

Biogenic Selenium Nanoparticles Loaded Alginate-Gelatin Scaffolds for Potential Tissue Engineering Applications

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Abstract

Selenium nanoparticles (SeNPs) were reported for its anticancer and antimicrobial properties. Alginate and gelatin scaffolds can act as an important biomaterial, more specifically in bone tissue engineering. Green synthesis of SeNPs from *Luffa cylindrica* (LC) and loading of SeNPs with alginate-gelatin scaffold and to check its biocompatibility. The SeNPs were prepared via the green synthesis method and loaded into an alginate-gelatin scaffold. Characterization studies such as UV-Vis spectroscopy, FTIR, and SEM were carried out in LC-SeNPs and Se-NPs loaded scaffold. The hydrophilicity of the scaffolds was determined using water contact angle measurements. Annexin V PI assay was conducted to determine the biocompatible nature of prepared SeNPs-loaded alginate-gelatin scaffolds. The UV-VIS spectrum gave an intense peak at 266 and 384 nm, whereas the FTIR gave a strong peak at 3500-500 cm^{-1} fingerprint regions. SEM images showed flower-shaped LC-SeNPs and their distribution of SeNPs on the surface of alginate-gelatin scaffolds. water contact angle measurement was found to be 29.21°. Cell viability results showed 78.11% viable cells following treatment with alg-gel-Se scaffold, revealing its biocompatibility towards peripheral blood mononuclear cells. Overall, it could be concluded that the SeNP-loaded alg-gel scaffold is a promising candidate for tissue engineering, but further studies are required to confirm its potential role.

Keywords- *Selenium nanoparticles, Alginate-gelatin scaffolds, tissue engineering, biocompatibility.*

Introduction

Selenium nanoparticles (SeNPs) are well known for their unique physical, chemical, and biological properties [1]. It also possesses less toxicity and more excellent biological activity. Due to its potent biological activity, SeNPs have been of great interest to researchers in recent years [2, 3]. SeNPs have been reported for anti-inflammatory activity. It is also used in various disease conditions like diabetes, liver fibrosis, etc [4–6]. Selenium nanoparticles with anticancer and antimicrobial properties that may contribute to human health, not only as dietary supplements, but also as therapeutic agents [7, 8]. General synthesis of SeNPs by physiochemical method has some adverse effects. On the other hand, green synthesis of

NPs is considered an eco-friendly method, as it utilizes plant extracts for synthesizing NPs [9, 10].

Luffa cylindrica (LC), also known as loofa is one of the medicinal plants that grows in warmer temperatures [11]. Traditionally, this plant has been used to treat various disease conditions such as asthma, carbuncles, jaundice, leprosy, etc. [12]. LC was reported to exhibit various pharmacological properties like anticancer, antioxidant, anti-inflammatory, etc [13]. The major phytochemical constituents present in LC are glycosides, flavonoids, triterpenoids, saponins, alkaloids, saponins, tannins, etc [14, 15]. So, LC is utilized in synthesizing SeNPs through the green synthesis method. Alginate (Alg) is a polymer extracted from natural

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seaweed and is used extensively in the medical field due to its nontoxicity and biocompatibility [16]. It is also a cost-effective biomaterial [17, 18]. Alg is also considered an excellent biomaterial for tissue engineering strategies and is used to fabricate scaffolds [19, 20].

Gelatin is a denatured collagen formed by the hydrolysis of collagen and possesses the properties of biodegradability, biocompatibility, stability, etc., for which it is utilized in medicinal applications [21]. Both the alginate and gelatin can be used to fabricate scaffolds [22]. Sodium alginate/ gelatin scaffolds are reported as suitable biomaterials for bone tissue engineering in a previous study [23].

The present study aims to prepare SeNPs using *Luffa cylindrica* leaf extract and load them into alginate-gelatin scaffolds (Alg- gel- SeNPs). The scaffolds are characterized using FTIR, SEM, and water contact angle measurements. The scaffolds are further investigated for biocompatibility using Annexin V PI assay to reveal their suitability in tissue engineering applications.

Experimental Section

Green Synthesis of SeNPs

Leaves of *Luffa cylindrica* (LC) were collected, shade-dried, and then powdered. 2g of powdered LC leaf was boiled in 50 ml of distilled water for 20 mins at 80°C and filtered. The resulting filtrate was used for synthesizing SeNPs [16].

