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Facile Synthesis Of Cnps From Mesosphaerum Sauveolens And Its Antimicrobial, Antioxidant And Anticancer Property

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Abstract
Carbon nanoparticles are widely used in biological application due to their biocompatibility. However, the characters of CNPs depends upon the method of synthesis and origin of the material. In this study, CNPs are synthesized from an aromatic, medicinal plant Mesosphaerum sauveolens through open incineration method in the form of biosoot. The biosoot thus collected were characterized for their nanoproperties using DLS, SEM, XRD, FTIR and BET. Further their biological activities like antimicrobial property were evaluated using few bacteria and fungal species. The antioxidant property of the CNPs of Mesosphaerum saueolens was evaluated using DPPH free radical. The anticancer ability was evaluated against the breast cancer cell line MFC-7. The study revealed the size of the biosoot of Mesosphaerum sauveolens falls between 121 to 289nm; 344nm to 818nm and 2313nm to 5500nm when studied through DLS, SEM revealed the size range between 30 to 50nm, The XRD revealed the nature of CNPs as both crystalline and amorphous in nature. FTIR revealed the presence of alkynes, Phosphines, Alkyl mercaptans, Aromatic hydrocarbon and secondary amines as its associated compounds. BET revealed the total pore volume as 2.5388^{-02}cc/gm and the mean pore size as 0.8nm. The antimicrobial study revealed that CNPs of Mesosphaerum sauveolens controlled all the bacteria studied except that of Pseudomonas aeruginosa and between the fungi, Aspergilus flavus was controlled when compared to Aspergillus niger. The EC_{50} value recorded for DPPH was at 32µg. The LD_{50} dosage for anticancer actvity against the breast cancer cell line recorded was 69.77µg. The study revealed the easiest method to synthesis CNPs from the medicinal plant. The synthesized CNPs of Mesosphaerum sauveolens can act as a potential antimicrobial, antioxidant and anticancer agent after proper study for their toxicity. These CNPs of Mesosphaerum sauveolens can be successfully used in cosmetic and pharmaceutical industry as an amendment to topical creams.
Keywords: *CNPs, Biosoot, Mesosphaerum sauveolens, Anticancer activity, Topical agent*

INTRODUCTION

Carbon nanoparticles (CNP) have gained significant attention in recent years due to their unique physiochemical properties, including biocompatibility, low toxicity and easy availability. They can also be used for binding, chemical reactions or absorption activities due to their porous nature (Yang et al. 2010). They can be synthesised using physical and chemical methods that involves consumption of larger amount of energy and lower usage of toxic chemicals (Dresselhaus et al. 1996). Traditional methods for synthesizing CNPs often involve complex processes that are resource- intensive, costly and potentially harmful to the environment (Dresselhaus et al. 1996). Thus it is important to find the alternative method which will be easy and economical in synthesis of CNPs.In contrast, the properties of CNPs depends on the source of the material. Depending upon the source, the CNPs may be toxic, hazardous to expose and pollutant to environment. However, the CNPs of biological origin may be safe when compared to the previous one and even they are found to be biocompatible. In this study plants are used in the synthesis of CNPs in the form of Biosoot. Further, Plants are rich in bioactive compounds such as alkaloids, flavonoids and terpenoids, which also impart additional biological functionalities (Fahim et al. 2010). Among the various plant-based methods, the use of Biosoot, a carbon rich by product generated from the incomplete combustion of organic materials, offers a simple and cost-effective approach for synthesizing CNPs (Udayaprakash and Bhuvaneswari, 2019). In our study, the biosoot of Mesosphaerum sauveolens, an aromatic medicinal plant was utilized as a natural carbon source for synthesizing CNPs. Mesosphaerum sauveolens is a widely recognized plant for its antimicrobial, antioxidant and therapeutic properties

(Worku et al. 2024), making its biosoot an excellent candidate for green nanotechnology applications. The controlled combustion of Mesosphaerum sauveolens biomass produces biosoot that retains the plant's functional groups, which contribute to the unique physiochemical characteristics of the resulting nanoparticles (Sindhupriya et al. 2023). By utilizing biosoot, this method offers a sustainable and innovative approach in producing CNPs while harnessing the intrinsic bioactive properties of Mesosphaerum sauveolens. The synthesized CNPs were subjected to various characterization techniques such as Dynamic Light Scattering spectroscopy (DLS), Scanning Electron Microscopy (SEM), X-ray Diffraction spectroscopy (XRD), Fourier Transform Infrared spectroscopy (FTIR), and Brunauer-Emmett-Teller (BET) analysis to ensure that they are nanoparticles. The biological activities of the CNPs were evaluated for their antimicrobial efficacy against a range of bacterial and fungal strains, antioxidant activity through the DPPH assay, and anticancer potential against the breast cancer cell line MCF-7. This study aimed to investigate the potential of biosoot derived CNPs from Mesosphaerum sauveolens as multifunctional agent with applications in pharmaceutical, cosmetic and therapeutic fields, emphasizing sustainable and green synthesis approaches.

