

ANTIBACTERIAL ACTIVITY OF SILVER NANOPARTICLES SYNTHESIZED USING *SYZYGIUM AROMATICUM*, *CINNAMONUM TAMALA*, *CINNAMONUM CASSIA* PLANT EXTRACT

ABSTRACT

Syzygium aromaticum (Clove), *Cinnamomum tamala* (Bay leaf), *Cinnamomum cassia* (Cinnamon bark) are well known plant in India. All three plants are rich source of secondary metabolites that use as antimicrobial agent, in pharmaceutical industry, cosmetics, food and agriculture industry. In regards of antimicrobial activity, prepared the green silver nanoparticle were synthesised by using these plants aqueous extract (25%w/v). For silver nanoparticle synthesis different concentration of plant extract mixed with AgNO₃ solution and exposed to sunlight and estimated by the UV-Visible spectrophotometer. Powdered silver nanoparticles (AgNPs) were dissolved in autoclaved distilled water at different concentration (20mg/ml, 10mg/ml, 5mg/ml, and 2.5mg/ml) and performed antimicrobial activity through agar well diffusion method. Silver nanoparticles of all three plants were showed antimicrobial activity against human bacterial pathogen *Escherichia coli* and *Bacillus subtilis*. The inhibition zone was increasing with increasing concentration of all three plants (*S. aromaticum*, *C. tamala* and *C. cassia*) AgNPs, so maximum inhibition zone was observed at 20mg/ml for both pathogens. At higher concentration, the inhibition zone of *E. coli* were 2.25 ±0.05, 1.7 ±0.1 and 1.85 ±0.05 cm in presence of *S. aromaticum*, *C. tamala* and *C. cassia* AgNPs respectively. Although, the presence of phytochemical terpenoids, tannin and glycosides are confirmed by the chemical reagent test. In modern era, the nanoparticle based medicine are exploring in pharmaceutical and medical science. Therefore this green synthesis of silver nanoparticle can be explored in pharmaceutical science.

KEYWORD: *Syzygium aromaticum*, *Cinnamomum tamala*, *Cinnamomum cassia*, *Escherichia coli* and *Bacillus subtilis*

1. INTRODUCTION

Nowadays nanotechnology is the most valuable field in modern science. It has great significance to create different applications in various fields of science. In nanotechnology, Nanoparticles size range between 1-100nm and this range can goes up to 1-1000nm in size. Nanoparticles have been used in medicine, human health care, textiles and cosmetics industry [1]. Among all metal nanoparticles, silver nanoparticles have created a remarkable deliberation due to their special characteristics like antimicrobial activity, catalytic activity, chemical stability and electrical conductivity [2].

The efficiency of silver nanoparticles has been used in to deliver drugs which is made it feasible in the field of medical science. Silver nanoparticles have been used in to resist infections, showing antibacterial and antifungal activity [3-4]. Silver nanoparticles nontoxic to the immune system, reproductive system and cardiovascular system and not considered to be carcinogenic [5]. Silver ion (Ag⁺) based compounds are relatively toxic to the microorganisms. Silver ion destroy the peptidoglycan layer of the bacterial cell wall, inhibit the bacterial growth and shatter the bacterial metabolism when they interact with macromolecules which are present in the bacterial cell wall like DNA and protein. Silver ion binds with DNA and inhibits the replication in bacteria [6].

Nanoparticles are synthesized by using different approaches like physical, chemical and biological approaches. Chemical mode of synthesis requires short period of time and large amount of nanoparticles. Chemicals which are used for nanoparticles synthesis are highly toxic and non-eco-friendly. So, biological synthesis of nanoparticles is seeking an extra ordinary as “green nanotechnology” [7].

Plants gives a best platform for nanoparticle synthesis because they are free from toxic compounds and they are very eco-friendly. Plants are having several biomolecules and those are containing different functional groups which aid to the reduction of silver ion and helps in the formation of silver nanoparticles. *Syzigium aromaticum* shows ample sources of phenolic compounds like phenolic acids (gallic acid), flavonol glucosides, phenolic volatile oils (eugenol, acetyl eugenol) and tannins [8]. Green synthesis of silver nanoparticles from *S. aromaticum* plant extract have been reported and showed antimicrobial activity [9-10].