About 2mL of the extract was added to 20 mL of 50 mM sodium selenite solution and, continuously stirred in a magnetic stirrer for 24 h, and centrifuged at 8000 rpm for 15 min [3]. The supernatant was discarded, and the resulting pellet was lyophilized and used for scaffold fabrication.

Fabrication of SeNPs Loaded Alg-Gel Scaffolds

The LC-SeNPs loaded Alg-gel scaffolds were prepared via gelation. Lyophilized LC-SeNPs powder was mixed with 10% chitosan

hydrochloride solution. To 500 μ L of chitosan solution containing LC-SeNPs, add the same quantity of 10% gelatin and mix well. The gel formed was subjected to lyophilization and used for further studies.

Characterization Studies

The synthesized LC-SeNPs were first characterized using the UV-Vis spectrophotometer. UV-Vis Spectrum of the LC-SeNPs scaffold was recorded in a wavelength range of 250 - 550 nm. The presence of different functional groups in LC-SeNPs and SeNPs loaded Alg-Gel scaffolds was analyzed by FTIR with the wavelength range of 3500-500 cm^{-1} . The surface morphology of LC-SeNPs and SeNPs loaded Alg-Gel scaffolds was determined by SEM [3].

The hydrophilicity of the scaffold was determined using water contact angle measurement [16].

Annexin V PI Assay

Annexin V PI assay was used to determine the biocompatible nature of Alg-Gel-SeNPs scaffolds. Approval from the Institute Human Ethical committee was received for collecting blood from donors. 2ml of histopaque was added over 2ml of blood and centrifuged at density gradient centrifuge at 2000 rpm for 40 min to isolate peripheral blood mononuclear cells (PBMCs). 100 μ l of Alg-Gel-seNPs scaffolds were added to Isolated PBMCs and cultured in RPMI media with 10% FBS (Fetal Bovine Serum), 1% amino acid L-glutamine and 1% PenStrep for 24 hrs. Cells without scaffold treatment were taken as control and the culturing was done in triplicate. For straining the cultured cells 5 μ l of Annexin V FITC and 5 μ l of Propidium iodide (PI) was added and incubate for 15 mins. Followed by incubation 400 μ l of 1X binding buffer were and observed for apoptosis using BD FACS lyric flow cytometer and analysis was done using FACSuite 4.1 Software [16].

Results

The UV-Vis spectrum of LC-SeNPs gave a strong absorption peak at 266 and 384 nm

confirmed the formation of SeNPs. The shift in SPR peak evidenced the nanoparticle formation. Figure 1 represents the UV-Vis spectrophotometer of LC-SeNPs.

