



**GREEN SYNTHESIS AND CHARACTERIZATION OF SILVER NANOPARTICLES  
FROM *Illicium verum* Hook. F AND THEIR ANTIOXIDANT AND ANTI DIABETICS  
ACTIVITY**

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**ABSTRACT**

The study conducted qualitative phytochemical screening of *Illicium verum* Hook. F dried fruit extracts, revealing varied concentrations of phytochemicals across solvents. Chloroform and ethanol extracts exhibited high glycosides, steroids, and terpenoids, while aqueous and acetone extracts showed lower phenols, flavonoids, and alkaloids. Green synthesis of silver nanoparticles from the fruit extract was characterized by UV spectrum analysis, indicating silver ion reduction. FT-IR spectrum analysis identified biomolecules responsible for ion reduction and revealed functional groups present. Scanning Electron Microscope (SEM) images exhibited nanoporous structures with potential applications in drug delivery and heavy metal adsorption. The antioxidant activity of dried fruit extracts, attributed to phenolic compounds, was evaluated using the DPPH-assay method, demonstrating concentration-dependent free radical scavenging effects surpassing standard ascorbic acid. The in vitro alpha amylase inhibitory assay indicated promising antidiabetic activity of the extract compared to standard acarbose, suggesting further investigation to isolate the active polyphenol. Overall, the study underscores the phytochemical richness and potential health benefits of *Illicium verum* Hook. F extracts, advocating for additional research to elucidate their therapeutic potential.

**Keywords: Phytochemical screening, chloroform, ethanol, aqueous and acetone**

## INTRODUCTION

Medicinal plants have long been a crucial source of therapeutic remedies for humanity. According to the World Health Organization, approximately 80% of the global population relies on medicinal plants for their primary healthcare needs [1]. These plants have been utilized since ancient times across various cultures, forming the basis of traditional healing practices. India, in particular, boasts a rich tradition of medicinal systems like Ayurveda, Siddha, and Unani, documented in ancient texts like the Vedas [2]. The utilization of medicinal plants extends beyond traditional medicine, with their extracts being integral components in industries like food and cosmetics. However, harnessing the therapeutic potential of these plants comes with challenges, primarily due to the variability in secondary compound production among different plant species and even within specific plant cells. The traditional approaches to standardizing medicinal plant products are becoming inadequate for the modern herbal market, necessitating the integration of advanced techniques from herbal drug technology [3]. This integration enables standardization, quality control, and the amalgamation of traditional knowledge with modern scientific methodologies. In recent years, there has been a global resurgence in the utilization of medicinal plant products, driven by growing interest in alternative medicines. This trend has led to the expansion of natural

product markets and a renewed focus on traditional herbal medicines. However, ensuring the safety and efficacy of herbal products relies heavily on standardized processes [4-5].

Phytochemicals, the biologically active compounds found in plants, serve as the foundation for medicinal agents [6]. These compounds, derived from various plant parts, possess a wide range of pharmacological activities, including antimicrobial, antioxidant, anti-inflammatory, and antidiabetic properties [7]. Furthermore, advancements in nanotechnology have opened up new avenues for utilizing plants in medicinal applications. Plant-mediated biological synthesis of nanoparticles is gaining importance due to its economic and eco-friendly nature. This approach allows for the synthesis of metallic nanoparticles from plant extracts, offering potential benefits across various fields, including pharmaceuticals, agriculture, and biotechnology [8]. This paper aims to explore the collection and extraction of active compounds from medicinal plants, focusing on the qualitative and quantitative analysis of phytochemicals. Additionally, it delves into the pharmacological activities of these compounds, particularly in the context of treating diseases like diabetes mellitus. The antioxidant potential of plant extracts is also investigated, considering their role in combating oxidative stress-related diseases.

## MATERIALS REQUIRED:

### Preparation of Plant Powder

Healthy plant samples, free from insect damage and fungal infections, were dried at room temperature for 5-8 days or until they easily broke by hand. Once completely dry, the plants were ground to a fine powder using an electronic blender. The resulting plant powder was stored in closed colored glass bottles at room temperature until needed.

