tincture was released from BSA-TMT nanoparticle by dissolving 20 mg of BSA-TMT in 15 ml artificial gastric juice (0.01 M PBS pH 2.0) and enzyme free intestinal juice (0.01 M PBS pH 7.4). The mixture was incubated at 37°C at 200 rpm. At specific time intervals (0, 1, 2, 3, 4, 5, 6, 12, 24, 48 and 72 hours), the mixture is sampled and centrifuged at 3000 rpm for 10 minutes. To assess the amount of Thuja Mother tincture released, the pellet was resuspended in 100 µL of ethanol and optical density (OD) is measured using a UV-vis spectrophotometer at predetermined λmax. All measurements were taken in three times independently[12].

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http://www.veterinaria.org
Article Received: Revised: Accepted:

Size distribution analysis of synthesized BSA-TMT nanoparticle.
The average size of the synthesised BSA-TMT nanoparticles was determined using dynamic light scattering analysis. The synthesised BSA-TMT were dissolved in deionized water and undergo ultra sonification. The prepared suspension was centrifuged at 5000 rpm for 30 minutes at 25˚C. The supernatant was then collected and was discretized six more times to conduct an outsize distribution analysis. The average size of the synthesised BSA-TMT nanoparticles in the liquid suspension was then determined using a computer-aided nanoparticle size analyser[13].

Scanning electron microscopy (SEM) coupled with energy-dispersive X-ray spectroscopy (EDX)
SEM coupled with EDX was employed to identify the morphology and elemental composition of the BSA nanoparticles. The nanoparticles were cleaned with ethanol to remove any surface contaminants. A thin layer of gold was then coated onto the samples to enhance conductivity for SEM imaging. SEM observations were conducted with an electron beam energy of 20 kV and a working distance of 11 mm. For the EDX analysis, the same setup was used, with the EDX spectrum acquired over a duration of 100 seconds.

Anticancer activity of synthesized BSA-TMT nanoparticles in gastric cancer cell line
The anticancer potential of synthesised BSA-TMT nanoparticles was assessed by cell proliferation assay by using MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) reagent in a human stomach cancer, AGS cell line. AGS cells were seeded into 96-well culture dishes at a density of 9,000 cells per well and allowed to adhere for 24 hours. The cells were then subsequently treated with six different concentrations of BSA-TMT nanoparticles, BSA nanoparticles without drug and Thuja mother tincture (5, 10, 20, 40, 80,100 µg/ml) respectively for 48 hours. After 48 hours of incubation, the culture media was removed and the MTT reagent was added. Following a 4-hour AGS treatment with MTT, formazan crystals were formed which was dissolved in DMSO. The absorbance was then measured at 570 and 630 nm. The measured data were normalised against a control group of cells treated with the vehicle control (ethanol), and the percentage of cell viability was plotted graphically. The experiments were replicated three times[14].

Antioxidant activity of synthesized BSA-TMT nanoparticles by DPPH assay
The antioxidant activity of synthesised BSA-TMT nanoparticles was measured using the DPPH (1,1-diphenyl-2-picrylhydrazyl) assay. The experiment was carried out in a 96-well plate. BSA-TMT nanoparticles, BSA nanoparticles without drug, and Thuja mother tincture solutions were used at six different concentrations (5, 10, 20, 40, 80, 100 µg/mL), with ascorbic acid solutions serving as positive controls. A 96-well plate was filled with 100 µL of each testing concentration, followed by 100 µL of DPPH solution (0.1 mM). In the negative control, 100 µL of ethanol was mixed with 100 µL of the DPPH solution. The plate was gently vortexed to ensure proper mixing and incubated in the dark at room temperature for 30 minutes. After incubation, the absorbance was measured at 517 nm. The data were normalised against a control group containing DPPH solution and ethanol, and the proportion of DPPH radical scavenging was plotted graphically. The experiments were replicated three times[15].