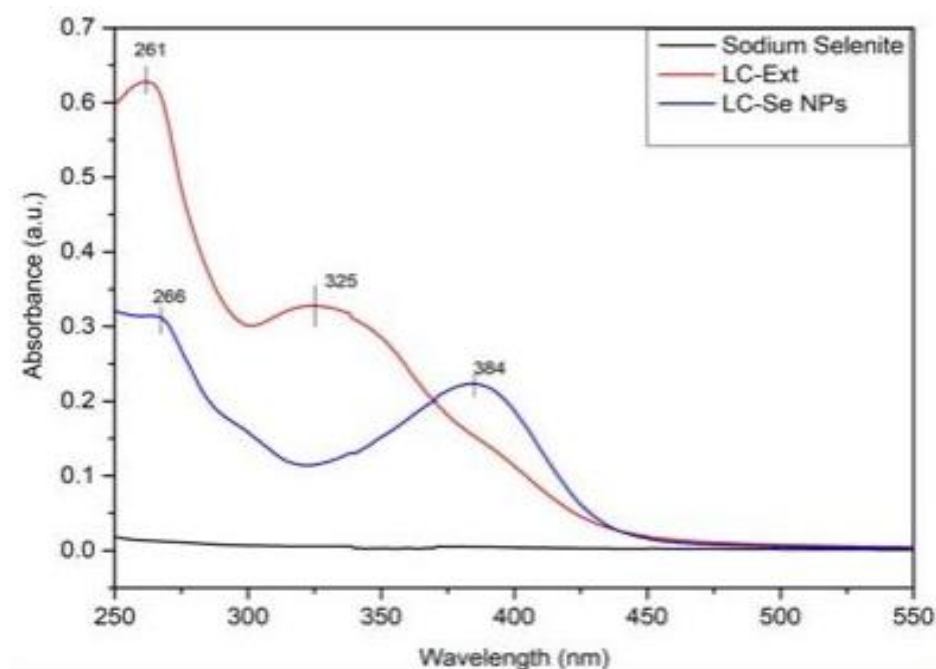


Figure 1. UV-Vis Spectra of LC Extract and LC-SeNPs

FTIR of LC-SeNPs gave intense peaks at 3193, 2906, 1661, 1374, 1001, 835, 701, and 623 cm^{-1} . 3193 cm^{-1} peak corresponds to O-H stretching, indicating the presence of the carboxylic acid/alcohol group. 2906 cm^{-1} peak corresponds to O-H / N-H stretching, indicating alcohol group/amine salt. 1661 cm^{-1} corresponds to C=C stretching indicating an alkene group. 1374 corresponds to O-H bending, indicating the phenol group. 1001 corresponds to C-F stretching indicating fluoro compound. 835 corresponds to C-Cl stretching indicating halo compounds. 701 corresponds to C=C bending / C-Cl stretching, indicating alkane group/halo

compound. 623 corresponds to C-Br stretching, indicating halo compound. FTIR of Alg-Gel-SeNPs scaffolds gave peaks at 2362, 1532, and 1232 cm^{-1} . 2363 cm^{-1} corresponds to C-O-C stretching, indicating glycoside linkage. 1532 cm^{-1} corresponds to N-O stretching indicating nitro compound. 1232 cm^{-1} corresponds to C=O / C-N stretching indicating the alkyl aryl ether/amine group. The formation of peaks at LC-SeNPs and Alg-Gel-LC-SeNPs confirms the presence of different functional groups. Fig 2 represents the FTIR of LC-SeNPs and Alg-Gel-LC-SeNPs.

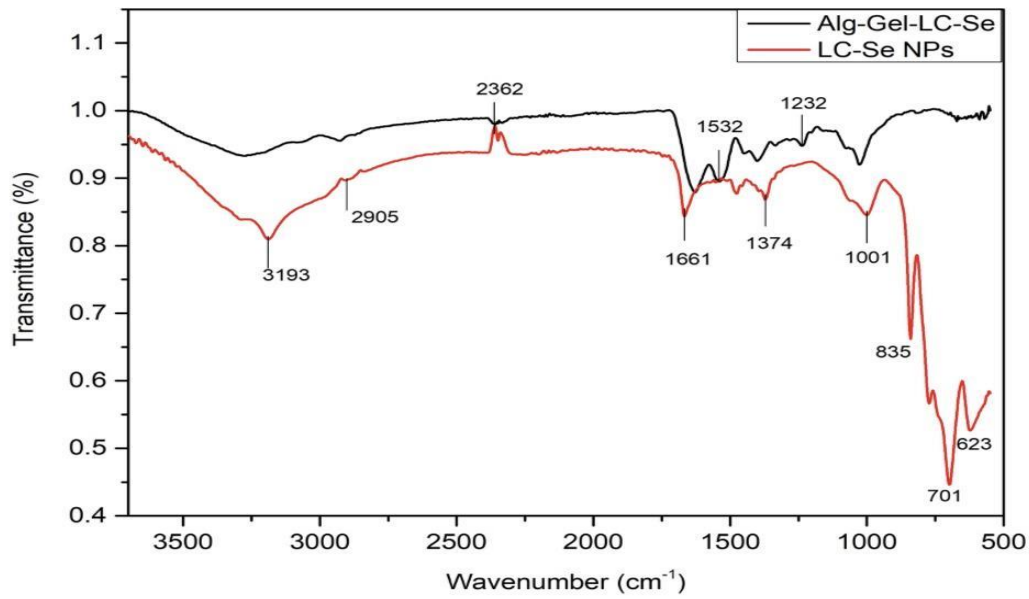


Figure 2. FTIR of LC-SeNPs and Alg-Gel-SeNPs

SEM analysis of LC-SeNPs was found to be flower-shaped. The SEM micrograph of Alg-Gel-SeNPs scaffolds showed the dispersion of SeNPs on the surface of the porous structure of the scaffolds. Fig 3 displays the SEM images of LC-SeNPs and Alg-Gel-SeNPs scaffolds. When

the contact angle of SeNPs loaded alginate-gelatin scaffold was measured it was found to be 29.21°, which confirmed the hydrophilic nature of the scaffold. Fig 4 represents the water contact angle measurement of the Alg-Gel-SeNPs scaffold.