MATERIALS AND METHODS

Plant collection

The leaf samples of Mesosphaerum sauveolens also called as Hyptis sauveolens were collected from Chengalpattu district of the state of Tamil Nadu in India, and shade dried. The shade dried plants were combusted and the soot developed were exposed to porcelain tiles. The soot deposited on the tiles were scraped off, collected and stored in vials for further use.

Characterization of Biosoot as Carbon Nanoparticles

The biosoot thus collected were characterized using Dynamic Light Scattering spectroscopy (DLS) (Nanotrac Wave II, Microtrac Inc. USA), Scanning Electron Microscope (SEM) (Model: Quattro S, Thermofischer Scientific, USA), X-ray diffraction spectrophotometer (XRD) (SmartLab SE X-ray, Rigaku, Japan), Fourier Transform Infra-Red spectrophotometer (FTIR) (Spectrum II FT-IR/Sp10 software, Perkin Elmer, USA) and Brunauer Emmett Teller (BET) BELSorp Max, Microtrac, BEL Corp, Japan).

Antimicrobial Property

The Biosoot collected were subjected to antimicrobial studies. The antibacterial property was evaluated against the bacterial standards of Bacillus subtilis (MTCC 121), Escherichia coli (MTCC 443), Pseudomonas aeruginosa (MTCC 424), Staphylococcus aureus (MTCC 96) and Streptococcus mutans (MTCC 497) and the antifungal property were analysed using the fungal strains, Aspergillus flavus (MLCT 0022) and Aspergillus niger (MLCT 0021). The concentration of 25µg, 50µg and 75µg of biosoot of Mesosphaerum sauveolens was evaluated using well diffusion method in triplicates. The average zone of inhibition was recorded against the individual bacteria and fungi used for the study.

Antioxidant Assay

The antioxidant property of the carbon nanoparticle of Mesosphaerum sauveolens was evaluated using 1,1-diphenyl-2-picryl-hydrazyl (DPPH) free radical assay. DPPH solution of 0.1 mM was studied against biosoot ranging from 10µg to 50µg. The biosoot sample was prepared using distilled water and mixed with 1 mL of 0.1 mM DPPH, and incubated for 30 min at room temperature in dark and after incubation, the absorbance was measured at 517 nm. Ascorbic acid wad used as a standard. Percent radical scavenging activity of the DPPH was calculated by the formula

$$\% \, RSA = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

IC_{50} of the value of the biosoot is presented.

Cytotoxic Assay

The anticancer ability was evaluated against the breast cancer cell line MFC-7 using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. The MTT (3-[4,5 dimethylthiazole-2-yl]-2,5-diphenyltetrazolium bromide) experiment was carried by trypsinizing the cell culture. The monolayer culture was adjusted to the cell count of 1 x 10^5 cells/ml. 0.1ml of diluted cell suspension was added to the wells of microtitre plate and treated with the Biosoot of aromatic, medicinal plant Mesosphaerum sauveolens with varying concentration ((20,40,60,80 and 100µg/ml). The plates were incubated at specified conditions as stated above for a period of 24 hours and the results were evaluated microscopically using phase contrast microscope and after staining with Ethidium Bromide in Flourescent microscope.

RESULTS

The dried leaves of the medicinal plant, i.e. Mesosphaerum sauveolens were openly incinerated and the emanating soot was collected using a porcelain tile. The collected soot were stored in eppendorf tubes and weighed for the yield. The yield was calculated as the initial weight of eppendorf tubes and the final weight after the collection of biosoot. The total yield of biosoot per 100gm of dried leaf is 188mg.

