Biomedical Applications Of Novel Green Biomaterial Synthesized From Endophytic Bacteria *Cronobacter Sakazakii*

Love Edet M  
B. S Abdul Rahman Crescent Institute of Science and Technology

Hemalatha S (✉ hemalatha.sls@bsauniv.ac.in)  
B. S Abdul Rahman Crescent Institute of Science and Technology  
https://orcid.org/0000-0002-8150-7721

Research Article

**Keywords:** Endophytic Bacteria, Cronobacter sakazakii, Nyctanthes arbor-tristis, Biomaterial, Antimicrobial activity, Reactive oxygen species (ROS), Anti-arthritis,

**Posted Date:** December 21st, 2021

**DOI:** https://doi.org/10.21203/rs.3.rs-1185108/v1

**License:** ☕️ This work is licensed under a Creative Commons Attribution 4.0 International License. Read Full License
Abstract

Endophytic bacteria from the flower of *Nyctanthes arbor-tristis* was isolated and identified as *Cronobacter sakazakii*. The supernatant of the biomass of this endophyte was utilized for biomaterial synthesis which was confirmed by a change in colour and characterized by using UV-Visible spectrophotometer, FTIR, FESEM, EDAX, Zeta potential and DLS. The antibacterial efficacy of the biomaterial against Gram positive and Gram negative bacteria was determined via agar well diffusion, MIC, MBC and biofilm formation assays. The antioxidant and anti-arthritic assays were also performed. The result reveals antibacterial effect of biomaterial against the multi-drug-resistant *E. coli*, *S. aureus* and *K. pneumonia* strains. This report is the first to reveal the isolation and identification of endophytic bacteria from the flower of *Nyctanthes arbor-tristis*, alongside its biomaterial synthesis, characterization and biomedical applications. Our result implies that the synthesized biomaterial can be utilized as an effective antimicrobial, antioxidant and anti-arthritic agent and can be incorporated in pharmaceutical formulation for drug development.

1. Introduction

The curiosity of man to understand and enjoy his natural habitat in a unique way has always led to diverse invention in all spheres of life. One of the most amazing technologies that have been of great benefits in various facets for over a decade now is Nanotechnology.

Nanotechnology is fast becoming, if not, the most dynamic area of research due to the shape and size of these structures at the nanoscale level, hence showing a completely novel characteristics. Metallic nanoparticles (MNPs) synthesized from various metals such as Copper (Cu), Silver (Ag), Aluminium (Al), Gold (Au), possess unique physiochemical properties which have drawn the attention of researchers due to their usefulness in various biotechnical application in biomedicine, agriculture, environmental remediation, optical and electronic field as well as usage in drug delivery and bioimaging [1, 2]. Of all MNPs, AgNPs have been outstanding as the most sought after noble metal due to its wide range of biological activities especially in their ability to combat a wide spectrum of bacteria and fungi without any harm to animal cells [3–6].

Bacteria or microorganisms found inside the internal organs or tissues of plants in a mutualistic relationship with little or no adverse effect to the host are called endophytes, many of which secretes biologically active compounds [7, 8], enabling the host plant to develop resistance against pathogens and are also used in pharmaceutical industries as antibiotics, anticancer, antiviral, anti-diabetics and other bioactive compounds [9].

*Nyctanthes arbor-tristis* (NAT) a deciduous tree from the family Oleacea, also generally known as ‘Night Jasmine’ or ‘Harsinghar’ due to the fact that its flowers emanate a very strong and pleasant fragrance during the whole night [11]. Various parts of the plant have showed quite a number of pharmacological activities such as, hepatoprotective, anti-leishmaniasis, anti-viral, antifungal, anti-pyretic, anti-histaminic,
anti-malarial, anti-bacterial, anti-inflammatory, anti-oxidant activities, immunomodulation, CNS modulatory anti-allergic, anti-cancer, diuretic as well as used as hair tonic [12].

The rapid emergence of multiple drug resistant strains of pathogens to current antimicrobial agents, leading to severe bacterial infections is due to their ability to counter the biocidal activity of antibiotics, therefore the urgent need to develop new antibiotics.

Statistics shows that about 1 percent of the world’s population suffers from rheumatoid arthritis (RA) with a reduction of life span by 10-15 years. This systemic autoimmune disorder is portrayed by severe joint inflammation, destruction of cartilage, synovial proliferation and protein tissue denaturation. Medications such as disease modifying anti-rheumatoid drugs (DMARDS) and non-steroid anti-inflammatory drugs (NSAIDS) such as diclofenac sodium and ibuprofen are commonly used as treatment of this disorder usually results in gastrointestinal ulcer, hepatotoxicity, renal failure among many others after a period of administration [13, 14].