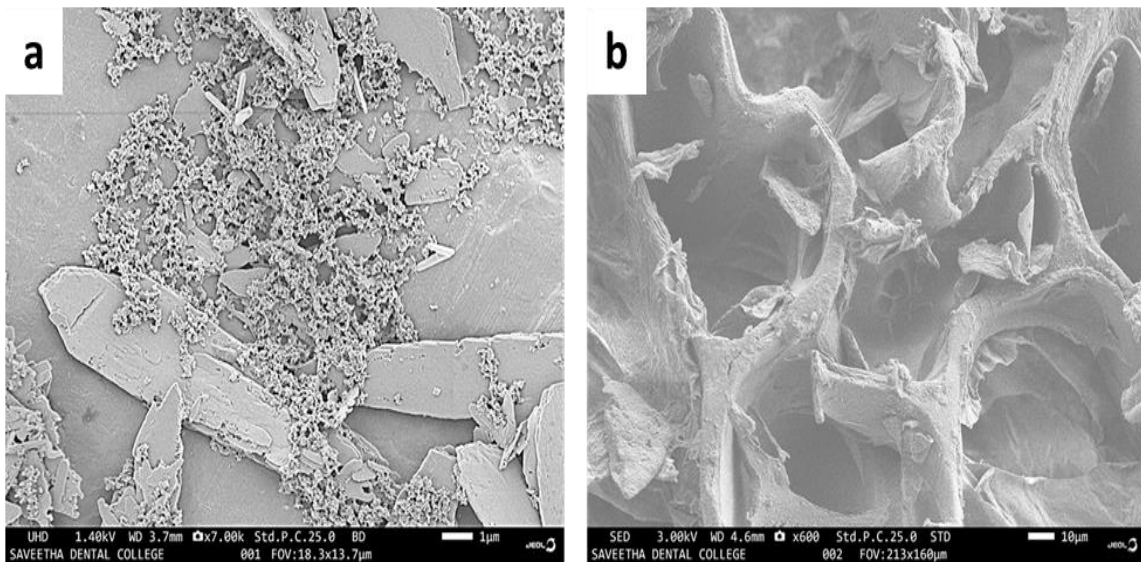


Figure 3. SEM Micrograph of LC-SeNPs (a) and SeNPs Loaded Alginate-Gelatin Scaffold

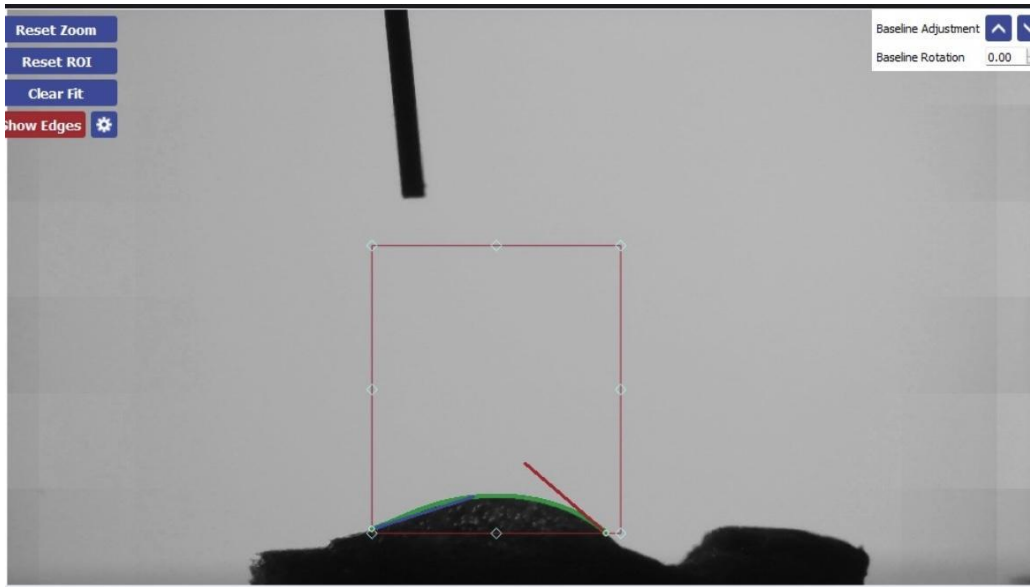


Figure 4. Water Contact Angle Measurement of Alg-Gel-SeNps Scaffolds

Biocompatibility Studies

Fig 5 represents the Biocompatibility assay of Alg-Gel-Se. The LL (Lower left) quadrant of the graph represents the percentage of viable cells while the LR (Lower right) quadrant represents the percentage of cells in early apoptosis, UR(upper right) represents the percentage of cells in late apoptosis and UL(Upper left) quadrant represents the percentage of cells that