### Solvent Extracts

Fifty grams of the dried plant powder (or dried fruits) were individually soaked with 300 ml of each solvent (at a ratio of 1:6 w/v), including aqueous, petroleum ether, chloroform, acetone, and ethanol, in a Soxhlet apparatus. The extraction process lasted for 48 hours at 310°C until complete extraction was achieved.

The extracted solutions were then filtered through Whatman No.1 filter paper to obtain the final pure extracts. These extracts were collected in labeled sterile universal bottles and stored in a refrigerator at 4°C until further use. Qualitative Phytochemical Screening Method was carried out by [9].

### Synthesis of Nanoparticles:

A 1 millimolar (mM) solution of silver nitrate ( $\text{AgNO}_3$ ) in water was prepared and employed for synthesizing silver nanoparticles. Specifically, 10 milliliters of dried fruit extract from *Illicium verum* Hook. F was combined with 90 milliliters of the 1 mM silver nitrate solution, as depicted in **Figure 1**, to facilitate the reduction of silver ions ( $\text{Ag}^+$ ) over a 24-hour period at room temperature [10].

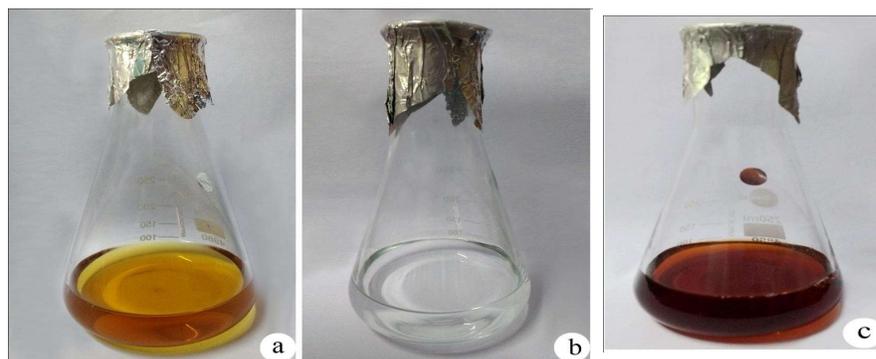


Figure 1: Synthesis of Silver Nanoparticles

- a) Plant extract
- b) 1mM Silver nitrate
- c) Synthesized Silver nanoparticles

### Characterization of Silver Nanoparticles:

Synthesis of silver nanoparticles was determined by the following spectroscopic and analytical methods:

#### UV- Visible Spectrophotometer:

The reduction of silver ions was confirmed by measuring the absorbance of the mixture at

regular intervals at a wavelength of 300- 800 nm.

#### SEM:

Scanning electron microscope analysis, the size and shape of the synthesized nanoparticles was determined by observing the mixture under SEM.

**FTIR:**

FTIR analysis was determined the functional groups present in the synthesized nanoparticles were determined by FTIR analysis. The solution containing nanoparticles was centrifuged at 60,000rpm and the pellet obtained was employed for the study [11].

**Antioxidant activity:**

The radical scavenging potential [12] of the nanoparticles was determined by using DPPH reagent. Solvent served as blank, ascorbic acid was used as control and the test sample were taken at five different concentrations.

DPPH was added to all the tubes and their absorbance was measured at 517nm.

**Anti-diabetics activity:**

The Alpha-Amylase Inhibitory Assay, following a modified procedure by [13], involved placing 1000  $\mu$ L of sample (20-100  $\mu$ g/ml) in a tube and adding 250 $\mu$ L of 0.02M sodium phosphate buffer (pH 6.9) containing  $\alpha$ -amylase solution (0.5mg/mL). After preincubation at 25°C for 10 min, 250 $\mu$ L of 1% starch solution in 0.02M sodium phosphate buffer (pH 6.9) was added at timed intervals and further incubated at 25°C for 10 min. The reaction was stopped by adding 500 $\mu$ L of dinitrosalicylic acid (DNS) reagent, followed by incubation in boiling water for 5 min and cooling to room temperature. Absorbance was measured at 540 nm using a spectrophotometer after dilution with 5mL distilled water. Percentage inhibition was calculated using  $[(\text{Abs control} - \text{Abs compounds}) / \text{Abs control}]$

x 100, with IC50 concentrations determined graphically.