ROS produced during biochemical processes plays important role in the prevalence of several disease conditions. Oxidative stress a common effect of ROS damages cell membrane such as the proteins and extracellular matrix resulting in inflammation [15]. Excessive release of these ROS such as superoxide anion (O2-) is found in high concentration in the site of inflammation [16]. Hence agents that can scavenge ROS as well as inhibits tissue protein denaturation and inflammations associated with RA are needed. The use of natural product in synergy with AgNPs has the potency to give a more effective and comparable result which is less toxic with little or no side effects.

The current study is focused on the isolation of endophytic bacterium from the flower part of *Nyctanthes arbor-tristis*, its identification, biogenic biomaterial synthesis, characterization as well as its biomedical applications as antimicrobial, antioxidant and anti-arthritic agents. Till date, there is no report on the endophytic isolation, identification and biomaterial synthesis from the flowers of *Nyctanthes arbor-tristis* making this the first report to relate this information.

2. Materials And Methods

2.1 Isolation and Identification of Endophytic bacteria

Flowers of *Nyctanthes arbor-tristis* were collected from BSAIST, Vandalur Chennai. After rinsing with distilled water, the flowers were surface sterilized with 70% ethanol for 1 minute followed by 0.1% Sodium hypochlorite solution for 1 minute then 70% ethanol again for 1 minute and finally washed with sterile distilled water and blot dried in laminar air flow chamber. After which the specimens were incubated into nutrient agar plate and incubated at 37°C in an incubator. Endophyte growth was observed after 24 hours, which was further subcultured in a Luria-Bertani broth and pure culture was maintained.

After an overnight incubation of bacteria culture in LB, DNA isolation was carried out by Lysis method [17]. PCR amplification of 16S rRNA was achieved by using gene specific primers using the isolated
genomic DNA as a template, while (5’AGAGTTTGATCC TGGCTCAG 3’ is used as forward primer and 5’ GGTTACCTTGTTACGACTT 3’ as reversed primers. The amplification reaction was carried out using a Master Cycler [18]. The PCR products were further resolved in 1.5% agarose gel, stained with 10 µg/ml ethidium bromide and ran at 65v for 20 minutes, then visualized in a gel documentation system. PCR amplicon purified, and sequenced and specie was identified by homology search using BLASTn (http://blast.ncbi.nlm.nih.gov/Blast.cgi) database at NCBI. The molecular phylogenetic analysis was done using MEGA X [19].

2.2 Synthesis and Characterization of Biomaterial

Isolated endophytic Cronobacter sakazakii was inoculated in 100ml of LB and kept in rotatry shaker for 24 hours at 37°C and 110rpm. The biomass was centrifuged at 10000rpm for 10 minutes after which the supernatant was collected and mixed with aqueous extract of Silver Nitrate (AgNO₃) solution in 1:2 ratio and incubated in dark condition for 2-5 days.

After formation of nanoparticles, the solution was centrifuged at 10000 rpm for 10 minutes, the pellets were collected and dried in hot air oven to obtain a fine powder for further studies. The synthesized biomaterial from Cronobacter sakazakii (CSB) was characterized by UV-Vis spectroscopy, FTIR, FESEM, Zeta potential, EDX, DLS to analyze different physiochemical features of the synthesized biomaterial CSB.

2.3 Antibacterial Screening

Antibacterial activity of CSB was performed via Agar well diffusion, Minimum inhibitory concentration (MIC), Minimum bactericidal concentration (MBC) and Biofilm assay, using Gram negative clinical strains of E. coli (strain E1 and E2) obtained from Tagore Medical College and Hospital, Chennai with proper ethical clearance (Ref. No. BSAU: REG-OFF: 2016/02SLS), E.coli ATCC (25922), K.pneumoniae ATCC (700603) and Gram positive S. aureus MTCC (1430), Methicillin-resistant Staphylococcus aureus (MRSA) ATCC (35591) and clinical strain of S. aureus (strain S1).1mg of the CSB was dissolved in 1000µl of distilled water, this solution was then used to carry out the antimicrobial assays. Ampicillin was used as standard drug.

2.4 Antioxidant Assay

2.4.1 DPPH Assay: The free radical scavenging activity of CSB under in-vitro condition was carried out on the basis of its scavenging activity of stable1,1-diphenyl-2-picrylhydrazyl (DPPH) assay [20]. Ascorbic acid was used as standard. After incubation, antioxidant activity was determined by measuring in absorbance at 517nm. The experiment was carried out in triplicates.

2.4.2 Fe reduction assay

To measure the reductive power of the synthesized AgNPs, we investigated the transformation of Fe³⁺ to Fe²⁺ using standard protocol [21]. The absorbance was measured at 700nm. Ascorbic acid was used as
standard in the same concentration and blank was done without sample. The experiment was carried out in triplicate.

2.5 Anti-Arthritic Activity

This assay was determined by evaluating the anti-inflammatory ability of the CSB through membrane stabilization assay and protein denaturation assay.

2.5.1 Membrane stabilization by Heat Induced Hemolysis

Following the procedure [22] with minor modification, diclofenac sodium (DFS) served as standard drug. Absorbance was measured at 560nm. The experiment was carried out in triplicate.