Dynamic light scattering spectroscopy (DLS)

The Dynamic light scattering studies on the biosoot of Mesosphaerum sauveolens showed that the particle size ranges from 121nm to 289nm, followed by second peak from 344nm to 818nm. Further the size of the particles were recorded at the size range of 2312nm to 5500nm i.e. 5.5µm. The characterization of biosoot of Mesosphaerum sauveolens revealed recording triple peak in their size range. Figure 1 shows the DLS spectrum recorded for the biosoot of Mesosphaerum sauveolens.

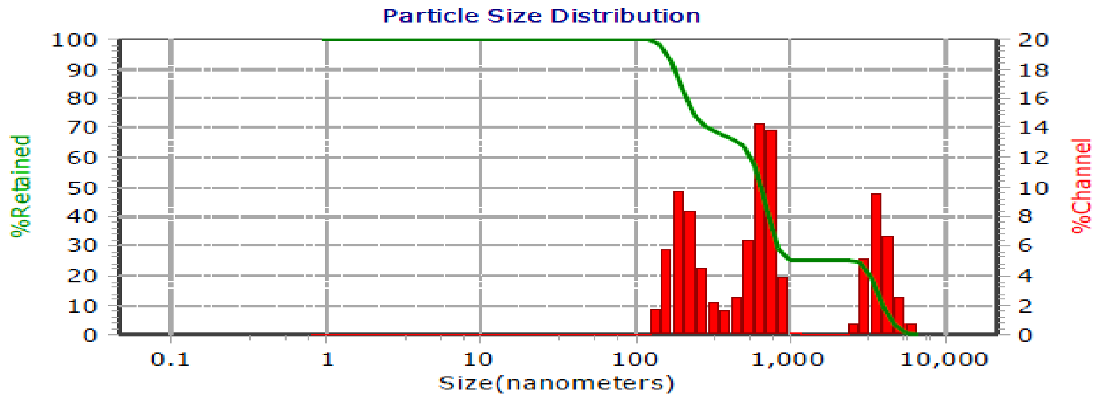

Fig. 1: Dynamic Light Scattering Spectrum recorded for the Biosoot of M. sauveolens

Scanning Electron Microscope

The biosoot of Mesosphaerum sauveolens was examined for their size distribution using Scanning electron Microscope (Figure 2). The study revealed the size of the carbon particles in the range of 30nm to 50nm in their size. The shape of the particles were recorded as spherical**.**

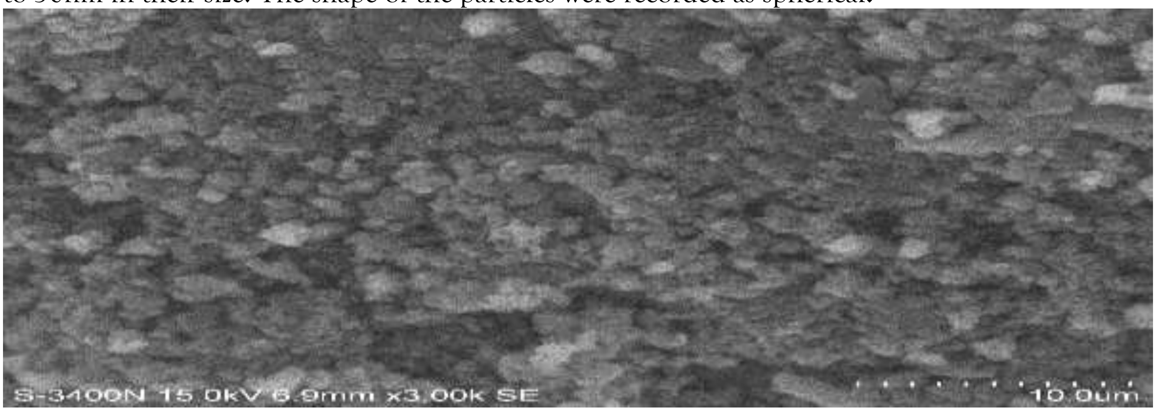

Fig. 2: Scanning Electron Microscope image of the Biosoot of M. sauveolens

XRD spectrum of Biosoot of Mesosphaerum sauveolens

The biosoot of Mesosphaerum sauveolens was subjected to X-ray crystallography to know about the crystalline nature of the biosoot (Figure 3). The study revealed that recording 2θ value ranges from 27 –

28° and also at 40°. This revealed that the biosoot of Mesosphaerum sauveolens is a mixture of amorphous carbon as well as crystalline graphite.