In *Cinnamomum tamala* phytochemicals, tannins, alkaloids, flavonoids and terpenoids are reported as major phytochemical constituent and showed pharmaceutical value [11]. Recently, Nahar and group reported the green synthesis of silver from *C. tamala* leaf extract and showed antibacterial activity on Gram-positive (*Bacillus subtilis* and *Staphylococcus aureus*) and Gram-negative (*Escherichia coli* and *Pseudomonas aeruginosa*) bacteria [12]. However, *Cinnamomum cassia* bark is rich source of lignin, terpenoids, flavonoids, phenylpropanoids, alkaloids and steroids photochemical [13]. Including *C. cassia* other species, *C. zeylanicum*, *C. tamala*, and *C. wilsonii*, are famous herbs and their bark used for treating cardiovascular, chronic gastrointestinal, and inflammatory diseases [14]. These three plant species are showing great effectiveness for pharmaceutical industry, cosmetics, food and agriculture industries as well as showing great antibacterial and antifungal activity [15]. So, the nanoparticle of these plants may be part of nanomedicine as antimicrobial agents. The main purpose of this research is to synthesize silver nanoparticle by using *Syzygium aromaticum* (Clove), *Cinnamomum tamala* (Bay leaf), *Cinnamomum cassia* (Cinnamon bark) extracts and to check their antibacterial effect.

2. MATERIALS AND METHODS

Plant material and chemical

Syzygium aromaticum (Clove), *Cinnamomum tamala* (Bay leaf), *Cinnamomum cassia* (Cinnamon bark) were purchased from local market, Calcutta, (West Bengal) India. Silver nitrate and antibiotics (Streptomycine) were obtained from SD fine chemical Ltd. All the reagents used in this study were analytical grade i.e. all the reagents were highly purified.

Preparation of plant extract

S. aromaticum (Clove), *C. tamala* (Bay leaf), *C. cassia* (Cinnamon bark) plant sample were cleaned with distilled water to remove dust particles followed by air dried at room temperature. Bay leaves, Clove and Cinnamon bark were separately crushed to make powder form using a grinder mixer. Five gram from each sample was added into 20ml of distilled water and heated for 30 minutes at 80°C. Then these three plant extracts were centrifuged for 5 min at 5000 rpm followed by filtered with using the whatman filter paper. These extracts were stored at refrigerator for further use.

Analysis of Phytochemical tests:

Presences of major class of phytochemical in extract were performed through chemical test as per described previous findings [16-17].

Test for tannins: Few drops of FeCl_3 (0.1%) was added in three different plant extracts to observe blue-black or brownish green colour [16].

Test for glycosides: 2ml of plant extract was dissolved in 2ml water and 1ml of NaOH solution was added. Appearance of yellow colour indicates the presence of glycosides [17].

Test for terpenoids by the Salkowski Test: 5ml of plant aqueous extract was mixed with 2ml of chloroform and then 3ml of conc. H_2SO_4 was added carefully. Appearance of reddish brown colour at the interface indicates the presence of terpenoids [16].

Test for cardiac glycosides by the Keller–Kiliani Test: 5ml of plant extract was mixed with 2ml of glacial acetic acid followed by a drop of FeCl_3 solution and followed by an addition of 1ml conc. H_2SO_4 . The appearance of a brown ring at the interface indicates the presence of cardenolides [16].

Synthesis of silver nanoparticles (AgNPs)

1mM silver nitrate (AgNO_3) solution was prepared in distilled water. 20 ml of AgNO_3 (1mM) solution was separately added with 0.5, 1.0, 2.0 and 4.0 ml of Bay leaves aqueous extracts. Same concentration used for Clove and Cinnamon bark extract as well. Thereafter, solutions were kept in sunlight for 2minutes for reaction. After sunlight incubation the colour was changed. The solutions were

centrifuged at 10,000 rpm for 20 min to collect nanoparticles and further kept in hot air oven at 60°C. Next day pellet was extracted as dried form and kept in an eppendorf tube for further experiments.

Characteristics of silver nanoparticles synthesized by UV-Vis spectrum analysis

The AgNPs were characterized by using a UV-visible spectrophotometer. The synthesis of silver nanoparticles was checked by recording the UV-visible spectra of solutions between 300–600 nm.

Agar well diffusion assay for antimicrobial activity of AgNPs

Antimicrobial activity of *S. aromaticum* (Clove), *C. tamala* (Bay leaf), *C. cassia* (Cinnamon bark) water extract and their Ag nanoparticles were tested on two isolated human bacteria pathogens *Bacillus subtilis*, and *Escherichia coli*. Antimicrobial activity was conducted through agar well diffusion method [18]. The bacterial organisms were grown in the nutrient broth overnight to attain the colony-forming unit (CFU) of $\sim 10^6$ per/ml. One-hundred microliters of each bacteria culture was spread on the Luria–Bertani agar plates. Dried AgNPs, dissolved in autoclaved distilled water to make 20mg/ml, 10mg/ml, 5mg/ml, and 2.5mg/ml. Agar wells (6 mm diameter) were punched with the help of sterilized cork borer and loaded with AgNPs, AgNO₃ solution, Antibiotic (Streptomycin-0.01mg/ml) which is used as a positive control. From each concentration, 20µl were added to well and plates were incubated for 24 h at 37°C, and diameters of zone of inhibition were recorded in centimetre.