2.5.2 Protein Denaturation

Inhibition of Egg Albumin Denaturation

With minor modification of the procedure [23], inhibition of egg albumin denaturation by CSB was assessed. DFS was used as standard and absorbance was measured at 660nm. The experiment was done in triplicate.

Inhibition of Bovine Serum Albumin (BSA) Denaturation

Experiment was done in triplicate [24] to check BSA denaturation by CSB. DFS was used as standard drug. Absorbance of the solution was measured spectrophotometrically at 660nm.

3. Results And Discussion

3.1 Identification of endophytic bacteria

The endophytic bacteria was isolated from NAT and identified by 16S rRNA sequencing as Cronobacter sakazakii (Accession no. MN814025) and was deposited in NCBI, GenBank. Fig. 1 shows the original phylogenetic tree of Cronobacter sakazakii with other closely related species. Evolutionary analysis was carried out by using MEGA X and the history inferred by neighborhood-joining method [25]. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Maximum Composite Likelihood method and are in the units of the number of base substitutions per site [19].

3.2 Synthesis and Characterization of Biomaterial

In this study, it was observed that the solution containing the supernatant of endophytic bacteria and AgNO3 gradually changed into brownish red (Fig. 2a), this change in colour intensity proven the reduction of metallic Ag to Ag ions and confirmed the synthesis of biomaterial CSB. Hence, colour change in the solution is a notable and important evidence to establish the formation of mediated CSB [26, 27].
Krishnaraj, C, Jagan, EG, Rajasekar, S, Selvakumar, P, Kalaichelvan, PT, Mohan, N: Synthesis of silver nanoparticles using Acalyphaindica leaf extracts and its antibacterial activity against water borne pathogens. Colloids Surf B Biointerfaces76,50–56 (2010)

35.Rajeshkumar, S, Kannan, C, Annadurai, G: Synthesis and characterization of antimicrobial silver nanoparticles using marine brown seaweed Padina tetrastromatica. Drug Invention Today 4, 511–513 (2012)

The biomaterial synthesis was also confirmed by UV-Vis absorption spectrum at 420nm (Fig. 2b), which was stable even after 1 month. Due to the abundance of free electrons, metallic nanoparticles move through conduction and valence bond responsible for surface plasmon resonance (SPR) absorption band [28]. SPR hence revealed the optical absorption spectrum which further exposes the band pattern likely associated with the size, shape, particle aggregation and distribution. The absorbance of synthesized CSB at 420nm is in compliance with earlier studies [29, 30].

This analysis revealed the diverse chemical bonds and organic compounds present in the CSB. The endophytic bacteria mediated CSB showed different absorbance ranging from 400 to 4000cm\(^{-1}\) (Fig. 2c). Major peak is seen at 3255cm\(^{-1}\), which is a broad band in the single bond area confirming the presence of hydrate (H2O), hydroxyl (OH), ammonium, or amino. Another predominate peak at 2921cm\(^{-1}\) confirmed aliphatic compounds. In the triple bond region, a peak is identified at 2111cm\(^{-1}\) confirmed the alkyne group. Also, presence of aromatic compounds is identified in the double bond region at peak 1992cm\(^{-1}\), 1883cm\(^{-1}\) and 1628cm\(^{-1}\). Peaks seen in the fingerprint regions are 1027cm\(^{-1}\), 635cm\(^{-1}\) and 427cm\(^{-1}\). From this analysis, the functional groups present in CSB are: hydroxyl (H bonded and OH stretched), alcohol (C-O stretch), carbonyl (C=O), alkene (>CH2, Methylene C-H asymmetric and Cyclohexane ring vibrations), alkyne (C≡C and C-H bend), amine (N-H), Isothiocyanate (-NCS), Open-chain azo (-N=N-), Thioethers, CH3-S-(C-S stretch) and transition metal carbonyls [31,32].

Synthesized CSB was also morphologically analyzed. A wide variety of information ranging from the sample surface, shape and size were obtained using a higher resolution and a much greater energy range via Field Emission Scanning Electron Microscopy (FESEM). In this technique the size of CSB was revealed a range from 1µm to 200nm with the shape being predominantly semi-spherical while some are seen as rod-shaped which confirmed the shape of a typical Cronobacter sakazakii mediated CSB (Fig. 2d)

EDAX analysis provides information on the relative presence and amount of elements that are found in the CSB. EDAX analysis of the synthesized CSB showed several peaks of Ag, C, O, N, Cl, Na and S at different percentage as showed in the fig. 2e and Table 1. Silver showed the highest peak at 3keV which is normal for AgNPs absorption due to SPR and further confirms the formation of CSB [33]. The
The percentage amount of C and O is a proof that organic substances are attached to the CSB in the form of bio-capping.