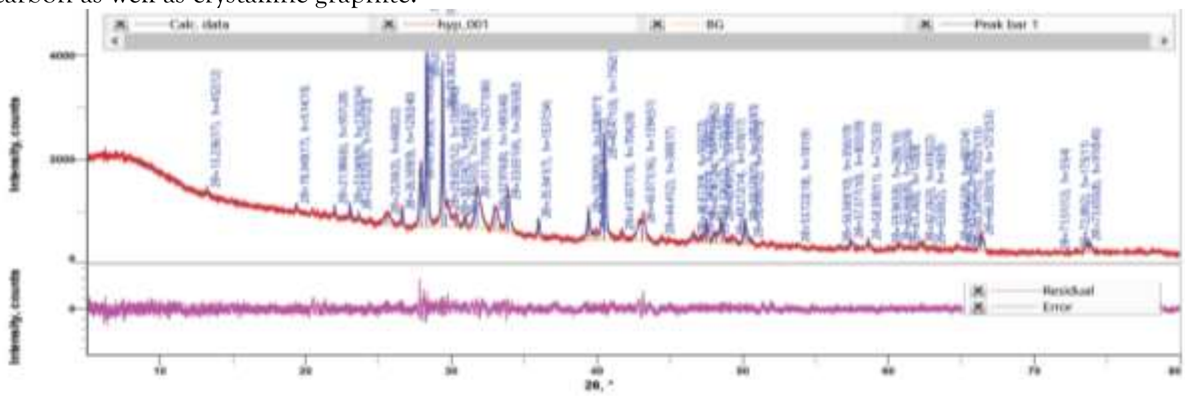

Fig. 3: X-ray Diffraction spectrum of the Biosoot of M. sauveolens

Fourier Transform Infra-Red Spectrum of the Biosoot of Mesosphaerum sauveolens

The FTIR spectral analysis of the biosoot of Mesosphaerum sauveolens (Figure 4) revealed the presence of C triple bond C alkynes, Phosphines, Alkyl mercaptans, Aliphatic compounds, aromatic hydrocarbons and N-H secondary amides with that of the biosoot of Mesosphaerum sauveolens.