RESULTS

Determination of entrapment and loading efficiency in BSA nanoparticles loaded with Thuja Mother Tincture
The entrapment efficiency and loading efficiency were assessed using the previously mentioned equations (1) and (2). The BSA-TMT nanoparticle produced using a ratio of 1:4 had a significantly higher entrapment efficiency than the ratios of 1:3 and 1:2. The entrapment efficiency were 95.97%, 49.35%, and 31.23%, respectively. Regarding loading efficiency, there were no significant differences among the different ratios. However, the BSA-TMT nanoparticle prepared using the ratio of 1:4 shows the highest percentage of loading efficiency, followed by the ratios of 1:2 and 1:3, which were 52%, 49.28%, and 36.56%, respectively. The result is graphically plotted and shown in the Figur 1.

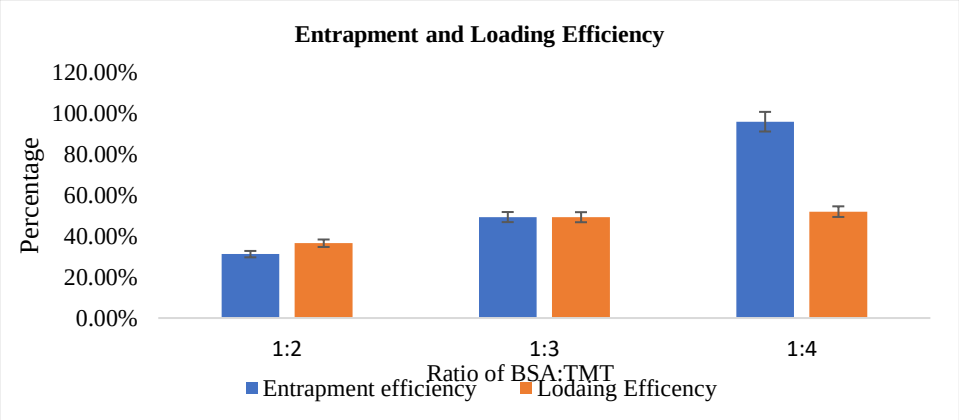

Figure 1. Entrapment and Loading Efficiency in BSA Nanoparticles Loaded with Thuja Mother Tincture.

BSA-TMT nanoparticles at a ratio of 1:4 exhibited significantly higher entrapment efficiency (95.97%) compared to ratios 1:3 (49.35%) and 1:2 (31.23%), with similar loading efficiencies.

Stability assessment of BSA nanoparticles loaded with Thuja Mother Tincture

The stability testing of BSA-TMT nanoparticles, was done with 1:4 ratio since it showed the best entrapment and loading efficiency. The BSA-TMT nanoparticles demonstrated very stable conditions over 12 hours. The stability percentages at 0.5, 1, 2, 3, 4, 5, 6, 12, 18, and 24 hours were found to be 95.26%, 88.70%, 73.6%, 67.23%, 61.9%, 57.0%, 54.21%, 50.86%, 33.85%, and 26.67%, respectively. The result is graphically plotted and shown in the Figure 2.