Table 1: Percentage weight and atom distribution of CSB in EDAX analysis

| Atoms | Weight% | Atom% |
|-------|---------|-------|
| Ag    | 40.61   | 8.52  |
| C     | 23.11   | 43.55 |
| O     | 16.34   | 23.11 |
| N     | 11.51   | 18.59 |
| Cl    | 5.28    | 3.37  |
| Na    | 2.28    | 2.24  |
| S     | 0.87    | 0.62  |

The hydrodynamic or intensity mean size (Z-average) and polydispersity (PDI) index value of the endophytic biomass mediated synthesized CSB was determined via DLS (Fig. 2f). The CSB has a size distribution of 1152nm with a PDI of 0.380. The scale for PDI ranges from 0-1 with 0 as monodispersed and 1 as polydispersed. PDI of the CSB is less than mid-value and can be considered as acceptable.

Zeta potential of the synthesized CSB is showed at -4.24mV (Figure 2g). This value implies an excellent degree of coagulation which increases the stability of the CSB [34].

3.3 Antibacterial Screening

In agar well diffusion assay, a clear zone of inhibition measured as 19mm, 20mm and 20.6mm was observed in Gram positive *S. aureus* strain 1, MRSA and MTCC *S. aureus* respectively, also 14.7mm, 14.7mm, 14mm and 12.3mm zone of inhibition was observed in Gram negative *E.coli* strains 1 and 2, ATCC *E.coli* and *K.pneumonia* respectively, all containing 100µg/ml CSB. The wells containing 25µg/ml and 50µg/ml of CSB also showed noticeable amount of inhibition as noted in Table 2. When compared to the wells containing standard drug, E2, *K.pneumoniae*, and all S1 strains were completely resistant (R) to the standard drug hence no inhibition was observed. Previous studies have assessed antibacterial potential of the flowers of NAT in terms of zone of inhibition using various extracts but at higher concentrations [35-37]. Therefore synthesized CSB can be considered as a potent antimicrobial agent as it is able to inhibit the growth of both positive and negative Gram bacteria.

Table 2: Zone of inhibition was studied in control and treated with CSB in Gram positive and Gram negative standard and pathogenic antibiotic resistant strains
In MIC assay, as shown in fig. 3a, CSB inhibited the growth of *E.coli* strains when compared to the standard drug Ampicillin. This study is in agreement with findings that showed the antibacterial activity of the flowers of NAT [35-37]. The lowest concentration at which visible growth of Gram negative human bacteria *E.coli* strains 1 and 2, ATCC *E.coli* and *K.pneumonia* were inhibited at 24 hours of culture was seen at 1.5625µg/ml, 12.5µg/ml, 25µg/ml and 6.25µg/ml which gave percentage inhibition of 47.5%, 65.3%, 57.1% and 55.6% respectively. While the standard drug at 25µg/ml inhibited human *E.coli* strain 1 at 41.9%, strain 2 at 18.7%, ATCC *E.coli* at 42.4% and *K. pneumonia* at 15.84%. Also, same fig. 3a showed minimal inhibition of Gram positive bacteria of human *S. aureus* strains 1, MRSA and MTCC *S. aureus* at concentration of 1.5625µg/ml, 6.25µg/ml with the percentage inhibition of 72%, 53% and 57.4% respectively. While the percentage inhibition of ampicillin at 25µg is calculated as 53%, 80.7% and 43.7% for human *S. aureus* strains 1, MRSA and MTCC *S. aureus* respectively. Furthermore when the concentration of CSB was increased to the same concentration with ampicillin, that is 25µg, an increase in inhibition was observed.

MBC assay revealed the concentration at which pathogenic bacteria are completely killed. Our result (Table 3) revealed that bactericidal concentration of CSB with *S. aureus* human strain 1 having the lowest concentration of 6.25µg/ml and MTCC *S. aureus* having the highest concentration of 100µg/ml. This is much more comparable to the standard drug that showed bactericidal effect for only human *E.coli* strain 1.

From the biofilm assay result (Fig. 3b) production of biofilm in the MIC is quite high yet comparable to the standard drug. High percentage of biofilm formation implies low inhibitory effects. Comparing the percentage formation of CSB at 25µg/ml to the same concentration of standard drug, it was observed that there is low percentage of biofilm formation in strains treated with CSB than the standard drug ampicillin.
From the above antibacterial screenings, it can be stated that the antibacterial effects of biogenic CSB is high when compared to standard, this can be attributed to the size and large surface area of the CSB which gives better interaction that enables it penetrate cell wall and nuclear content of bacteria [38, 39]. Release of silver ions on to the surface of bacterial cell membrane is also known to disrupt the permeability of the membrane and DNA replication [33]. Also, studies have established that nanoparticles especially silver demonstrate a strong inhibition for most drug resistant Gram-positive and Gram-negative bacteria [40-42]. It is important to also note that increase in the concentration of CSB proffered significant increase in antibacterial activity. According to our research no previous work has been done to establish Cronobacter sakazakii or its synthesized nanoparticle as a potent source of antibiotics, making this study the first while opening the ground for further research. The ability of novel CSB to inhibit the production of biofilm formed by pathogenic bacteria and its potential as antibacterial agent in different assays established its multifaceted ability to combat microbial infectious hence useful for anti-biolm drug development.