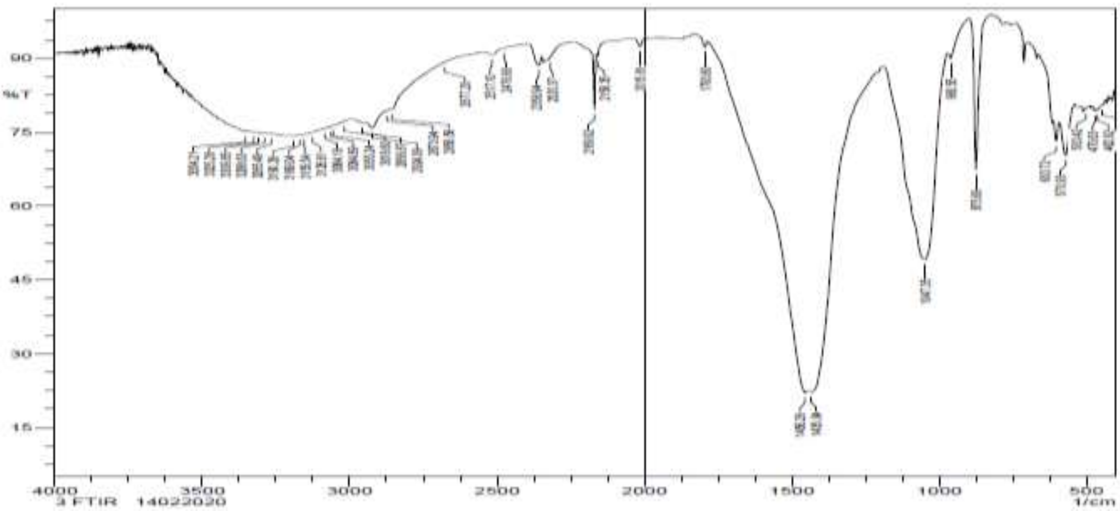

Fig. 4: FTIR spectrum of the Biosoot of M. sauveolens

Brunauer–Emmett–Teller (BET) Analysis

The BET analysis of the biosoot of Mesosphaerum sauveolens revealed the mean pore size as 37.60nm and the total pore volume as 2.5388^{-02} cubic centimetre / gram. The corresponding BET plot is presented in Figure 5a. The mesoporous properties of the biosoot were investigated using the Barrett–Joyner–Halenda (BJH) and Dubinin–Harkins (DH) methods. The BJH analysis showed a total pore volume of 2.4709×10^{-2} cm^3/g, a specific surface area of 0.7387 m^2/g, and a pore radius peak at 7.99 nm. Similarly, the DH method revealed a total pore volume of 2.4613×10^{-2} cm^3/g, a specific surface area of 0.2608 m^2/g, and a pore radius peak at 7.97 nm. The BJH and DH plots illustrating the mesoporous adsorption characteristics are presented in Figures 5b and 5c, respectively. The micropore distribution analysis showed that the mean difference in pore size was 0.8 nm. The specific surface areas were determined as 2.8752 m^2/g (a1) and 0.7614 m^2/g (a2). The total pore volume was calculated as 2.7650×10^{-4} cm^3/g, with the peak pore diameter at 0.8 nm. The micropore distribution is depicted in the MP plot in Figure 5d.

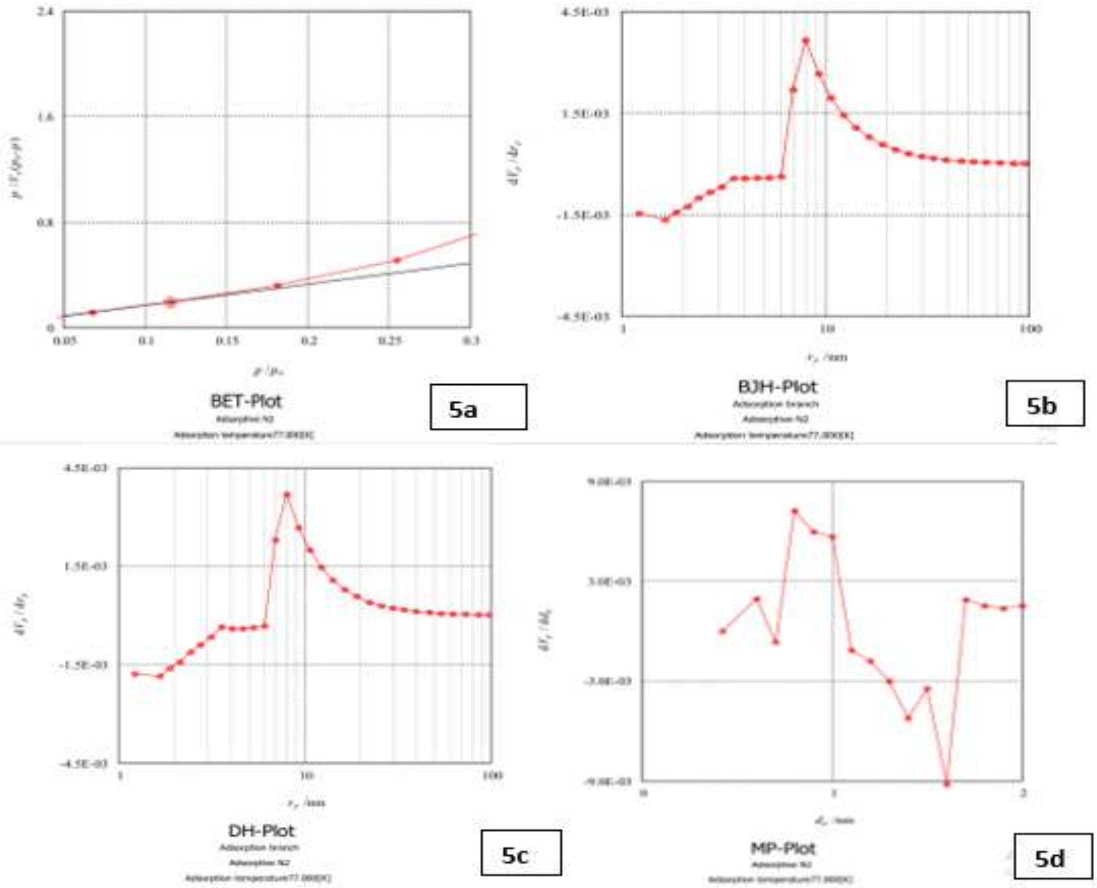

Fig. (5a): BET Plot Showing the Pore Size and Total Pore Volume (5b): BJH Plot Depicting Mesoporous Adsorption Characteristics (5c): DH Plot Illustrating Mesoporous Adsorption Characters (5d): MP Plot Showing Micropore Distribution and Pore Size Characteristics of Biosoot of M. sauveolens

The characterization studies on the biosoot of Mesosphaerum sauveolens revealed that the biosoot generated are clearly of Carbon Nanoparticles. Thus, the Carbon Nanoparticles (CNPs) are further evaluated for their bioactivities.