**RESULTS AND DISCUSSION:****Phytochemical Analysis****Qualitative phytochemical screening**

The qualitative phytochemical tests revealed the presence of various phytochemicals in aqueous, acetone, ethanol and chloroform dried fruits extracts of *Illicium verum* Hook. F the chloroform and ethanol dried fruits extracts were showed a high amount of glycosides, steroids, terpenoides. The aqueous and acetone extracts were exhibited a low amount of phenol, flavonoids and alkaloids.

In the present study, the ethanol extracts of *Illicium verum* Hook. F has higher amount of phenolic compounds, saponin and steroids as shown in (Table 1). Presence of alkaloid, steroids, flavonoids, saponines, and triterpinods were earlier reported by [14-15] were found to be alkaloids, flavonoids, and phenolics *etc.* The extraction is the most important step in the analysis of constituents which are presented in herbal preparations. In addition, the strengths and weaknesses of different extraction techniques are discussed.

**Green Synthesis of Silver Nanoparticles****UV Spectrum**

When the desired compound absorbs UV radiation, the absorbed energy molecules cause excitation of the electron, in both atoms and molecules, from lower energy to higher energy

orbital. The plant extract is pale yellow in colour and appeared turbid soon after adding sample. After keeping the solution at the room temperature, the intensity of the colour increased gradually from pale yellow to dark brown at the end of the experiment. Reduction of silver ions present in the aqueous solution of silver nitrate during the reaction with the ingredients of *Illicium verum* Hook. F plant extract has been seen by the UV spectroscopy ranging from 200 to 1100 nm. The maximum absorption was obtained at 398.75 and 450.80 wavelength the proper baseline as shown in **Figure 2**. The UV analysis results are earlier reported in *Solanum nigrum* [16], *Cassia auriculata* [17] and in *Aloe vera* [18].

#### FT- IR Spectrum

The FT-IR measurements were to identify the possible biomolecules responsible for the reduction of the  $\text{Ag}^+$  ions synthesized by plant dried fruits extract of *Illicium verum* Hook. F based on the peaks values in the region of infrared radiation. The biologically synthesized AgNPs were mixed with the potassium bromide to make a pellet. The spectra of the extracts taken after the biosynthesis of nanoparticles were analysed. Absorptions due to stretching and bending of covalent bonds in molecules visible ranges from 400 to 4000  $\text{cm}^{-1}$  nm by using BRUKER optik GmbH spectrometer and the characteristic peaks were recorded. The visible wavelengths typically expressed in nanometres ( $1 \text{ nm} = 1 \times 10^{-9} \text{ m}$ ). In this study

showed different stretches of bonds at different peak values from 500  $\text{cm}^{-1}$  to 4000  $\text{cm}^{-1}$  the spectrum as indicated in **(Figure 3)** indicates major peak at 3856.55  $\text{cm}^{-1}$  The C-Cl stretch group of alkyl halides intensity is strong, O-H stretch group of alcohols intensity is medium, C=O stretch group of ketones intensity is strong, C=O stretch group of amides intensity is medium and O-H stretch group of carboxylic acids compounds intensity is strong, in the extract. It may be concluded that the presence of the higher percentage of alkyl halides group of molecules are responsible for the reduction process and the amides and carboxylic acids linkages in protein are responsible for the stabilization of the particles. The FT- IR analysis results are earlier reported in *Citrullus colocynthis* [19], *Acalypha indica* [20] and *Ocimum sanctum* [21].

#### Scanning Electron Microscope (SEM)

The SEM images of silver nanoparticles were formed due to interactions of hydrogen bond and electrostatic interactions between the bioorganic capping molecules bound to the AgNPs. The nanoparticles were not in direct contact even within the aggregates, indicating stabilization of the nanoparticles by a capping agent [22]. During the process of vaporization, owing to the gas convection, the vaporized smog will rapidly lose its energy and condenses into nanoparticles due to its collision with the

atoms of inert-gas. Along with the nanoparticles will then rush rapidly towards the surface of the very low-temperature collector, which then condense and form into nanoparticles.