Table 3: Minimum inhibitory concentration and Minimum bactericidal concentration of bacteria strains treated with CSB

| Bacteria Strains          | Concentration (µg/ml) | MIC    | MBC    |
|---------------------------|-----------------------|--------|--------|
| *E.coli* strain 1         | 1.5625                | 25     |        |
| *E.coli* strain 2         | 12.5                  | NA     |        |
| *E.coli* ATCC (25922)     | 25                    | 50     |        |
| *S.aureus* strain 1       | 1.5625                | 6.25   |        |
| MRSA ATCC (35591)         | 6.25                  | 12.5   |        |
| *S. aureus* MTCC (1430)   | 6.25                  | 100    |        |
| *K. Pneumoniae* ATCC (700603) | 6.25               | 50     |        |

3.4 Antioxidant Assay

3.4.1 DPPH Assay: The DPPH scavenging ability of AgNPs and standard ascorbic acid was determined spectrophotometrically. CSB showed DPPH scavenged ability of 81.03% which is comparable with ascorbic acid 99% at the highest concentration of 300µg/ml. (Fig. 4a). In this study, synthesized CSB demonstrated to possess antiradical activity which can be attributed to diverse bioactive metabolites present in endopytes acting as capping agent in nanoparticles [20]. DPPH is a stable free radical known for playing role in the reduction of accepting hydrogen or electron from donors.

3.4.2 Reducing Power: The reducing power of CSB and standard ascorbic acid is determined by an increase in absorbance (y-axis) as concentration (x-axis) also increases (Fig. 4b). Values of absorbance
are seen in a dose-dependent manner, at 100µg/ml, AgNPs gave an absorbance of 0.817 which is comparable to that of ascorbic acid. This means that the synthesized CSB is able to react with potassium ferricyanide (Fe$^{3+}$) to form potassium ferrocyanide (Fe$^{2+}$), which in turn reacts with ferric chloride to form ferric ferrous complex [21].

Natural plants are known to possess polyphenolic compounds responsible for antioxidant activity and previous phytochemical screening showed the presence of these polyphenolic compounds in the flowers of NAT [37]. Different flower extracts of NAT has also showed antioxidant activity [37, 43]. These bioactive compounds in plant and endophytes can donate hydrogen to free radicals thereby disrupt the free radical chain reaction by acting as CSB-capping [44], making the synthesized CSB served as hydrogen donors and reducing agents as seen in this study. This further contributes to the high antioxidant potency of CSB in low concentration making CSB fulfilled one of the factors for a substance to be called antioxidant [45].

3.5 Anti-Arthritic Assay

3.5.1 Membrane Stabilization: Stabilization of membrane red blood cells by CSB was observed in a dose dependent manner (Fig. 5a). At low concentration of (50µg/ml), CSB exhibited 41.6% inhibition while diclofenac sodium exhibited 35.7% inhibition. However, at higher concentration of 300µg/ml AgNPs exhibited 79.6% inhibition while diclofenac sodium exhibited 77.8%. Membrane of the red blood cell is analogous to the membrane of the lysosome, this suggests that the ability of the CSB to stabilize the red blood cell is an indicator that it can also stabilize the lysosomal membrane in inflammatory condition. Hence, the in-vitro anti-inflammatory assay by heat induced method showed that CSB has the ability to act as a lysosomal membrane stabilizer therefore can limit the process of inflammation and in turn can prevent the release of neutrophils which causes tissue irritation and damage [14]

3.5.2 Protein Denaturation: Inhibition of BSA protein denaturation by CSB was observed in a dose dependent manner at low concentration (50µg/ml), CSB exhibited 25% inhibition while diclofenac sodium exhibited 42.5% inhibition. However, at higher concentration (300µg/ml) CSB exhibited 72.5% inhibition same as the standard drug (Figure 5c). In egg albumin denaturation, percentage inhibition at 300µg/ml was noted as 70% which is higher than that of the standard drug (58.5%) (Fig. 5b). One of the major causes of inflammation in RA condition is tissue protein denaturation, which results in the destabilization of the structures especially the secondary and tertiary structure of protein leading to severe joint pain [46]. After denaturation, antigens are expressed resulting in type III hypersensitivity leading to RA [14]. Hence, agents that prevent denaturation can be useful for anti-arthritis drug development. The synthesized CSB can be incorporated in RA drug formulation to prevent denaturation and ultimately help to cure RA without any side effects.