Antibacterial and Antifungal Activity of CNPs of Mesosphaerum sauveolens

The CNPs of Mesosphaerum sauveolens exhibited antibacterial activity (Table 1) against all tested bacteria, with the maximum zone of inhibition recorded for Escherichia coli (23 mm, 27 mm, and 32 mm at 25 µg, 50 µg, and 75 µg concentrations, respectively). The minimum zone of inhibition was observed for Pseudomonas aeruginosa, which displayed a zone of only 7 mm at 75 µg concentration. Comparable zones of inhibition were noted for Streptococcus mutans, Staphylococcus aureus, and Bacillus subtilis. The CNPs also demonstrated antifungal activity (Table 1) against Aspergillus niger and Aspergillus flavus. Zones of inhibition were recorded for 25 µg, 50 µg, and 75 µg concentrations, with activity evident against both fungal species.

Table 1: Antibacterial and Antifungal activity of CNPs of M. sauveolens

Microorganism	**25 µg**	**50 µg**	**75 µg**
Escherichia coli	23 mm	27 mm	32 mm
Pseudomonas aeruginosa	-	-	7 mm
Streptococcus mutans	14 mm	18 mm	22 mm
Staphylococcus aureus	16 mm	20 mm	25 mm
Bacillus subtilis	15 mm	19 mm	24 mm
Aspergillus niger	10 mm	14 mm	18 mm
Aspergillus flavus	11 mm	15 mm	19 mm

DPPH free radical scavenging activity

The antioxidant potency of the CNPs of Mesosphaerum sauveolens was evaluated against the free radical DPPH (Figure 6). The IC_{50} value recorded for Mesosphaerum sauveolens was 32µg. The radical scavenging activity increased with higher concentrations of the CNPs.

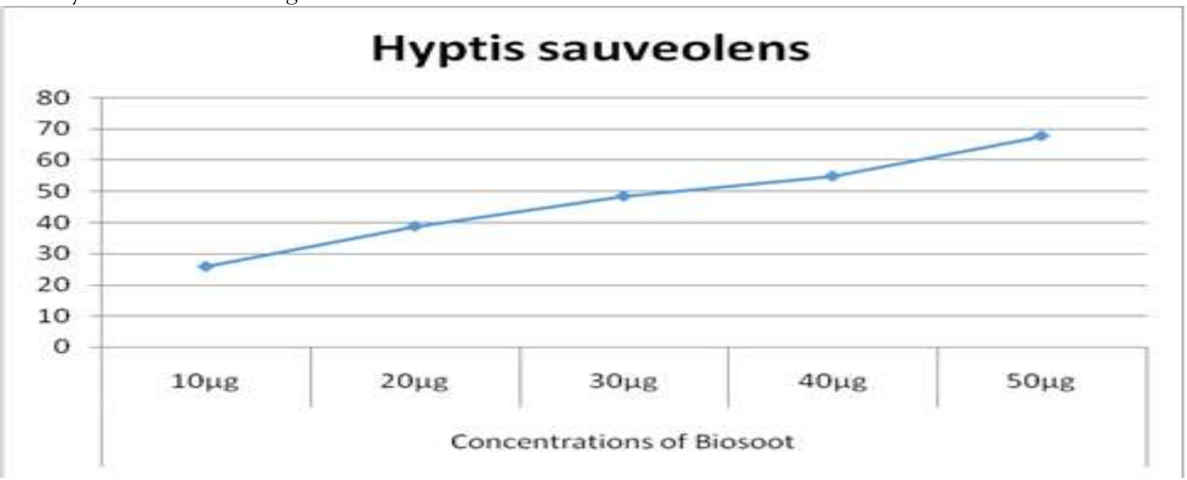

Fig. 6: The DPPH radical scavenging activity of CNPs of M. sauveolens

Anticancer Evaluation of CNPs of Mesosphaerum sauveolens

The anticancer activity of the CNPs of Mesosphaerum sauveolens was evaluated against the MCF-7 breast cancer cell line using the MTT assay. The study revealed a Lethal Dosage 50 (LD_{50}) value of 69.77 µg/ml, indicating its cytotoxic potential. The detailed viability data across various concentrations is depicted in Figure 7. Additionally, morphological changes in the MCF-7 cell line were observed and documented (Figure 8).