undergo necrosis. It was found that 78.11 % of cells(LL) were found to be viable post-treatment with Alg-Gel-Se and 21.79% of cells were found to be in early apoptosis stage (LR), 0.07% of cells were in late apoptosis(UR), and 0.02% of cells were in necrosis(UL). These results were found to be concordant with control. From this result, it was evident that the Alg-Gel-SeNps scaffold was found to be biocompatible.

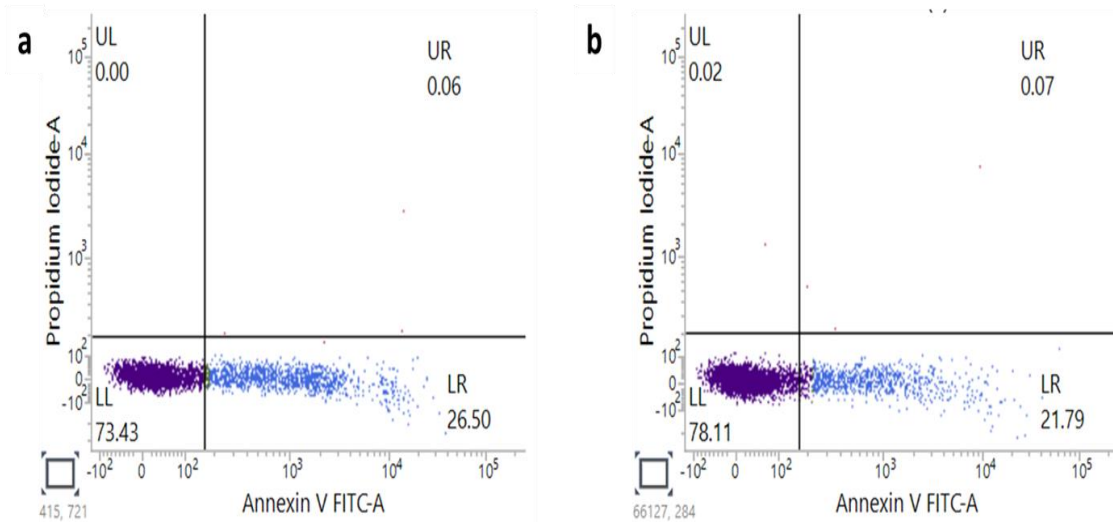


Figure 5. Biocompatibility Assay of Control Cells (a) and Cells Treated with Alg-Gel-SeNPs Scaffold

Discussion

Green synthesis of Nanoparticles (NPs) was considered as a safe and eco-friendly method for NPs synthesis. NPs are well known for their

shape, structural morphology, and physicochemical properties [24]. NPs synthesized by green synthesis have varying properties compared with physical and chemical methods [25].

In this study, for the first time, we have prepared LC-SeNPs using LC leaf extract and fabricated them into a Se-loaded Alg-gel scaffold. A previous study reported the green synthesis of SeNPs using *Withania somnifera* leaf extract [26]. The UV-visible spectrum of LC-SeNPs in our study showed a strong, intense peak at 384 nm, which was concordant with the previously reported UV peaks of Se obtained from *Clausena dentata* at 420 nm [27]. Similarly, FTIR of LC-SeNPs gave strong peaks at 3193, 2906, 1661, 1374, 1001, 835, 701, and 623 cm^{-1} and Alg-Gel-Se gave peaks at 2362, 1532, and 1232 cm^{-1} . and both the results were found to be concordant with the result reported previously [28, 29].

SEM images of LC-SeNPs showed a flower-shaped morphology which are in line with the results of Se NPs reported previously [30]. Annexin V PI assay confirms the biocompatible nature of Ala-Gel-SeNPs scaffolds. Biogenically synthesized SeNPs loaded alginate gelatin scaffolds acts as a promising candidate for tissue engineering. But further *in vitro* and *in vivo* studies are required to confirm its mechanism of action in tissue engineering.