4. Conclusion
Isolation of endophytic bacteria *Cronobacter sakazakii* from the flower of NAT was carried out for the first time alongside its AgNPs biosynthesis which was stable and confirmed via change in colour, UV-visible spectrophotometer, FTIR, SEM, EDAX and Zeta potential. The antibacterial effect of the synthesized CBS against the strains of Gram positive and Gram negative bacteria demonstrates that it can be used to control human pathogens as an alternative to antibiotics. Also this *in-vitro* antioxidant and anti-inflammatory studies revealed the potency of synthesized CSB as an agent to combat severity of ROS and inflammation. Further studies to determine its effects against enzymes and cytokines mediating inflammation process will be done in the future.

**Statements And Declarations**

**Acknowledgement**

LEM acknowledges MediScience International, UK, for providing scholarship for study. All authors thank B.S.Abdur Rahman Crescent Institute of Science and Technology for providing the facilities.

**Funding**

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors

**Authors’ contribution**

SH conceived the idea, edited the manuscript. LEM conducted the experiment, analyzed data and prepared manuscript

**Declaration of Competing Interest**

The authors declared no conflict of interest

**References**

1. M. Bilal, T, Rasheed, H.M.N. Iqbal, H. Hu, X. Zhang, Silver nanoparticles: Biosynthesis and antimicrobial potentialities. Int. J. Pharmacol. 13, 832–845 (2017). https://doi.org/10.3923/ijp.2017.832.845

2. A. Sharma, S. Sharma, K. Sharma et al., Algae as crucial organisms in advancing nanotechnology: a systematic review. J. Appl. Phycol. 28, 1759–1774 (2015). https://doi.org/10.1007/s10811-015-0715-1
3. J.L. Elechiguerra, J.L. Burt, J.R. Morones, A. Camacho-Bragado, X. Gao, H.H. Lara, M.J. Yacaman, Interaction of silver nanoparticles with HIV-1. J. Nanobiotechnol. 3, 6 (2005). https://doi.org/10.1186/1477-3155-3-6

4. S.Y. Yeo, H.J. Lee, S.H. Jeong, Preparation of nanocomposite fibers for permanent antibacterial effect. J. Mater. Sci. 38, 2143–2147 (2003). https://doi.org/10.1023/A:1023767828656

5. A.B. Lansdown, A pharmacological and toxicological profile of silver as an antimicrobial agent in medical devices. Adv. Pharmacol. (2010). https://doi.org/10.1155/2010/910686

6. K. Chaloupka, Y. Malam, A.M. Seifalian, Nanosilver as a new generation of nanoproduct in biomedical applications. Trends Biotechnol. 28, 580–588 (2010). https://doi.org/10.1016/j.tibtech.2010.07.006

7. N.K. Ravindra, C.V. Vijay, S. Gary, E. David, The endophytic fungal complex of Catharanthus roseus (L.) G. Don. Current Science 95, 228–233 (2008). https://www.jstor.org/stable/24103051

8. O. Liarzi, P. Bucki, S. Braun Miyara, D. Ezra, Bioactive Volatiles from an Endophytic Daldinia cf. concentrica Isolate Affect the Viability of the Plant Parasitic Nematode Meloidogyne javanica. PLoS One 11, e0168437 (2016). https://doi:10.1371/journal.pone.0168437

9. M. Singh, A. Kumar, R. Singh, K. D. Pandey, Endophytic bacteria: a new source of bioactive compounds. 3 Biotech 7, 315 (2017). https://doi:10.1007/s13205-017-0942-z

10. D.J. Newman, G.M. Cragg, Natural products as sources of new drugs over the last 25 years. J. Nat. Prod. 70, 461–477 (2007). https://doi.org/10.1021/np068054v

11. K. Ashwani, R. Beenu, T. Vani, Systemic Review on Anti-Sciatica Plant “Night Jasmine” (Nyctanthes arbor tristis Linn.). Int. J. Curr. Microbiol. App. 6, 101 – 1035 (2017). https://doi.org/10.20546/ijcmas.2017.606.118

12. J. Agrawal, A. Pal, Nyctanthes arbor-tristis Linn—a critical ethnopharmacological review. J. Ethnopharmacol. 146, 645–658 (2013). https://doi.org/10.1016/j.jep.2013.01.024

13. D.D. Obiri, N. Osafo, P.G. Ayande, A.O. Antwi, Xylopia aethiopica (Annonaceae) fruit extract suppresses Freund's adjuvant-induced arthritis in Sprague-Dawley rats. J. Ethnopharmacol. 152, 522-31 (2014). https://doi.org/10.1016/j.jep.2014.01.035

14. R.A. El-Shiekh, M.A. Salem, S.M. Mouneir, A. Hassan, E. Abdel-Sattar, A mechanistic study of Solenostemma argel as anti-rheumatic agent in relation to its metabolite profile using UPLC/HRMS. J. Ethnopharmacol. 265, 113341 (2021). https://doi.org/10.1016/j.jep.2020.113341

15. C.A. Hitchon, H.S. El-Gabalawy, Oxidation in rheumatoid arthritis. Arthritis Res. Ther. 6, 265-78 (2004). https://doi.org/10.1186/ar1447

16. A. Mirshafiey, M. Mohsenzadegan, The role of reactive oxygen species in immunopathogenesis of rheumatoid arthritis. Iran J. Allergy Asthma Immunol. 7, 195-202 (2008).