The SEM images of synthesized silver nanoparticles from dried fruits extract of *Illicium verum* Hook. F the pores were circular with the same size and pores ranging from 200 to 300 nm diameter with pore to pore distance were clearly observed an average size were estimated to be approximately 95.02 nm. The internal and external structures of all the spherical triangle and truncated triangles were different in pore arrangements. AgNPs were analysed in different magnifications 1  $\mu$  and 2  $\mu$  as indicated in **Figure 4**.

The present study showed that high-resolution SEM results revealed the silver nanostructure with nanoporous material exhibited interesting application in drug delivery and heavy metal adsorbing. The SEM analysis results are earlier demonstrated in *Magnolia kobus* and *Diopyros kaki*, *Terminalia arjuna* and *Marine diatoms* [23].

### Antioxidants Activity

Antioxidants protect cells against damage caused by molecules known as free radicals. The antioxidant effect of dried fruits extracts of *Illicium verum* Hook. F is mainly due to the presence of phenolic compounds such as flavonoids, phenolic acids and tannins.

Numerous studies with plant phytochemicals with antioxidant activity may reduce risk of cancer and improve the health. The dried fruits extract of *Illicium verum* and standard ascorbic acid tested for *in vitro* antioxidant activity using the DPPH –assay method. The IC50 was calculated the graph obtained by plotting the percentage scavenging against concentrations used. The result is shown in **(Table 2)** the free radical scavenging effect of dried fruits extracts and standard on the DPPH was found to be concentration dependent and was in the order of ascorbic sample. The sample showed the antioxidant activity of IC50 value of 122.335  $\mu$ g/ml. The result indicates that the antioxidant activity of sample is higher than the standard ascorbic acid. The extracts showed a higher antioxidant activity than quercetin that could be explained by the presence of other phenolic compounds (phenolic acids, flavanols and flavones), also endowed with a significant antioxidant activity were earlier reported by [24].

### In Vitro Alpha Amylase Inhibitory Assay

The result showed dried fruit extract of *Illicium verum* Hook. F that the sample had better percentage inhibition at high concentrations when compared with standard acarbose **(Table 3)**. The compound showed 75 % activity at concentration 100  $\mu$ g/ml while ascorbic acid gave 79.46 % at the same concentration [25]. were reported the *in vitro*  $\alpha$ -glucosidase inhibitory activity of *Illicium verum* Hook. F dried fruits extract was

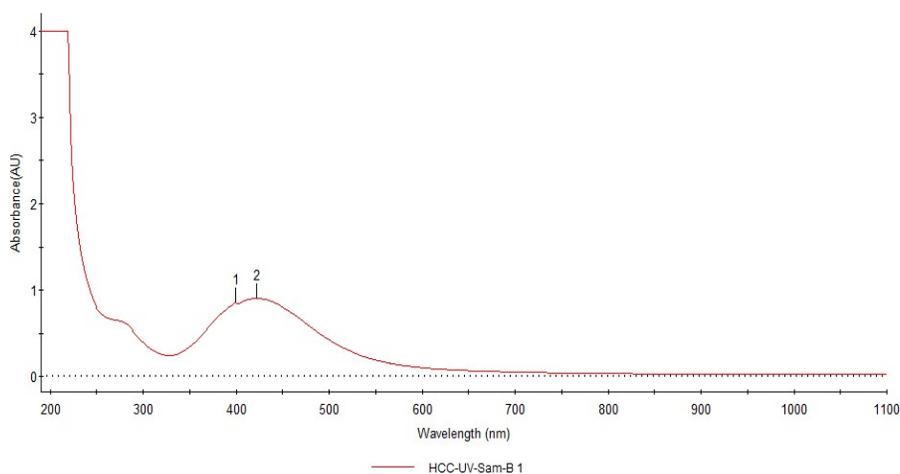
studied as explained in methods. It was found that, there is increase in percentage inhibitory activity with the increase in dosage against  $\alpha$ -glucosidase. As a standard drug, Acarbose was

used with similar dosage to compare inhibitory capacity of the *Illicium verum* Hook. F dried fruits extract.