References

- [1] Johnson, J., Shanmugam, R., and Lakshmi, T. 2022. A review on plant-mediated selenium nanoparticles and its applications. *Journal of population therapeutics and clinical pharmacology*, 28(2): e29–e40.
- [2] Ingole, A. R., Thakare, S. R., Khati, N. T., Wankhade, A. V., and Burghate, D. K. 2010. Green synthesis of selenium nanoparticles under ambient condition. *Chalcogenide Lett*, 7(7): 485–489.
- [3] Naaziya, M., Biju, T. S., Francis, A. P., Veeraraghavan, V. P., Gayathri, R., and Sankaran, K. (2023). Synthesis, Characterization and *in-vitro* Biological Studies of Curcumin decorated Biogenic Selenium Nanoparticles. *Nano LIFE*. <https://doi.org/10.1142/s1793984423500137>
- [4] Zhao, L., Wang, H., and Du, X. 2021. The therapeutic use of quercetin in ophthalmology: recent applications. *Biomedicine & Pharmacotherapy*. <https://doi.org/10.1016/j.biopha.2021.111371>
- [5] Khurana, A., Tekula, S., Saifi, M. A., Venkatesh, P., and Godugu, C. 2019. Therapeutic applications of selenium nanoparticles. *Biomedicine & pharmacotherapy 111*: 802–812.
- [6] Jayavarsha, V., Rajeshkumar, S., Lakshmi, T., and Sulochana, G. 2022. Green synthesis of selenium nanoparticles study using clove and cumin and its anti-inflammatory activity. *Journal of complementary medicine research*, 13(5): 84.
- [7] Skalickova, S., Milosavljevic, V., Cihalova, K., Horoky, P., Richtera, L., and Adam, V. 2017. Selenium nanoparticles as a nutritional supplement. *Nutrition*, 33: 83–90.
- [8] Sneka, and Santhakumar, P. 2021. Antibacterial Activity of Selenium Nanoparticles extracted from *Capparis decidua* against *Escherichia coli* and

Conclusion

We have synthesized SeNPs using LC leaf extract and fabricated SeNPs-loaded Alg-Gel scaffolds. Based on the results from characterization studies and biocompatible assay, we conclude that Alg-Gel-Se could be an ideal candidate for tissue engineering applications. However, in-depth research was required in the future to identify its molecular action.

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Conflict of Interest

The authors declare no conflict of interest.

Author Contribution

Priyam Bharathidasan has conducted the literature collection, study design, experimental work and written the manuscript. Vishnu Priya Veeraraghavan and Gayathri R have reviewed and edited the manuscript.