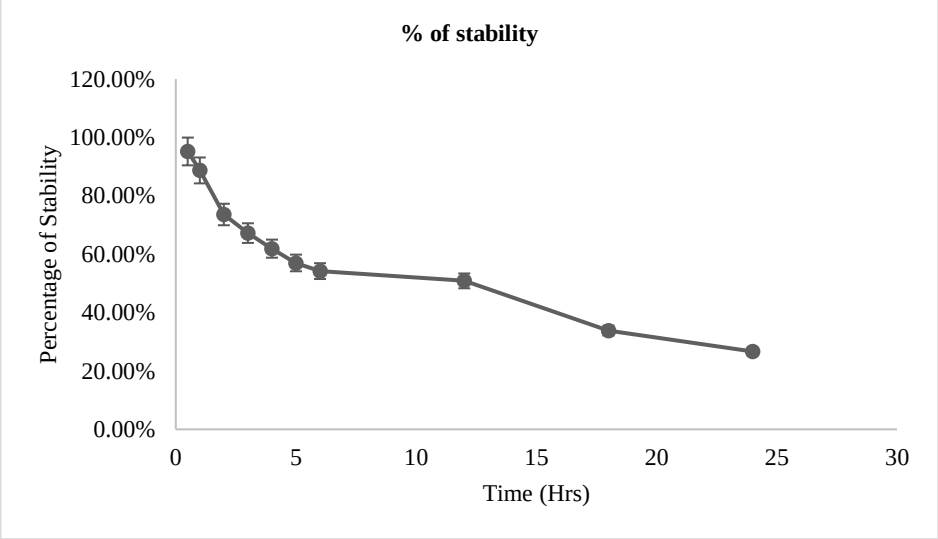

Figure 2. Stability of BSA-TMT in PBS (pH 7.4) at 37°C over a period of 24 hours.

The BSA-TMT nanoparticles demonstrated very stable conditions over a period of 12 hours.

Evaluation of in vitro release of Thuja Mother Tincture from BSA nanoparticles

The in-vitro release study of Thuja mother tincture from BSA nanoparticles revealed a controlled release profile over 72 hours. The release profile of Thuja mother tincture from BSA nanoparticles showed an initial burst release followed by a sustained release over time. By 72 hours, approximately 80% of the tincture was released, demonstrating the efficacy of BSA nanoparticles in prolonged and controlled release of bioactive compounds. The result is graphically plotted and shown in the Figure 3.

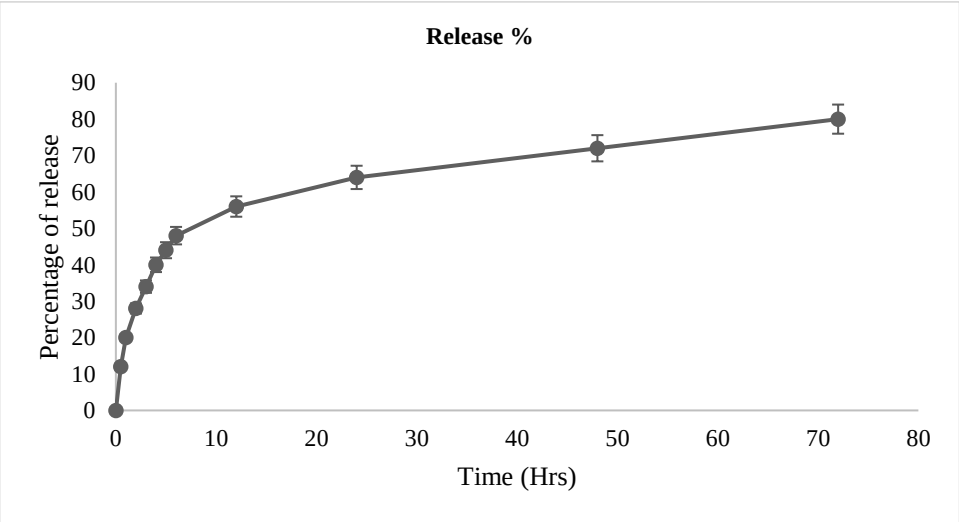

Figure 3. Release rate of Thuja Mother Tincture from BSA-TM in vitro in PBS.

In-vitro release profile of Thuja mother tincture from BSA nanoparticles over 72 hours, demonstrating an initial burst release followed by sustained release.

REDVET - Revista electrónica de Veterinaria - ISSN 1695-7504
Vol 25, No. 1 (2024)
http://www.veterinaria.org
Article Received: Revised: Accepted:

In vitro release kinetics of thuja mother tincture from BSA nanoparticles in simulated gastric conditions

The release profile showed a sustained release of Thuja mother tincture over 72 hours in enzyme-free intestinal juice (0.01 M PBS, pH 7.4), with the percentage released increased gradually and reaching approximately 66.67% at 72 hours. BSA is most stable at pH 7. However, in artificial gastric juice (0.01 M PBS, pH 2.0), BSA rapidly degrades by aggregation and hydrolysis. Consequently, BSA-TMT nanoparticles release the encapsulated tincture effectively in the acidic environment, highlighting their potential application as an oral medicine due to their ability to release at very low pH. The result is shown in Figure 4.