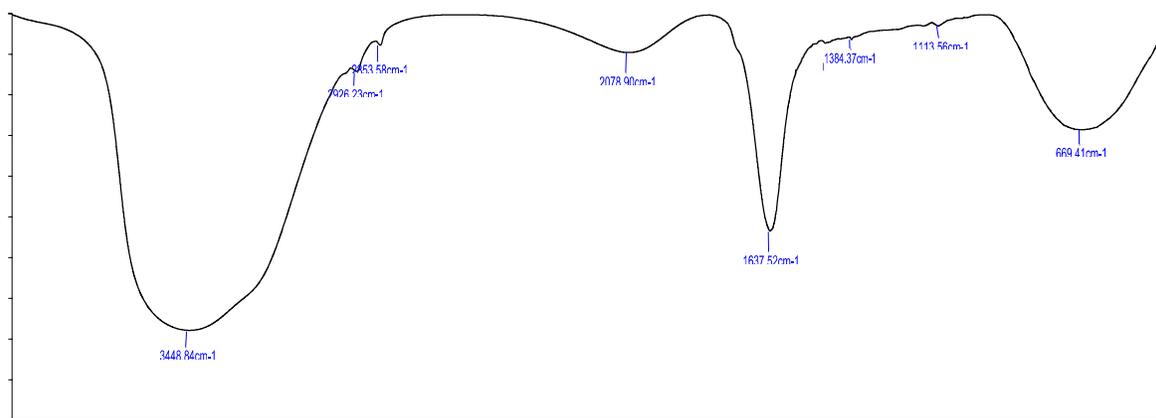
**Table 1: Qualitative phytochemical screening of *Illicium verum* Hook. F**

S. No	Test Name	Solvents			
		Aqueous	Acetone	Ethanol	Chloroform
1.	Alkaloids	+	++	++	+++
2.	Glycosides	+++	+	+++	+++
3.	Saponin	+++	+	+++	+++
4.	Reducing Sugar	+++	+++	++	++
5.	Phenol	+	+++	+++	+++
6.	Steroids	++	+++	+++	+++
7.	Terpenoides	+++	+++	+++	+++
8.	Flavonoids	+++	+++	+	+++

Note: + : Presents of compounds; - : Absents of compounds



**Figure 2: UV Spectrum of Synthesized Silver Nanoparticles**



**Figure 3: FT-IR Spectrum of synthesized silver nanoparticles**

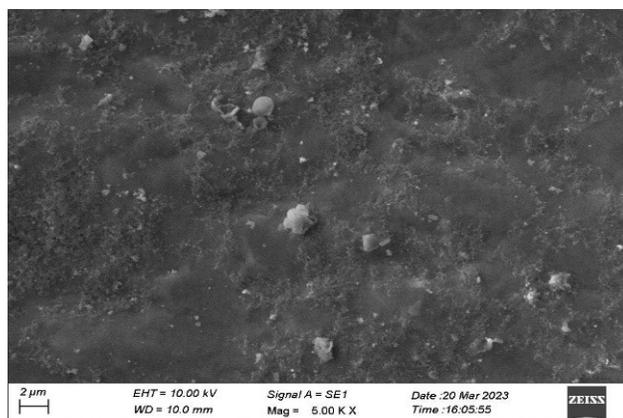


Figure 4: SEM images of Synthesized Silver Nanoparticles

Table 2: Antioxidant activity of the sample using DPPH method and comparison with standard absorbance

S. No.	Concentration( $\mu\text{g/ml}$ )	Standard Absorbance	Sample Absorbance	Sample % of DPPH Scavenged
1.	5	0.281	0.231	17.79
2.	10	0.281	0.225	19.92
3.	20	0.281	0.220	21.71
4.	40	0.281	0.194	30.96
5.	80	0.281	0.168	40.21
IC <sub>50</sub> Value				122.335