17. A.M. Shariq, R. Soundhararajan, T. Akther, M. Kashif, J. Khan, M. Waseem, H. Srinivasan, Biogenic AgNPs synthesized via endophytic bacteria and its biological applications. Environ. Sci. Pollut. Res. 26, 26939-26946 (2019). https://doi.org/10.1007/s11356-019-05869-6
18. M. Umar, N. Fathia, M.S.H.N. Mohammed, S. Hemalatha, Modified cement composites for protection against microbial induced concrete corrosion of marine structures. Biocatal. Agric. Biotechnol. 20, 101192 (2019). https://doi.org/10.1016/j.bcab.2019.101192.

19. S. Kumar, G. Stecher, M. Li, C. Knyaz, K. Tamura, MEGA X: Molecular Evolutionary Genetics Analysis across computing platforms. Mol. Biol. Evol. 35, 1547-1549 (2018). https://doi.org/10.1093/molbev/msy096

20. T. Akther, M. Vabeiryureilai, S.K. Nachimuthu, M. Davoodbasha, H. Srinivasan, Fungal-mediated synthesis of pharmaceutically active silver nanoparticles and anticancer property against A549 cells through apoptosis. Environ. Sci. Pollut. Res. Int. 26, 13649-13657 (2019). https://doi.org/10.1007/s11356-019-04718-w

21. N.R. Bhalodia, P.B. Nariya, R.N. Acharya, V.J. Shukla, in vitro antioxidant activity of hydro alcoholic extract from the fruit pulp of Cassia fistula Linn. Ayu. 4, 209-14 (2013). https://doi.org/10.4103/0974-8520.119684

22. N. Sen, L. Bulbul, F. Hussain, M.T. Amin, Assessment of thrombolytic membrane stabilizing potential and total phenolic content of Typha elephantine Roxb. J. Med. Plant Res. 10, 669-75 (2016). https://doi.org/10.5897/JMPR2016.6154

23. R. Gogoi, R. Loying, N. Sarma, T. Begum, S.K. Pandey, M. Lal, Comparative Analysis of In-Vitro Biological Activities of Methyl Eugenol Rich Cymbopogon khasianus Hack., Leaf Essential Oil with Pure Methyl Eugenol Compound. Curr. Pharm. Biotechnol. 21, 927-938 (2020). https://doi.org/10.2174/1389201021666200217113921

24. P. Sneha, S. Sheela, D.B. Manickam, in-vitro studies of bio-silver nanoparticles in cytotoxicity and anti-inflammatory. J. Complement Med. Alt. Healthcare 7, 555718 (2018). https://doi.org/10.19080/JCMAH.2018.07.555719

25. K. Tamura, M. Nei, Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. Mol. Biol. Evol. 10, 512-526 (1993). https://doi.org/10.1093/oxfordjournals.molbev.a040023

26. A.L. González, N. Cecilia, J. Beránek, A.S. Barnard, Size, Shape, Stability, and Colour of Plasmonic Silver Nanoparticles. J. Phys. Chem. C 118, 9128-9136 (2014). https://doi.org/10.1021/JP5018168

27. S. Ponarulselvam, C. Panneerselvam, K. Murugan, N. Aarthi, K. Kalimuthu, S. Thangamani, Synthesis of silver nanoparticles using leaves of Catharanthus roseus Linn. G. Don and their antiplasmodial activities. Asian Pac. J. Trop. Biomed. 2, 574–580 (2012) https://doi.org/10.1016/S2221-1691(12)60100-2

28. P. Mulvaney, Surface plasmon spectroscopy of nanosized metal particles. Langmuir 12, 788–800 (1996). https://doi.org/10.1021/la9502711

29. R. Ubaid, K.M. Saroj, S. Hemalatha, Effect of biosynthesized copper nanoparticle(CuNPs) on growth and biofilm formation in fluconazole resistant Candida albicans. J. Microbiol. Biotechnol. Food Sci. 9, 21-24 (2019). https://doi.org/10.15414/jmbfs.2019.9.1.21-24
30. J.L Huang, B.Q. Li, D.H. D.H. Sun, Y.H. Lu, Y.B. Su, X. Yang, H.X. Wang, Y.P. Wang et al., Biosynthesis of silver and gold nanoparticles by novel sundried cinnamomum camphora leaf. Nanotechnology 18, 1-11 (2017). https://doi.org/10.1088/0957-4484/18/10/105104

31. J. Coates, Interpretation of infrared spectra, a practical approach. Encyclopedia of analytical chemistry 12, 10815-10837 (2000) https://doi.org/10.1002/9780470027318.a5606