Statistical Analysis

We used Graph Pad prism, version 5.01 (GraphPad Software, San Diego, CA) for calculation of one-way ANOVA analysis with a Dunnett multiple test. The statistical significance of differences between aqueous extract and AgNPs sample was tested at different p value.

3. RESULTS AND DISCUSSION

Detection of phytochemicals by colour test

Existence of secondary metabolites in any plant samples are mainly used for medicinal activities. They are mainly phenolic compounds, alkaloids, tannins, carbohydrates, glycosides, terpenoids, flavanoids, steroids, etc. which are found among all over the plant kingdom. Here, we found the presence of tannin, glycosides and terpenoids in all three plant extract as shown in table 1. However, Cardiac glycosides, a digitalis compound was absent in all three plant extract. Color appearance of the respective compound test has been shown in figure 1. Previously, the presence of tannin, glycosides and terpenoids have been reported in *S. aromaticum* and played role as pharmacological value in various tradition remedy [19]. Although, *Cinnamomum* species retains the all three compound

and their extract have been used as immunomodulatory, anti-inflammatory, antimicrobial, antioxidant, and anticancer activities [20].

Experiment	Observation	Inference		
		<i>S. aromaticum</i>	<i>C. tamala</i>	<i>C. cassia</i>
Test for Tannins	Blueish-black colour was appeared.	Tannins are present	Tannins are present	Tannins are present
Test for Glycosides	Yellow colour was appeared.	Glycosides are present	Glycosides are present	Glycosides are present
Test for Terpenoids	Reddish brown colour was appeared.	Terpenoids are present	Terpenoids are present	Terpenoids are present
Test for Cardiac glycosides	Brown ring was not formed	Cardiac glycosides are absent	Cardiac glycosides are absent	Cardiac glycosides are absent

Table 1. Result of Phytochemicals tests for *Syzygium aromaticum*, *Cinnamomum tamala*, *Cinnamomum cassia*.

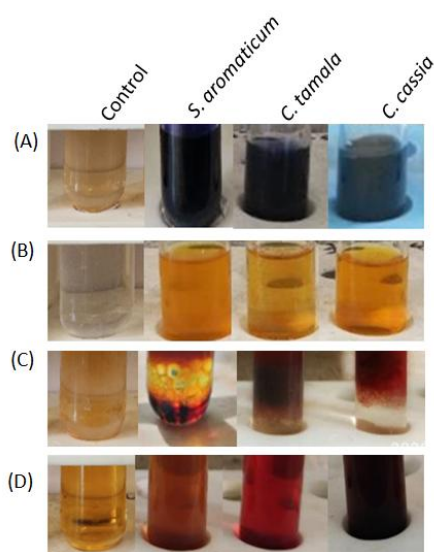


Figure 1. Phytochemical test of *Syzygium aromaticum*, *Cinnamomum tamala*, *Cinnamomum cassia* (A) tannins (B) glycosides (C) terpenoids (D) cardiac glycosides.

Synthesis of silver nanoparticles using plant extract

Till now many research have been published on green synthesis of nanoparticle using plant extract. Biosynthesis of silver nanoparticles of *S. aromaticum* (clove) have been reported as, significant antimicrobial and cytotoxic activity [10, 21]. In this study, the aqueous extract of *S. aromaticum* (Clove), *C. tamala* (Bay leaf), *C. cassia* (Cinnamon bark) were tested for synthesis of AgNPs. Aqueous extract of three plants were separately added to AgNO₃ solution and the changes in colour

were observed after sunlight exposure (Figure 2). Different amount of plant extract (0.5 ml, 1.0 ml, 2.0 ml and 4.0 ml) were added in silver nitrate solution and taken absorbance. The change in color of silver nitrate revealed the formation of silver nanoparticle through the reduction process [22]. But maximum colour changes and spectrophotometer absorption was observed in 4ml extract added silver nitrate solution as shown in figure 2.