Table 3: *In vitro* anti diabetic activity of the sample using alpha amylase method and comparison with standard drug acarbose

S. No.	Concentrations	Alpha amylase (%)	
		Sample B	Acarbose
1.	20 ( $\mu\text{g/ml}$ )	41.96	55.35
2.	40 ( $\mu\text{g/ml}$ )	46.42	59.82
3.	60 ( $\mu\text{g/ml}$ )	48.21	66.07
4.	80 ( $\mu\text{g/ml}$ )	58.92	70.53
5.	100 ( $\mu\text{g/ml}$ )	63.39	79.46
IC <sub>50</sub> Value		53.73	4.89

## CONCLUSION

The findings of this study reveal that the phytochemical analysis of synthesized silver nanoparticles from *Illicium verum* Hook. F fruit extract indicates a high concentration of phenolic compounds. Additionally, the *in vitro* antidiabetic potential of dried fruit extract from *Illicium verum* Hook. F was confirmed through the assessment of its percentage inhibition compared to standard acarbose. Moreover, the methanolic extract of silver nanoparticles from *Illicium verum* was evaluated for its *in vitro* antioxidant activity

using the DPPH-assay method. The concentration-dependent free radical scavenging effect of the silver nanoparticle extract on DPPH was observed. Therefore, further purification to identify the active polyphenol responsible for the antidiabetic activity is warranted, along with conducting *in vivo* studies.

## REFERENCES

- [1] Lahlou, M. 2004. Essential oils and fragrances compounds: bioactivity and mechanisms of action. Flavor Frag J, 19: 159 - 165.

- [2] Cragg, G.M. and Newman, D.J. 2001. Medicinals for the millennia. Ann. New York Acad. Sci, 953: 3 - 25.
- [3] Abosi, A. O., & Raseroka, B. H. (2003). *In vitro* antimalarial activity of *Vernonia amydalina*. *British Journal of Biomedical Scie*, 60, 89-91.
- [4] Jayapriya, E. and Lalitha, P. 2013. Synthesis of silver nanoparticles using leaf aqueous extract of *Ocimum basilicum* (L.) *International Journal of Chetek Research*, 5(6): 2985 -2992.
- [5] Leela, A. and Vivekanandan, M. 2008. Tapping the unexploited plant resources for the synthesis of silver nanoparticles. *African Journal of Biotechnology*, 7: 31662 - 3165.
- [6] Tiwari, P., Kumar, B., Kaur M., Kaur, G. and Kaur, H. 2011. Phytochemical screening and extraction, a review: *Internationale Pharmaceutica Scientia*1, (1): 98 - 106.
- [7] Ravinder S, Devendar Rao K, Shashidhar B, Jayaprakash Reddy G, Ajay D. 2011. Evaluation of antidiabetic activity of *Annona squamosal* linn seed in Alloxan induced diabetic rats. *International Journal of Preclinical Research*, 2(2):100- 106.
- [8] Soniya Choudhary, Babeet Singh Tanwer, Rekha Vijayvergia. Total Phenolics, flavonoids and antioxidant activity of *Tricosanthes cucumeerena* Linn[J] *Drug Inven Today*. 2012;4(5):368–370.
- [9] Brindha, P., Saraswathy, A. and Saraswathy, R. 1981. Phytochemical composition of Pentatropis, Oldenlandia and Plumeria. In proceeding of the National Seminar on Recent Trends in Natural Products Chemistry, 30 - 31.
- [10] Mashau, M. E., Mamagau, T., Foforane, K., Nethathe, B., Ramphinwa, M. L., & Mudau, F. N. (2023). Biosynthesis of Silver Nanoparticles from Fermented Bush Tea (*Athrixia phylicoides* DC) Leaf Extract and Evaluation of Their Antioxidant and Antimicrobial Properties. *Fermentation*, 9(7), 648.
- [11] Latha, M. B., Mani, N., Kavikala, N., Karthika, S., & Rajasudha, V. (2023). Evaluation of Secondary Metabolites of *Ageratina adenophora* and Synthesis of Silver Nanoparticles for its Antibacterial and Antioxidant activity. *Oriental Journal of Chemistry*, 39(1), 102.
- [12] Alothman, M., *et al.* "Antioxidant capacity and phenolic content of selected tropical fruits from Malaysia, extracted with different solvents." *Food Chemistry* 115.3 (2009): 785-788.
- [13] McCue and Shetty, (2004). Inhibitory effects of rosmarinic acid