- Lactobacillus Species. *Journal of advanced pharmaceutical technology & research*, 14(8): 4452–4454.
- [9] Ndwandwe, B. K., Malinga, S. P., and Kayitesi, E. 2021. Advances in green synthesis of selenium nanoparticles and their application in food packaging *International Journal of Food Science & Technology* <https://doi.org/10.1111/ijfs.14916>
- [10] Ali, S. J., Preetha, Jeevitha, Prathap, L., and Rajeshkumar. 2020. Antifungal Activity of Selenium Nanoparticles Extracted from Capparis decidua Fruit against *Candida albicans*. *Journal of evolution of medical and dental sciences*, 9(34): 2452–2455. <https://doi.org/10.14260/jemds/2020/533>
- [11] Partap, S., Kumar, A., Sharma, N. K., and Jha, K. K. 2012. Luffa Cylindrica : An important medicinal plant. *J. Nat. Prod. Plant Resour.*, 2(1):127-134
- [12] Al-Snafi, A. E. 2019. Constituents and pharmacology of Luffa cylindrica-A review. *IOSR Journal of Pharmacy*. 9(9): 68-79
- [13] Akinwumi, K. A., Eleyowo, O. O., and Oladipo, O.O. 2021. A review on the ethnobotanical uses, phytochemistry and pharmacology effects of Luffa cylindrica. *Natural Drugs from Plants*. <https://doi.org/10.5772/intechopen.98405>
- [14] Aboh, M. I., Okhale, S. E., & K., I. (2012). Preliminary studies on Luffa cylindrica: Comparative phytochemical and antimicrobial screening of the fresh and dried aerial parts. *African journal of microbiology research*, 3088, 3091. <https://doi.org/10.5897/AJMR11.301>
- [15] Balakrishnan, N., and Sharma, A. 2013. Preliminary phytochemical and pharmacological activities of Luffa cylindrica L. fruit. *Asian journal of pharmaceutical and clinical research*, 6(2): 113–116.
- [16] Soni, M., Gayathri, R., Sankaran, K., Veeraraghavan, V. P., and Francis, A. P. 2023. Green synthesis of selenium nanoparticles using *luffa cylindrica* and its biocompatibility for potential biomedical applications. *Nano*. 18(6): 2350042
- [17] Cuadros, T. R., Erices, A. A., and Aguilera, J. M. 2015. Porous matrix of calcium alginate/gelatin with enhanced properties as scaffold for cell culture. *Journal of the mechanical behavior of biomedical materials*, 46: 331–342.
- [18] Wang, L., Zhang, H. J., Liu, X., Liu, Y., Zhu, X., Liu, X., and You, X. 2021. A Physically Cross-Linked Sodium Alginate–Gelatin Hydrogel with High Mechanical Strength. *ACS Applied Polymer Materials*, 3(6): 3197–3205.
- [19] You, F., Wu, X., and Chen, X. 2017. 3D printing of porous alginate/gelatin hydrogel scaffolds and their mechanical property characterization. *International Journal of Polymeric Materials and Polymeric Biomaterials*, 66(6): 299–306.
- [20] Serafin, A., Murphy, C., Rubio, M. C., and Collins, M. N. 2021. Printable alginate/gelatin hydrogel reinforced with carbon nanofibers as electrically conductive scaffolds for tissue engineering. *Materials science & engineering. C, Materials for biological applications*, 122: 111927.
- [21] Afjoul, H., Shamloo, A., and Kamali, A. 2020. Freeze-gelled alginate/gelatin scaffolds for wound healing applications: An in vitro, in vivo study. *Materials science & engineering. C, Materials for biological applications*, 113, 110957.
- [22] Luo, Y., Li, Y., Qin, X., Wa, Q. 2018. 3D printing of concentrated alginate/gelatin scaffolds with homogeneous nano apatite coating for bone tissue engineering. *Materials & design*, 146: 12–19.
- [23] Bose, S., Roy, M., and Bandyopadhyay, A. 2012. Recent advances in bone tissue engineering scaffolds. *Trends in biotechnology*, 30(10): 546–554.
- [24] Gour, A., and Jain, N. K. 2019. Advances in green synthesis of nanoparticles. *Artificial cells, nanomedicine, and biotechnology*, 47(1): 844–851.
- [25] Hussain, I., Singh, N. B., Singh, A., Singh, H., and Singh, S. C. 2016. Green synthesis of nanoparticles and its potential application. *Biotechnology letters*, 38(4), 545–560.
- [26] Alagesan, V., and Venugopal, S. 2019. Green Synthesis of Selenium Nanoparticle Using Leaves Extract of *Withania somnifera* and Its Biological Applications and Photocatalytic Activities. *BioNanoScience*, 9(1): 105–116.
- [27] Sowndarya, P., Ramkumar, G., and Shivakumar, M. S. 2017. Green synthesis of selenium nanoparticles conjugated *Clausena dentata* plant leaf extract and their insecticidal potential against mosquito vectors. *Artificial cells, nanomedicine, and biotechnology*, 45(8): 1490–1495.
- [28] Fardsadegh, B., and Jafarizadeh-Malmiri, H.

2019. Aloe vera leaf extract mediated green synthesis of selenium nanoparticles and assessment of their in vitro antimicrobial activity against spoilage fungi and pathogenic bacteria strains. *Green Processing and Synthesis*, 8(1): 399–407.

[29] Sharma, A., Puri, V., Kumar, P., and Singh, I. 2020. Rifampicin-Loaded Alginate-Gelatin Fibers Incorporated within Transdermal Films as a Fiber-in-

Film System for Wound Healing Applications. *Membranes*, 11(1):7

[30] Leena, R. S., Vairamani, M., and Selvamurugan, N. 2017. Alginate/Gelatin scaffolds incorporated with Silibinin-loaded Chitosan nanoparticles for bone formation in vitro. *Colloids and surfaces. B, Biointerfaces*, 158, 308–318.