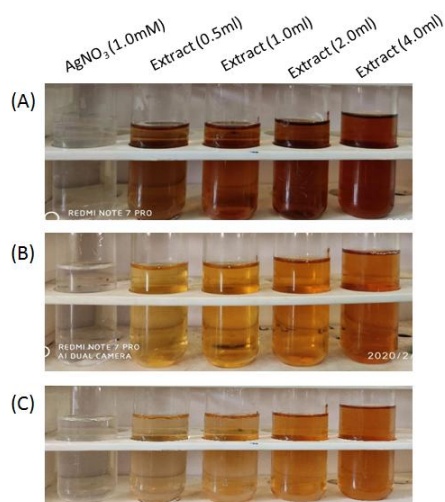


Figure 2. Ag Nanoparticles synthesis from aqueous extract of (A) *Syzygium aromaticum*, (B) *Cinnamomum tamala* (C) *Cinnamomum cassia* under sunlight exposure.

Recently, published research article on *C. tamala* AgNPs which was synthesised at 70 °C temperature for 30 min and shown antibacterial activity [23]. Similarly, Silver nanoparticles were synthesized using aqueous extract of cinnamon berks using conventional heating, microwave heating method and proved their role as anti-bacterial activity [24]. Silver nanoparticle from other medicinal plant extract also have been reported and tetsted for their antimicrobial activity [25-26].

UV-Visible Analysis of AgNP Synthesis

The primary characterization of *S. aromaticum* (Clove), *C. tamala* (Bay leaf), *C. cassia* (Cinnamon bark) AgNPs synthesis using the aqueous extract was done by UV-visible spectroscopy. After amendment of the *S. aromaticum* (Clove), *C. Tamala* (Bay leaf), *C. cassia* (Cinnamon bark) aqueous extract to silver nitrate solution, the colour of silver nitrate solution changed to dark brown after 2 min exposure to sun light. This change in colour of solution indicates the formation of silver nanoparticles and validated through spectrophotometer absorption [22]. Change in colour by addition of plant extract demonstrates the reducing ability of the plant extract for synthesis of AgNPs. UV-visible spectra of the silver nanoparticle's colloidal solution synthesised using extract of the *S. aromaticum* (Clove), *C. tamala* (Bay leaf), *C. cassia* (Cinnamon bark) showed the concentration dependent need

for silver nitrate reduction. Spectrophotometric absorbances of maximum color developed plant extract of all three plants are shown in figure 3.

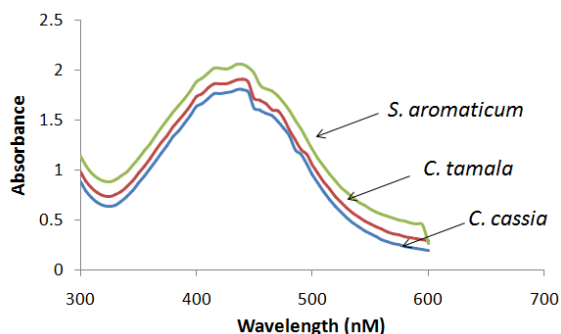
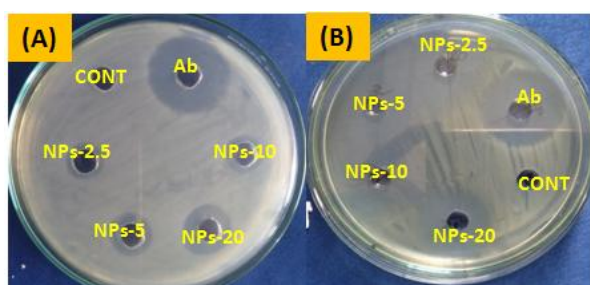


Figure 3. UV-Visible Spectrophotometer of *Syzygium aromaticum*, *Cinnamomum tamala* and *Cinnamomum cassia* AgNPs

Antimicrobial activity of *S. aromaticum* AgNPs

Prior to check antimicrobial activity of AgNPs, we tested antimicrobial activity against *E. coli* and *B. subtilis* (using the agar well diffusion method) of these three *S. aromaticum* (Clove), *C. tamala* (Bay leaf), *C. cassia* (Cinnamon bark) sample aqueous extract at different concentration (2.5, 5, 10 and 20 mg/ml) (Data not shown here). All sample showed antibacterial activity against tested pathogen but maximum inhibition zone was 20mg/ml concentration which considered as reference.

20µl from each concentration of *S. aromaticum* AgNPs were loaded in agar well along with positive control as antibiotics as shown in figure 4a,b. Inhibition zones were decreasing with decreasing the concentration of *S. aromaticum* AgNPs in case of both pathogens(Figure 4c,d). Maximum inhibition zone for *E. coli* and *B. subtilis* were 1.7 and 1.75cm respectively, which was followed by positive control antibiotics inhibition zone (0.01mg/ml) (Figure 4c,d). Water extract of respective plant sample was taken as reference to compare with AgNPs inhibition. Ajitha et al., (2019) have been reported *S. aromaticum* AgNPs as antimicrobial against the fungal and bacterial pathogens. Several other research published on green AgNPs as for the antibacterial against *Escherichia coli* (ATCC 25922), *Staphylococcus aureus* (ATCC 29213), *Pseudomonas aeruginosa* (ATCC 27853), *Azotobacter chroococcum* WR 9, and *Bacillus licheniformis* (MTCC 9555) [9,24,27].