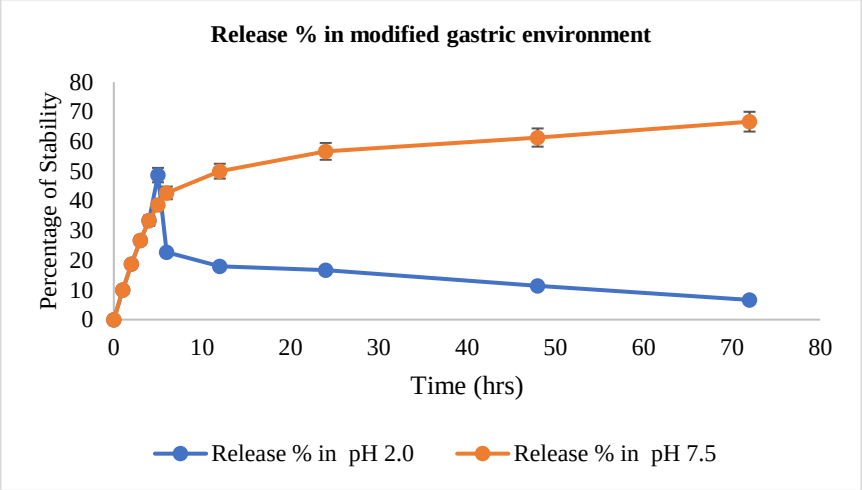

Figure 4. Release rate of Thuja mother tincture from BSA-TMT in vitro in artificial gastric juice at pH 2.0 and artificial intestinal juice at pH 7.4 at 37°C over period of 72 hours.

Size distribution analysis of BSA-encapsulated Thuja Mother Tincture nanoparticles

Dynamic Light Scattering (DLS) analysis revealed that the average size of the synthesized BSA-Thuja nanoparticles was approximately 20 nm, with a narrow size distribution, which demonstrating the uniformity and consistency of the nanoparticle size within the sample. The result is showed in the Figure 5.

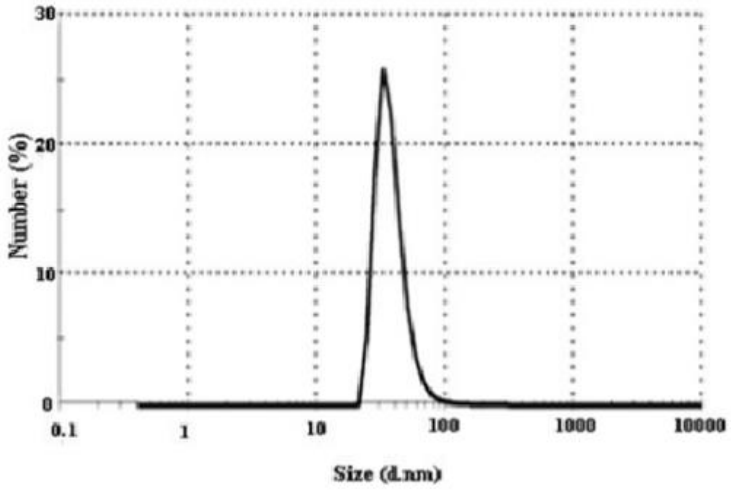

Figure 5. Particle size analysis of BSA-Thuja nanoparticles by DLS.

DLS analysis indicates that the average size of the synthesized iron-Thuja nanoparticles is approximately 40 nm

SEM-EDX Analysis of Synthesized BSA-Thuja Nanoparticles

The SEM images revealed that the nanoparticles were predominantly spherical with a smooth surface texture. The size of the nanoparticles was uniformly distributed, with an average diameter of approximately 10-100 nm (Figure 6). The spherical morphology and uniform size distribution suggest that the synthesis process of BSA-TMT nanoparticles was effective and reproducible. The EDAX analysis of BSA-TMT nanoparticles revealed characteristic peaks corresponding to the elements carbon (C), nitrogen (N), oxygen (O), and sulphur (S). The elemental composition reflects the constituents of both the BSA protein and the Thuja mother tincture and it is shown in the Figure 7.