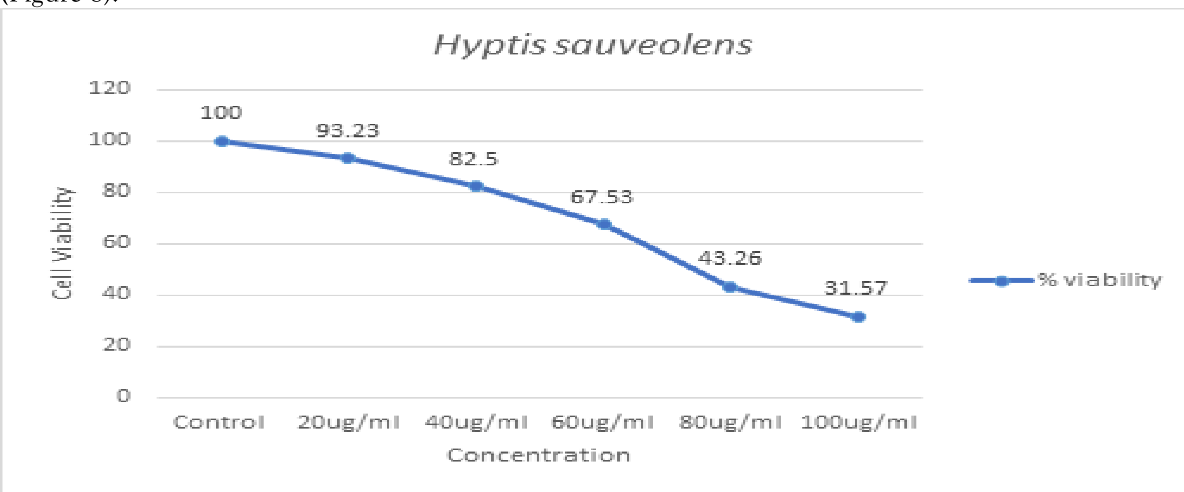

Fig. 7: Percent viability of cell line MCF-7 against the CNPs of M. sauveolens

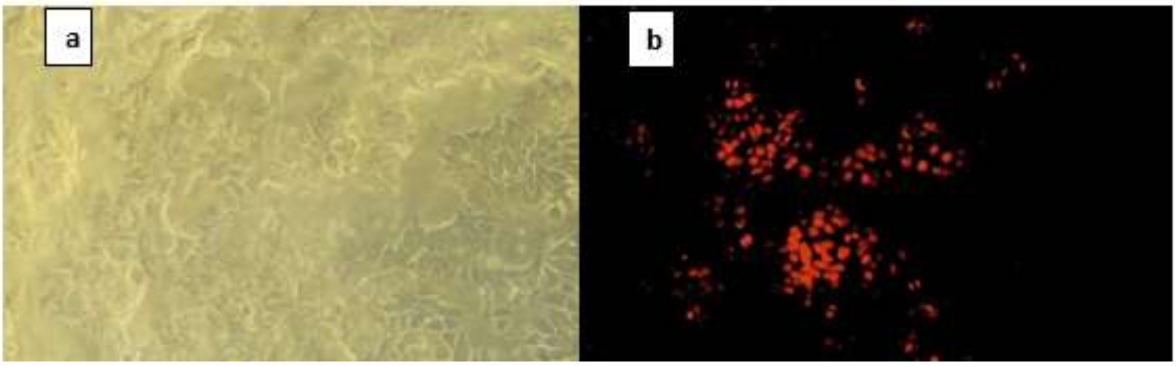

Fig. 8: Images of MCF – 7 cell line treated with CNPs of M. sauveolens (a) phase-contrast microscope (b) fluorescent microscope (20x magnification)

DISCUSSION

Production of biosoot from Mesosphaerum suaveolens leaves is a novel approach toward a significant step in the use of natural plant material for the synthesis of nanomaterials. Unlike conventional methods of carbon nanoparticle synthesis, which generally require chemical precursors and highly energetic processes, this incineration method has proved to be an easy, environment-friendly approach. With such minimal resources required for its production, the overall yield of biosoot is as high as 188 mg per 100 g of dried leaves.DLS analysis showed a wide range of particle sizes that include nanoscale particles, 121–289 nm, and larger aggregates, 5.5 µm. The SEM also supported the appearance of spherical nanoparticles within the size range of 30–50 nm. In a similar investigation into carbon nanoparticle synthesis using other plant material, Chromolaena odorata (Kumar et al.2023), found that nanoparticles varied from 20–60 nm in size. However, the triple-peaked size distribution in this study reveals the heterogeneity of biosoot, possibly because of the open incineration process. High-temperature combustion may partially aggregate nanoparticles, leading to the larger particle sizes.