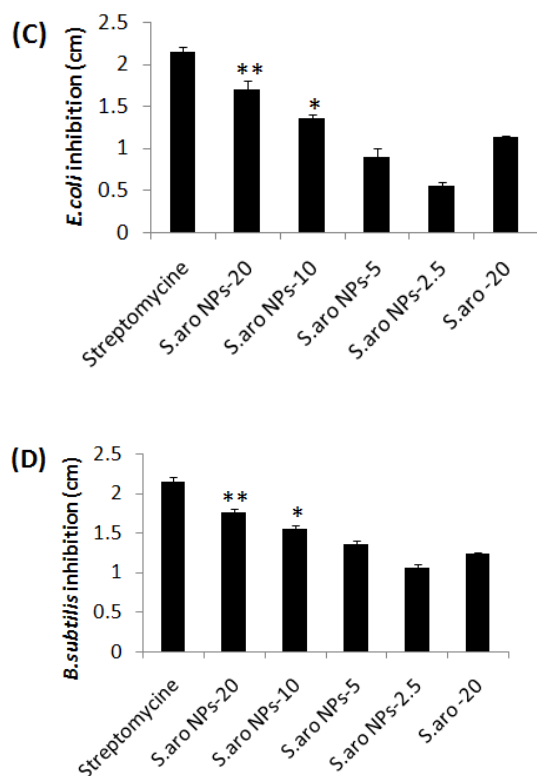


Figure 4. Antimicrobial activity of *Syzygium aromaticum* AgNPs at different concentration on (A) *E. coli*, (B) *B. subtilis* (C) Inhibition zone of *E. coli* compared with antibiotic & water extract (D) Inhibition zone of *B. subtilis* compared with antibiotic & water extract. NPs-20 (Ag-Nanoparticles-20mg/ml), NPs-10 (Ag-Nanoparticles- 10mg/ml), NPs-5 (Ag-Nanoparticles- 5mg/ml), NPs-2.5 (Ag-Nanoparticles- 2.5mg/ml) (S.aro-20; *S. aromaticum* water extract 20mg/ml) (S.aro; *Syzygium aromaticum*). Asterisks indicate a significant difference from the water extract at p value $* < 0.1$, $** < 0.05$

Antimicrobial activity of *C. cassia* AgNPs

C. cassia AgNPs were prominently inhibiting the growth of both *E. coli*, and *B. subtilis* (Figure 5a,b). In case of *E. coli*, at 10 and 20 mg/ml concentration of *C. cassia* AgNPs were substantially inhibited higher (2.25cm) than streptomycin antibiotics. However, *B. subtilis* was less sensitive and inhibition zone was lesser than positive control antibiotics (Figure 5c,d). Abdalla and group have been proved the antibacterial activity of *C. cassia* AgNPs against *E. coli* [24]. In another experiments, substantially inhibited the growth of *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Bacillus cereus* by 19 mm, 13 mm, 20 mm and 11 mm respectively after application of *C. cassia* AgNPs [28].

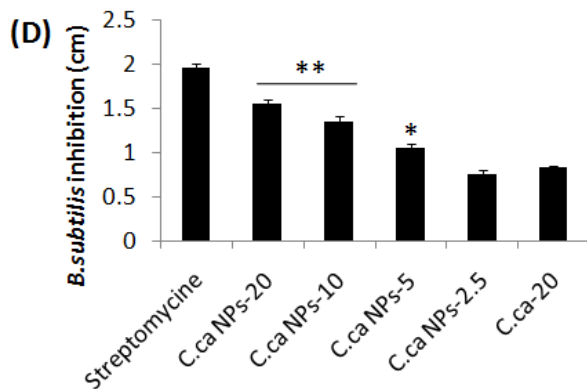
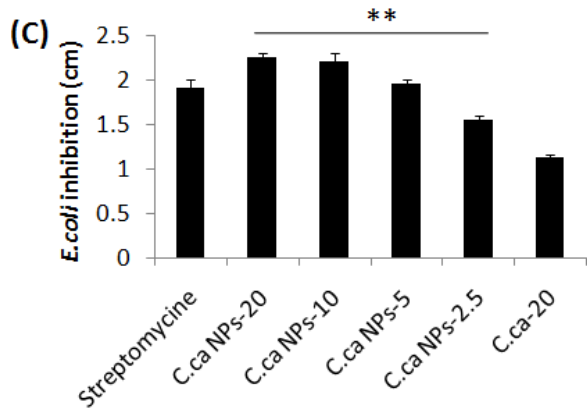
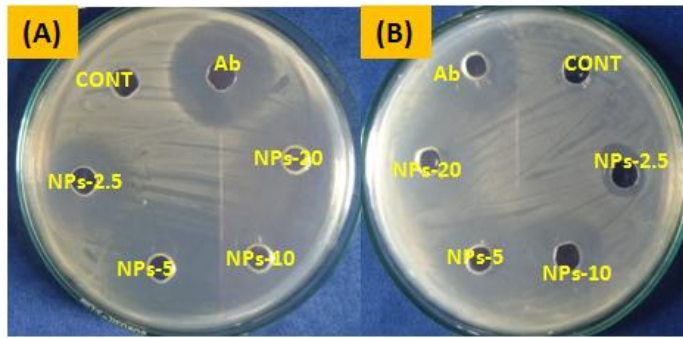