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Vol 25, No. 1 (2024)
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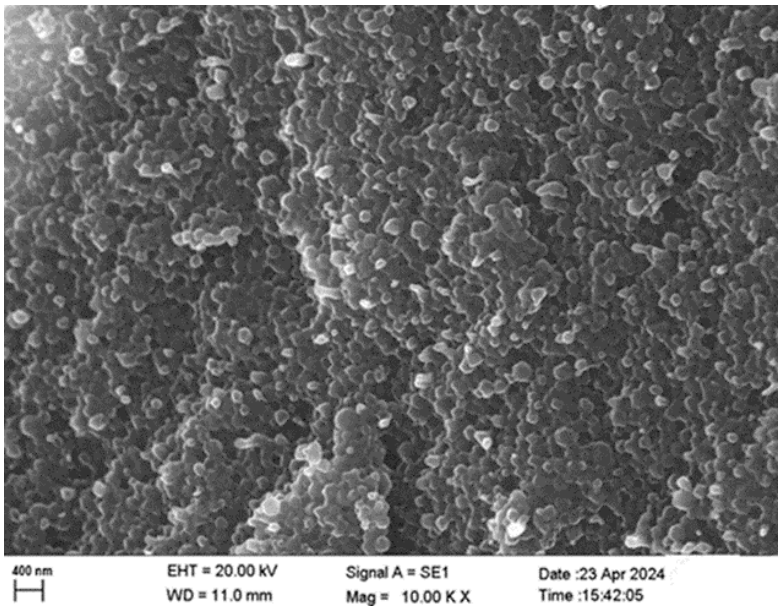

Figure 6. SEM image of BSA-Thuja nanoparticles.

Images confirming the spherical morphology of synthesized nanoparticles.

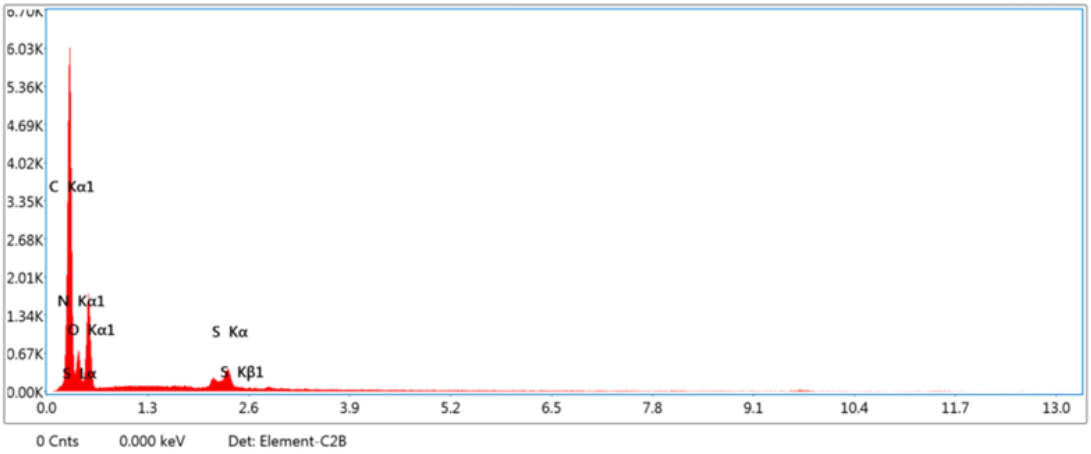

Figure 7. EDX analysis of Thuja Mother-Fe nanoparticles.

Anticancer Activity of BSA Nanoparticles, Thuja Mother Tincture, and BSA-Encapsulated Thuja Mother Tincture

The anticancer activity of BSA nanoparticles, Thuja Mother Tincture, and BSA-encapsulated Thuja Mother Tincture was evaluated using the MTT assay. BSA nanoparticles without drug loading showed no significant anticancer activity, indicating that the nanoparticles alone did not affect cell viability. In contrast, Thuja Mother Tincture exhibited anticancer effects. The BSA-encapsulated Thuja Mother Tincture displayed enhanced anticancer activity, with an IC_{50} value of 20 µg/ml, indicating a significantly improved potency compared to the free Thuja Mother Tincture. The result is showed in the Figure 8.