32. B.D.N. Asep, O. Rosi, R. Risti, How to Read and Interpret FTIR Spectroscope of Organic Material. Indones. J. Sci. Technol. 4, 97-118 (2019). https://doi.org/10.17509/ijost.v4i1.15806

33. S. Ghosh, S. Patil, M. Ahire, R. Kitture, S. Kale, K. Pardesi et al., Synthesis of silver nanoparticles using Dioscorea bulbifera tuber extract and evaluation of its synergistic potential in combination with antimicrobial agents. Int. J. Nanomed. 7, 483–496 (2012). https://doi.org/10.2147/IJN.S24793

34. V.S. Kotakadi, S.A. Gaddam, S.K. Venkata, P.V. Sarma, D.V. Sai Gopal, Biofabrication and spectral characterization of silver nanoparticles and their cytotoxic studies on human CD34 +ve stem cells. 3 Biotech 6, 216 (2016). https://doi.org/10.1007/s13205-016-0532-5

35. N.A. Khatune, M.A. Mosaddik, M.E. Haque, Antibacterial activity and cytotoxicity of Nyctanthes arbor-tristis flowers. Fitoterapia 72, 412–414 (2001). https://doi.org/10.1016/s0367-326x(00)00318-x

36. K. Priya, G. Deepak, Antibacterial Activities and Phytochemical Analysis of Different Plant Parts of Nyctanthes arbor-tristis (Linn.). Res. J. Phytochem. 1, 61-67 (2007). https://doi.org/10.3923/rjphyto.2007.61.67

37. M.M Haque, N. Sultana, S.M.T. Abedin, N. Hossain, S.E. Kabir, Fatty acid analysis, cytotoxicity, antimicrobial and antioxidant activities of different extracts of the flowers of Nyctanthes arbor-tristis L. Bangladesh J. Sci. Ind. Res. 55, 207-214 (2020). https://doi.org/10.3329/bjsir.v55i3.49394

38. L. Kvitek, A. Panacek, J. Soukupova, M. Kolar, R. Vecerova, R. Prucek et al., Effect of surfactants and polymers on stability and antibacterial activity of silver nanoparticles (NPs). J. Phys. Chem. C. 112, 5825–5834 (2008). https://doi.org/10.1021/jp711616v

39. C.N. Lok, C.M. Ho, R. Chen, Q.Y. He, W.Y. Yu, H. Sun et al., Proteomic analysis of the mode of antibacterial action of silver nanoparticles. J. Proteome Res. 5, 916–924 (2006). https://doi.org/10.1021/pr0504079

40. G. Franci, A. Falanga, S. Galdiero, L. Palomba, M. Rai, G. Morelli et al., Silver nanoparticles as potential antibacterial agents. Molecules 20, 8856–8874 (2015). https://doi:10.3390/molecules20058856

41. B. Le Ouay, F. Stellacci, Antibacterial activity of silver nanoparticles: A surface science insight. Nano Today 10, 339-354 (2015). https://doi.org/10.1016/j.nantod.2015.04.002

42. R.R.R. Kannan, R. Arumugam, D. Ramya, K. Manivannan, P. Anantharaman, Green synthesis of silver nanoparticles using marine macroalga Chaetomorpha linum. Appl. Nanosci. 3, 229–233 (2013). https://doi.org/10.1007/s13204-012-0125-5

43. M. Khanapur, R.K. Avadhanula, O.H. Setty, In vitro antioxidant, antiproliferative, and phytochemical study in different extracts of Nyctanthes arbor tristis flowers. Biomed. Res. Int.
Figures

Figure 1

Phylogenic tree constructed for isolated Endophytic Bacteria from NAT plant flowers
Figure 2
(a) Colour Change was Observed in solution used for biomaterial synthesis (b) UV-Vis Absorption Spectrum of Biosynthesized CSB (c) FTIR Analysis of CSB (d) FESEM analysis of CSB (e) EDX Analysis of CSB (f) DLS Analysis of CSB (g) Zeta potential analysis of CSB

![Graph showing percentage inhibition and biofilm formation](image)

Concentration (μg/ml) of bacteria strains: E1(1.5625), E2(12.5), ATCC E. coli (25), K. pneumoniae (6.25), S1(1.5625), MRSA(6.25), MTCC S. aureus (6.25)

Figure 3
(a) Percentage growth inhibition of bacterial strains by CSB at MIC compared to Ampicillin (b) Percentage of biofilm formation in control and treated with CSB in various strains of pathogenic bacteria
Figure 4

(a) DPPH Scavenging Activity of CSB and standard Ascorbic acid (b) Reducing power activity of (i) Synthesized CSB (ii) Ascorbic acid
Figure 5

(a) Percentage of membrane stabilization in RBC when treated with CSB and Diclofenac sodium (standard). (b) Percentage of protein denaturation inhibition in egg albumin (c) Percentage of protein denaturation inhibition in BSA