Figure 5. Antimicrobial activity of *Cinnamomum cassia* AgNPs at different concentration on (A) *E. coli* and (B) *B. subtilis* (C) Inhibition zone of *E. coli* compared with antibiotic & water extract (D) Inhibition zone of *B. subtilis* compared with antibiotic & water extract. NPs-20 (Ag-Nanoparticles-20mg/ml), NPs-10 (Ag-Nanoparticles- 10mg/ml), NPs-5 (Ag-Nanoparticles- 5mg/ml), NPs-2.5 (Ag-Nanoparticles- 2.5mg/ml) (C.ca-20; *C. cassia* water extract 20mg/ml) (C.ca; *Cinnamomum cassia*). Asterisks indicate a significant difference from the water extract at p value $* < 0.1$, $** < 0.05$

Antimicrobial activity of *C. tamala* AgNPs

Aqueous extract of *C. cassia* independently showed the antibacterial activity against above mentioned pathogens, but its AgNPs showed better antimicrobial activity. Naturally, this plant known for the antimicrobial activity, while formation of nanoparticle is another area to be explored [11]. Dash and

group, recently reported the antimicrobial activity of *C. tamala* AgNPs against the multidrug-resistant bacterial strains such as *Escherichia coli* (EC-1), *Klebsiella pneumonia* (KP-1), and *Staphylococcus aureus* (SA-1) [23].

Antimicrobial of *C. Cassia* AgNPs against *E. coli* and *B. subtilis* is less than antibiotic but better than their aqueous extract (Figure 6a,b). Maximum inhibition zone of *E. coli* was 1.85 and 1.55 cm at 10mg/ml and 20mg/ml concentration of *C. Cassia* AgNP respectively, but higher than their respective plant aqueous extract (1.1cm) (Figure 6c). In case of *B. subtilis*, maximum inhibition was 1.65 and 1.35 cm at 10mg/ml and 20mg/ml concentration of *C. Cassia* AgNPs respectively. Overall, in present results at below 10 mg/ml concentration of AgNPs was showing less inhibition zone in all three plants.

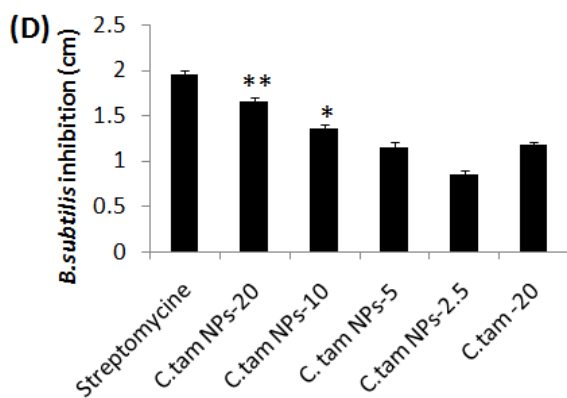
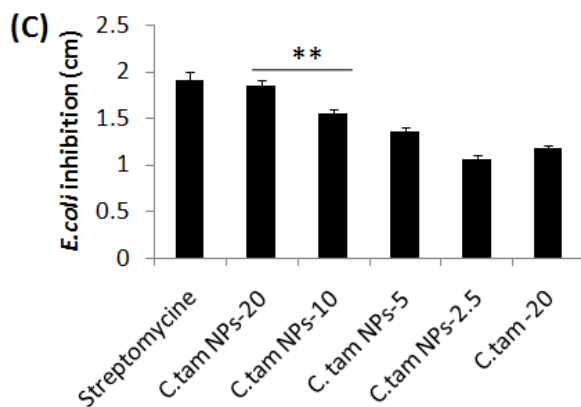
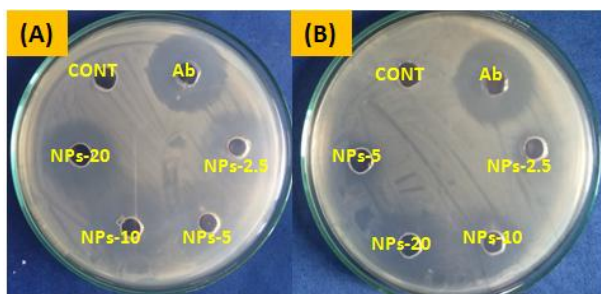