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Vol 25, No. 1 (2024)
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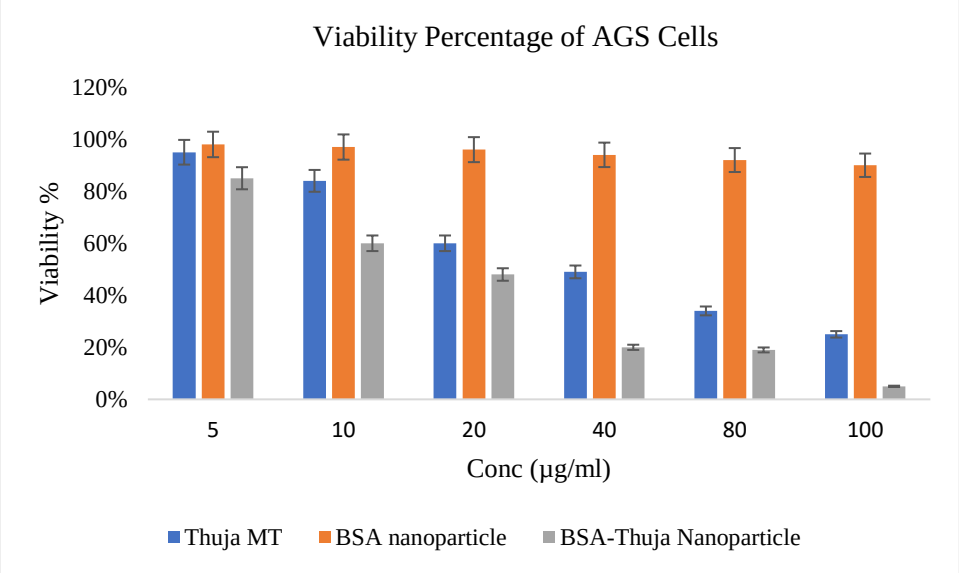

Figure 8. Anticancer Activity of BSA Nanoparticles, Thuja Mother Tincture, and BSA-Encapsulated Thuja Mother Tincture in AGS Cell Lines.

Thuja Mother Tincture showed moderate activity, while BSA-encapsulated Thuja Mother Tincture demonstrated enhanced anticancer effects with an IC_{50} value of 20 µg/ml**.**

Anitoxidant activity of BSA Nanoparticles, Thuja Mother Tincture, and BSA-Encapsulated Thuja Mother Tincture
BSA nanoparticles without drug loading displayed no antioxidant activity. Thuja Mother Tincture alone showed moderate antioxidant effects while the BSA-encapsulated Thuja Mother Tincture nanoparticles exhibited significantly enhanced antioxidant activity. At a concentration of 80 µg/ml, the antioxidant activity of the BSA-encapsulated nanoparticles was comparable to that of the positive control, ascorbic acid. This result demonstrates that the BSA-encapsulated formulation offers potent radical scavenging capabilities, similar to the well-established antioxidant ascorbic acid. The result is showed in the Figure 9.