- extracts on porcine pancreatic amylase *in vitro*, *Asia Pac J Clin Nutr*.13 (1):101-6.
- [14] Vadnere Gautam, P., Pathan Aslam, R. and Kulkarni Bharti, U. 2013. *Diplocyclos palmatus* a Psychopharmacological review. International Journal of Research in Pharmacy and Chemistry, 3(1): 157 - 159.
- [15] Alya Maimoona, Fareeha Iqbal, Qurat-Ul-Ain Syed and Salman Ahmed (2016) Phytochemical analysis and anti-emetic activity of *Illicium verum* Hook. f. fruit by chick emesis model, *Journal of Pharmacognosy and Phytochemistry*,5(6): 185-188
- [16] Asra Praveen, Aashis, S. Roy and Srinath, Rao, 2012. Biosynthesis and characterization of silver nanoparticles from *Cassia auriculata* leaf extract and *in vitro* evaluation of antimicrobial activity. *Int. J. Applied. Biol and Pharma Tech*, 3(2): 222 - 228.
- [17] Rache Deva Kirubai, S., Vedha, B., Panneerselvam, T. and Mukesh Kumar, D.J. 2016. Preparation, characterization and antibacterial studies of silver nanoparticles synthesized by *Solanum nigrum* leaf extract. *The bioscan*, 11(1): 57 – 60
- [18] Chandran, S.P., Chaudhary, M., Pasricha, R., Ahmad, A. and Sastry, M. 2006. Synthesis of gold and silver nanoparticles using *Aloe vera* plant extract. *J. Biotechnol. Prog*, 22: 577 - 583.
- [19] Satyavani, K., Gurudeeban, S., Ramanathan, T. and Balasubramanian, T. 2011. Biomedical potential of silver nanoparticles synthesized from calli cells of *Citrullus colocynthis* (L.) Schrad. *Echrad. Asian J. Biotechnol*, 9: 43.
- [20] Krishnaraj, C., Jagan, E. G., Rajasekar, S., Selvakumar, P., Kalaichelvan, P. T. and Mohan, N. 2010. Synthesis of silver nanoparticles using *Acalypha indica* leaf extracts and its antibacterial activity against water borne pathogens. *Colloids and Surfaces B: Biointerfaces*, 76: 50 - 56.
- [21] Ramteke, C., Chakrabarti, T., Sarangi, B. K. and Pandey, R.A. 2013. Synthesis of silver nanoparticles from the aqueous extract of leaves of *Ocimum sanctum*. *J. Chem*, 7.
- [22] Priya, M.M., Selvia, B.K. and Paul, J.A.J. 2011. Green synthesis of silver nanoparticles from the leaf extracts of *Euphorbia hirta* and *Nerium indicum*. *Dig J Nanomater Bios*; 6: 869 - 877.

- [23] Gnanamoorthy, P., Karthikeyan, V. and Ashok Prabu, V. 2014. Field emission scanning electron microscopy (FESEM) characterization of the porous silica nanoparticulate structure of marine diatoms. *J. Porous. Mater*, 21: 225 - 233.
- [24] Ibrahim S Majali (2022) Antioxidant and Anti-Inflammatory Activity of Star Anise (*Illicium Verum*) in Murine Model. *Biomed Pharmacol J*; 15(2).
- [25] Kalaivanam K.N, Dinesha R, Santhosh Kumar N (2018) Antioxidant and Anti-Diabetic activities of Polyphenol-enriched Star Anise (*Illicium verum*) seeds extract, *International Journal of Biotechnology and Biochemistry* ISSN 0973-2691 Volume 14, Number 2 (2018) pp. 77-84.