Figure 6. Antimicrobial activity of *Cinnamomum tamala* AgNPs at different concentration on (A) *E. coli* and (B) *B. subtilis* (C) Inhibition zone of *E. coli* compared with antibiotic & water extract (D) Inhibition zone of *B. subtilis* compared with antibiotic & water extract. NPs-20 (Ag-Nanoparticles- 20mg/ml), NPs-10 (Ag-Nanoparticles- 10mg/ml), NPs-5 (Ag-Nanoparticles- 5mg/ml), NPs-2.5 (Ag-Nanoparticles- 2.5mg/ml) (C.tam-20; *C. tamala* water extract 20mg/ml) (C.tam; *Cinnamomum tamala*). Asterisks indicate a significant difference from the water extract at p value * < 0.1, ** < 0.05

4. CONCLUSION

All three plants *S. aromaticum* (Clove), *C. tamala* (Bay leaf), *C. cassia* (Cinnamon bark) pertains the tannin, terpenoids, and glycosides in their respective tissue. All three plants showed reducing potential of Ag⁺ and leads to formation of silver nanoparticles in solution. This experiment also revealed that aqueous extract of *S. aromaticum* (Clove), *C. tamala* (Bay leaf), *C. cassia* (Cinnamon bark) showed antimicrobial activity. However, their silver nanoparticle showed better inhibition zone than their respective aqueous extract.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

REFERENCE

1. Khezri K, Saeedi M, Maleki Dizaj S. Application of nanoparticles in percutaneous delivery of active ingredients in cosmetic preparations. *Biomed Pharmacother* 2018;106:1499-1505
2. Sharma VK, Yngard RA, Lin Y. Silver nanoparticles: green synthesis and their antimicrobial activities. *Adv Colloid Interface Sci* 2009;145:83-96.
3. Prasath Alias Surendhar. S. Microwave assisted rapid biosynthesis and anticancer activity of silver oxide nanoparticles. *Journal of Critical Reviews* 2020;7:1688-1696. doi:10.31838/jcr.07.06.259
4. Sorbiun M, Mehr ES, Ramazani A, Malekzadeh AM. Biosynthesis of metallic nanoparticles using plant extracts and evaluation of their antibacterial properties. *Nanochem Res* 2018;3:1-16.