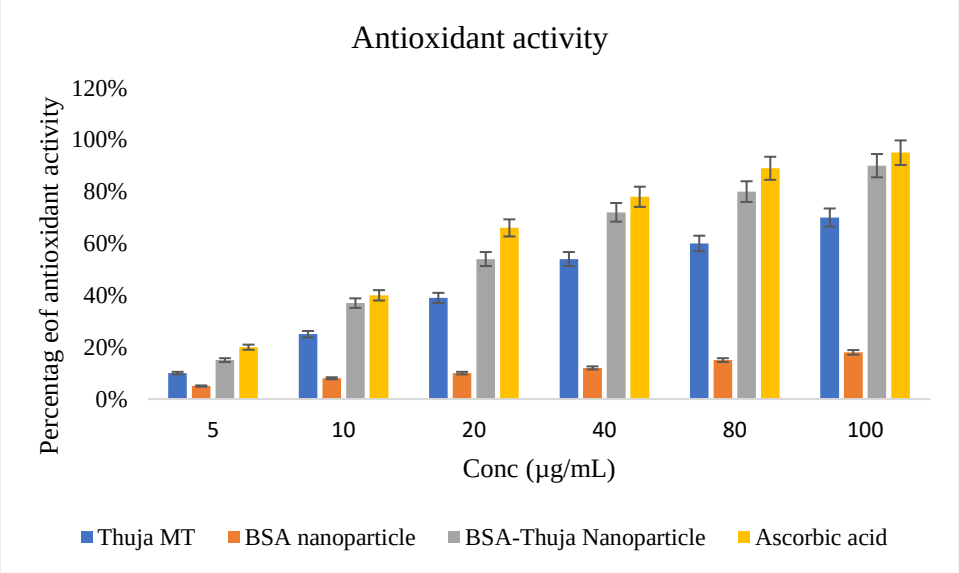

Figure 9. Antioxidant Activity of BSA Nanoparticles, Thuja Mother Tincture, and BSA-Encapsulated Thuja Mother Tincture.

The BSA-encapsulated Thuja Mother Tincture demonstrated superior antioxidant activity, with 80 µg/mL showing similar radical scavenging effects to the positive control, ascorbic acid.

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DISCUSSION

To enhance therapeutic efficacy and targeted delivery of therapeutic compounds, encapsulating in biocompatible nanoparticles has emerged as a promising strategy. Among these the utilization of bovine serum albumin (BSA) as a carrier for encapsulating drugs provide potential implications for therapeutic applications. Various research has established that BSA nanoparticles offer several advantages, including high biocompatibility, low toxicity, and the ability to encapsulate a range of bioactive substances[16]. For instance, encapsulating anti-cancer drugs within BSA nanoparticles has improved bioavailability and targeted delivery, demonstrating enhanced anticancer activity compared to free drugs[17]. The encapsulation of Thuja Mother Tincture, a traditional homeopathic drug known for its potential medicinal properties, into BSA nanoparticles is an innovative approach. Thuja Mother Tincture is prepared from the fresh aerial parts of *Thuja occidentalis* using a process of maceration and extraction with alcohol. *Thuja occidentalis* exhibits various pharmacological activities, including antioxidant, anti-inflammatory, anticancer, and antimicrobial properties[18]. The study sought to evaluate the entrapment efficiency, stability, release kinetics, and biological activity of BSA-encapsulated TMT nanoparticles, comparing them with free Thuja mother and BSA nanoparticles without drug loading.

The entrapment and loading efficiencies of BSA-TMT nanoparticles were markedly influenced by the ratio of Thuja Mother Tincture to BSA. The entrapment of the drug in BSA is done by desolvation process as it shows high dispercity as well as biocompactability[19]. The nanoparticles were cross-linked with glutaraldehyde solution to stabilize the structure and enhance the encapsulation efficiency of the Thuja Mother Tincture within the BSA nanoparticles. The ratio of 1:4 yielded the highest entrapment efficiency of 95.97% compared to the 1:2 and 1:3 ratios. This highest efficiency at the 1:4 ratio was because of the optimal balance between drug and protein, which enhances the encapsulation process[20]. The loading efficiency did not show significant variation among the different ratios, which suggests that once the tincture is encapsulated, the loading capacity remains relatively constant regardless of the initial ratio.

The stability of BSA-TMT nanoparticles was evaluated over a 24-hour period, with the nanoparticles demonstrating high stability for up to 12 hours. This stability is crucial for maintaining the therapeutic efficacy of the nanoparticles during storage and application. The decline in stability post 12 hours could be attributed to potential protein degradation and nanoparticle aggregation[21]. The results highlight the effectiveness of BSA as a stabilizing agent for nanoparticles. The controlled release profile of Thuja Mother Tincture from BSA nanoparticles over 72 hours, with an initial burst followed by sustained release, underscores the potential of BSA-TMT nanoparticles for prolonged drug delivery. This release pattern is beneficial for maintaining therapeutic levels of the drug over extended periods. The controlled release mechanism shows that protein-based nanoparticle systems has sustained release capabilities with previous findings on protein-based nanoparticle systems[22]. Moreover, the release of Thuja Mother Tincture in simulated gastric conditions of pH 2.0 and intestinal juice of pH 7.4 reveals the nanoparticle's ability to effectively release the drug in acidic environments. The effective release of the encapsulated tincture in low pH environments suggests that BSA-TMT nanoparticles are well-suited for oral administration, where they can release their payload in the acidic stomach and subsequently in the neutral pH of the intestines (Liu et al., 2021).