XRD analysis suggested that the biosoot consisted of amorphous carbon and crystalline graphite, with 2θ peaks located at 27–28° and 40°. A similar two-phase structure was seen in carbon materials, which have been enhanced by the existence of crystalline graphite, a factor that increased electrical conductivity, and which made these carbon materials useful in electrochemical applications (Xu et al. 2024).The Fourier Transform Infrared (FTIR) analysis of the biosoot revealed functional groups such as alkynes, aromatic hydrocarbons, and secondary amides, suggesting its potential for diverse applications, including catalysis, biosensing, and drug delivery. A similar study reported the presence of phytocompounds and their bioactivities of the leaves of Chromolaena odorata (Kumar et al. 2023), which indicates that these associated compounds are generally considered as Reactive Oxidative Species (ROS).BET analysis resulted in the average pore diameter as 37.6 nm with a total pore volume of 2.5388×10^{-2} cm^3/g. Mesoporous nature, observed by BJH and DH analysis, indicates large surface area and thus high capacity of adsorption that is good for energy storage and environmental remediation. By comparison, carbon materials synthesized from Chromolaena odorata through pyrolysis possessed a specific surface area in the range 0.8–1.2 m^2/g (Kumar et al. 2023). The latter was slightly greater than the biosoot values of Mesosphaerum suaveolens. It can be attributed to variations in the synthesis methods, plant biochemistry, and combustion conditions. The study clearly demonstrated the synthesis of Carbon Nanoparticles (CNPs) in the form of Biosoot.The study confirmed that Carbon Nanoparticles can be easily synthesized by open pyrolysis method. Previous the Carbon Dots were prepared in the form of Biosoot from the plant Datura metel (Priyanka et al., 2025).The CNPs thus prepared showed remarkable activity against both bacterial and fungal, with maximum zone of inhibition obtained against Escherichia coli at 32 mm and the activity was mild against Aspergillus. This is quite consistent with many earlier studies based on plant-originated nanoparticles whose surface functional groups played a prominent role in interrupting microbial cell membranes (Li et al.2014, Verma et al. 2021). The antioxidant activity of the biosoot indicated by a DPPH IC_{50} value of 32 µg/mL is comparable to green-synthesized Silver nanoparticles from Azadirachta indica (Roy et al. 2017).

The biosoot showed promising cytotoxicity against MCF-7 breast cancer cells with a LD_{50} value of 69.77 µg /mL. Similar cytotoxicity has been reported for nanoparticles synthesized from Moringa oleifera extracts (Adebayo-Tayo B et al. 2019) where the presence of bioactive compounds improved anti-cancer efficacy. The morphological changes observed in treated cells confirm further the potential of biosoot as an anti-cancer agent possibly due to the combined effects of its carbon structure and surface functional groups. The study strongly suggests that the naturally synthesized CNPs from the aromatic, medicinal plant Mesosphaerum sauveolens can be exploited in pharmaceutical industry and cosmetic industry (Priya et al., 2023) after proper evaluation for their toxicity.

CONCLUSION

The study demonstrated a simple and efficient method for synthesizing carbon nanoparticles from the medicinal plant Mesosphaerum sauveolens. The synthesized carbon nanoparticles exhibited significant antimicrobial and antioxidant properties, making them promising candidates for applications in the cosmetic and pharmaceutical industries, particularly in the formulation of topical creams. Furthermore, the findings suggest that the carbon nanoparticles of Mesosphaerum sauveolens possess potential anticancer activity, indicating their utility as a therapeutic agent following comprehensive toxicity evaluations.

Author Contribution Statement

N. K. Udaya Prakash, S. Bhuvaneswari and K.P. Kannan –Conceptualization and Review, Selvakumar Priyanka and Sanjeev Kumar – Experimental, Investigation, Writing and Vardhana Janakiraman - Editing and Review.

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Conflicts Of Interest

The authors declare that there is no conflict of interest

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