5. Bahadar H, Maqbool F, Niaz K, Abdollahi M. Toxicity of Nanoparticles and an Overview of Current Experimental Models. *Iran Biomed J*2016; 20:1-11.
6. Dakal TC, Kumar A, Majumdar RS, Yadav V. Mechanistic Basis of Antimicrobial Actions of Silver Nanoparticles. *Front Microbiol* 2016;7:1831.
7. Thuesombat P, Hannongbua S, Akasit S, Chadchawan S. Effect of silver nanoparticles on rice (*Oryza sativa* L. cv. KDML 105) seed germination and seedling growth. *Ecotoxicol Environ Saf* 2014;104:302-309.
8. Cortes-Rojas DF, de Souza CR, Oliveira WP. Clove (*Syzygium aromaticum*): a precious spice. *Asian Pac J Trop Biomed* 2014;4:90-96.
9. Ajitha B, Reddy YAK, Lee Y, Kim MJ, Ahn CW. Biomimetic synthesis of silver nanoparticles using *Syzygium aromaticum* (clove) extract: Catalytic and antimicrobial effects. *Applied Organometallic Chemistry*, 2019; e4867. doi:10.1002/aoc.4867
10. Bansal H, Kaushal J, Chahar V, Shukla G, Bhatnagar. Green Synthesis of Silver Nanoparticles Using *Syzygium aromaticum* (Clove) Bud Extract: Reaction Optimization, Characterization and Antimicrobial Activity. *Journal of Bionanoscience* 2018; 12:378-389. 10.1166/jbns.2018.1531.
11. Hassan W, Kazmi SNZ, Noreen H, Riaz A, Zaman B. Antimicrobial Activity of *Cinnamomum tamala* Leaves. *J Nutr Disorders Ther* 2016;6:190. doi:10.4172/2161-0509.1000190
12. Nahar K, Aziz S, Bashir MS, Haque MdA, Al-Reza SMd. Synthesis and characterization of Silver nanoparticles from *Cinnamomum tamala* leaf extract and its antibacterial potential. *Int. J. Nano Dimens.*, 2020; 11 : 88-98.
13. Rao PV, Gan SH. Cinnamon: A Multifaceted Medicinal Plant. *Evidence-Based Complementary and Alternative Medicine*2014:642942.
14. Zhao J, Ma JS. Phytochemicals and biological activities of the genus *Cinnamomum*. *Journal of Pharmacognosy and Phytochemistry*, 2016;4 : 27–34
15. Qais FA, Shafiq A, Khan HM, Husain FM, Khan RA, Alenazi B, Alsalmeh A, Ahmad I. Antibacterial Effect of Silver Nanoparticles Synthesized Using *Murraya koenigii* (L.) against Multidrug-Resistant Pathogens. *BioinorgChemAppl* 2019;4649506.
16. Zhang C, Fan L, Fan S, Wang J, Luo T, Tang Y, et al. (2019) *Cinnamomum cassia* Presl: A Review of Its Traditional Uses, Phytochemistry, Pharmacology and Toxicology. *Molecules* 2019;24.
17. Odebiyi OO, Sofowora EA. Phytochemical screening of Nigerian medicinal plants II. *Lloydia* 1978;41:234-246.

18. Qais FA, Samreen, Ahmad I. Broad-spectrum inhibitory effect of green synthesised silver nanoparticles from *Withania somnifera* (L.) on microbial growth, biofilm and respiration: a putative mechanistic approach. *IET Nanobiotechnology* 2018;12: 325–335.
19. Batiha GE, Alkazmi LM, Wasef LG, Beshbishy AM, Nadwa EH, Rashwan EK. *Syzygium aromaticum* L. (Myrtaceae): Traditional Uses, Bioactive Chemical Constituents, Pharmacological and Toxicological Activities. *Biomolecules* 2020;10.
20. Wu M, Ni L, Lu H, Xu H, Zou S, Zou X. Terpenoids and Their Biological Activities from *Cinnamomum*: A Review. *Journal of Chemistry* 2020:5097542.
21. Varadarajan R, Hirani B, Prashant CK, Jacqueline B, Nair D, Jubi M, et al. Green synthesis of silver nanoparticles using aqueous extract of *Syzygium aromaticum* and its cytotoxic and antibacterial activity. *I.J.S.N.* 2018; 9 :328-334
22. Gurunathan S, Kalishwaralal K, Vaidyanathan R, Venkataraman D, Pandian SR, Muniyandi J, et al. Biosynthesis, purification and characterization of silver nanoparticles using *Escherichia coli*. *Colloids Surf B Biointerfaces* 2009;74:328-335.
23. Dash SS, Samanta S, Dey S, Giri B, Dash SK. Rapid Green Synthesis of Biogenic Silver Nanoparticles Using *Cinnamomum tamala* Leaf Extract and its Potential Antimicrobial Application Against Clinically Isolated Multidrug-Resistant Bacterial Strains. *Biological Trace Element Research* 2020. doi: 10.1007/s12011-020-02107-w.
24. Abdalla KH, Hannan FA, Ali A, Henari F. Green Synthesis of Silver Nanoparticles using Cinnamon (*Cinnamomum cassia*), Characterization and Antibacterial Activity. *International Journal of Science and Research.* 2019; 6:965- 971
25. Ahmed S, Ahmad M, Swami BL, Ikram S. A review on plants extract mediated synthesis of silver nanoparticles for antimicrobial applications: A green expertise. *J Adv Res* 2016; 7:17-28.
26. Shah M, Fawcett D, Sharma S, Tripathy SK, Poinern GEJ. Green Synthesis of Metallic Nanoparticles via Biological Entities. *Materials (Basel)* 2015;8:7278-7308.
27. Prasad R, Swamy VS. Antibacterial Activity of Silver Nanoparticles Synthesized by Bark Extract of *Syzygium cumini*. *Journal of Nanoparticles* 2013;431218.
28. Premkumara J, Sudhakara T, Dhakala A, JeshanBabu S, Krishnakumara S, Balashanmugam P. Synthesis of silver nanoparticles (AgNPs) from cinnamon against bacterial pathogens. *Biocatalysis and Agricultural Biotechnology* 2018;15: 311–316.