In the characterisation process, the DLS analysis confirmed that the synthesized BSA-Thuja nanoparticles had an average size of approximately 20 nm. This size is optimal for cellular uptake and biological interaction. The narrow size distribution indicates uniformity in the particle preparation, which is crucial for consistent drug delivery and efficacy (Salatin et al., 2015). The SEM and EDX analyses provided visual and elemental confirmation of the nanoparticle's morphology and composition. It confirms the spherical shape and uniform size distribution, alongside the presence of carbon, nitrogen, oxygen, and sulphur. Carbon, nitrogen, and oxygen are prevalent in the organic components of both the tincture and the protein matrix whereas the Sulphur is indicative of the presence of cysteine residues in BSA, confirming its role in the nanoparticle structure (Banerjee et al., 2016). This elemental composition highlights the successful incorporation of Thuja's bioactive compounds within the BSA nanoparticles.

Biological activity, including antioxidant and anticancer activities, plays a pivotal role in evaluating the therapeutic potential of nanoparticles. Thuja Mother Tincture exhibits potent anticancer activity, attributed to its rich phytochemical profile and biological effects (Manivannan et al., 2024). But the anticancer activity of BSA-encapsulated Thuja Mother Tincture was significantly high compared to free Thuja Mother Tincture. The IC_{50} value of 20 µg/ml for BSA-encapsulated nanoparticles highlights their superior potency against cancer cells. This improvement in activity can be attributed to the efficient delivery of active compounds within the cancer cells, facilitated by the BSA nanoparticles, as described by various articles and research studies (Wan et al., 2020; Esim et al., 2021; Hao et al., 2013). The antioxidant activity of BSA-encapsulated Thuja Mother Tincture was significantly superior to that of the free tincture and BSA nanoparticles without drug loading. At a concentration of 80 µg/mL, the antioxidant activity of the encapsulated formulation matched that of ascorbic acid, a well-known antioxidant. This indicates that BSA-encapsulated nanoparticles retain the antioxidant properties of Thuja Mother Tincture and provide enhanced radical scavenging capabilities.

In the summery this study highlights the significant potential of BSA nanoparticles as a delivery system for enhancing the therapeutic efficacy of Thuja Mother Tincture. The encapsulation of Thuja mother tincture in BSA nanoparticles using the desolvation technique demonstrated notable improvements in drug stability and controlled release. The BSA-TMT nanoparticles exhibited high entrapment efficiency, with a favourable release profile that supports prolonged bioavailability of TMT. This method effectively addresses challenges associated with the stability and delivery of

bioactive compounds. Our results indicate that BSA nanoparticles not only stabilize TMT but also enhance its anticancer and antioxidant activities, reflecting their utility in optimizing therapeutic outcomes. The encapsulation process enhances the bioavailability of TMT, ensuring that therapeutic compounds are efficiently delivered to target cells. This study confirms the applicability of nanoparticle-based systems in improving the efficacy of traditional remedies and bioactive substances. Overall, BSA nanoparticles present a promising approach for the development of advanced drug delivery systems, offering improved stability, controlled release, and enhanced therapeutic potential of encapsulated compounds. These findings contribute to the evidence supporting the use of nanoparticles in enhancing the effectiveness of therapeutic agents.

Data availability

All data generated or analysed during this study are included in this published article and are available from the corresponding author upon request.

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Author contributions

Chandana Yesudas: Data analysis, figure preparation and manuscript writing. Uma Maheshwari: Review and editing. Illakkiam Devaraj: Supervision, review and editing.

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