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

ABSTRACT

Syzygiumaromaticum(Clove), *Cinnamonumtamala* (Bay leaf), *Cinnamonum 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 nanoparticlewere synthesised by using these plants aqueous extract (25%w/v). For silver nanoparticlesynthesis differentconcentration of plant extract mixed with $AgNO_3$solution and exposedto sunlight and estimated by the UV-Visible spectrophotometer. Powderedsilver 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 bacterialpathogen *Escherichia* coli and *Bacillus subtilis*. The inhibition zone wasincreasing with increasing concentration of all three plants (*S. aromaticum, C. tamala*and *C. cassia*) AgNPs, so maximum inhibition zone was observedat 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:*Syzygiumaromaticum, Cinnamonumtamala, Cinnamonum 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. Innanotechnology,Nanoparticles size range between 1-100nm andthisrange can goes up to 1-1000nm in size.Nanoparticles have been used in medicine, human health care, textilesand cosmetics industry[1].Among all metal nanoparticles, silver nanoparticles have createda remarkable deliberation due to their special characteristicslike antimicrobial activity, catalytic activity, chemical stability and electrical conductivity[2].

Comment [CdM1]: Please, use lower case here.

Comment [CdM2]: In this sentence you can include some examples of food application, such as food packaging. https://doi.org/10.1016/j.colsurfa.2020.125125

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,biologicalsynthesis of nanoparticles is seeking an extraordinaryas "green nanotechnology"[7].

Comment [CdM3]: I believe that in this paragraph you can include more details of biological synthesis of silver nanoparticles.

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.*Syzigiumaromaticum* shows ample sources of phenolic compounds like phenolic acids (gallic acid), flavonolglucosides, 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].

Comment [CdM4]: IN this section, you can elucidate that essential oils and plants extracts can be used to the synthesis.
I suggest the following paper:
https://iopscience.iop.org/article/10.1088/2053-1591/ab6c63/meta

In *Cinnamonumtamala*phytochemicals, tannins, alkaloids, flavonoids and terpenoidsare reportedas 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,*Cinnamonum cassia* bark is rich source of lignin, terpenoids, flavonoids, phenylpropanoids, alkaloidsand steroidsphotochemical[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 industryas wellas showing great antibacterial and antifungal activity[15].So,thenanopartcle of these plants may be part of nanomedicine as antimicrobial agents. The main purpose of this research is to synthesize silver nanoparticle by using *Syzygiumaromaticum*(Clove), *Cinnamonumtamala* (Bay leaf), *Cinnamonum cassia* (Cinnamon bark) extracts and to check their antibacterial effect.

Comment [CdM5]: Use "Nahar et al. (YEAR)" instead of Nahar and group

Comment [CdM6]: I believe that is better to create a new paragraph for the aim of the study.

2. MATERIALS AND METHODS

Plant material and chemicals

Syzygiumaromaticum(Clove), *Cinnamonumtamala* (Bay leaf), *Cinnamonum 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 cleanedwith distilled water to remove dust particles followed by air dried at room temperature. Bay leaves, Clove and Cinnamon bark wereseparately crushed to make powder form using a grinder mixer. Five gram fromeach 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:

The qualitative analysis of ~~Presences of major class~~ of phytochemical in the plants extract were performed through chemical test as ~~per~~ previouslydescribed ~~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 glycosidesby theKeller–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 previously prepared.~~ 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

Comment [CdM7]: Include the Country of the company here.

Comment [CdM8]: This sentence can be removed.

Comment [CdM9]: In all the manuscript you are repeating the scientific name of the plants. It is not necessary. Please, correct this along all the text.

Comment [CdM10]: Include the time that plants were dried and the average temperature. Is It 25°C?

Comment [CdM11]: Include the average temperature.

Comment [CdM12]: There is a redundancy in this sub-title. I suggest to use "Qualitative phytochemical tests"

Comment [CdM13]: Just in 2 minutes the reaction occurred?

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 byUV-Vis ~~spectrum analysis~~spectroscopy

The AgNPswere 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 testedon 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, *Cinnamonum* species retains the all three compound and

Comment [CdM14]: In the section "Synthesis of silver nanoparticles (AgNPs)" the authors described that the nanoparticles were centrifuged, dried, and then, a powder was obtained. However, the UV-Vis analysis should be conducted with the AgNPs in the aqueous solution. Please, include a sentence regarding this statement.

Comment [CdM15]: Change to AgNPs

Comment [CdM16]: Use "100µL"

Comment [CdM17]: Why the dried AgNPs were used? This analysis can be conducted with the aqueous-AgNPs.

Comment [CdM18]: You dried and then dissolved in water....

Comment [CdM19]: In this section, the authors should conduct a deep discussion related the presence of bioactive compounds. For this, I suggest the following paper:

https://doi.org/10.1002/jsfa.9961

IN the section "Bioactive compounds and antioxidant capacity in uvaia pulp" there are a range of information to include in this discussion.

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

Experiment	Observation	Inference		
		S. aromaticum	*C. tamala*	*C. cassia*
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 *Syzygiumaromaticum, Cinnamonumtamala, Cinnamonum cassia.*

Comment [CdM20]: This table should be remake. In the "nference" include the symbols +, ++, +++, -, instead the text. I believe that will be better to observe the results.

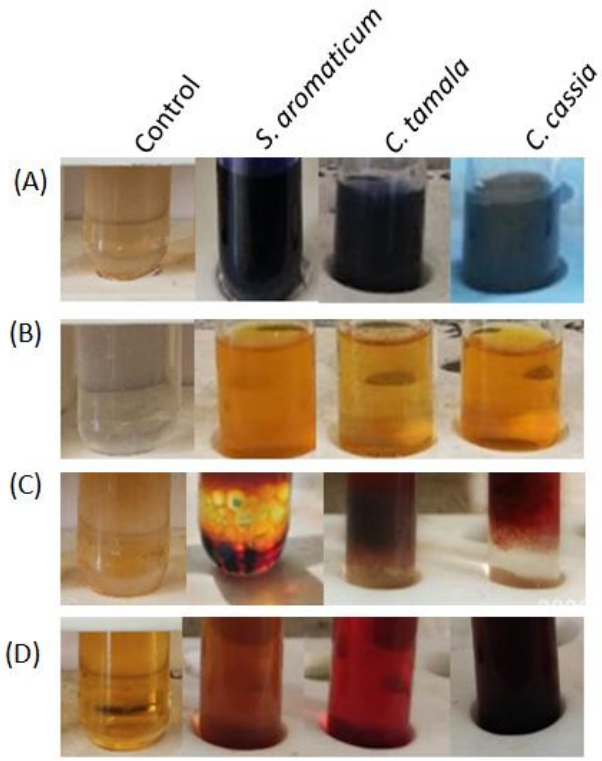


Figure 1. Phytochemical test of *Syzygiumaromaticum, Cinnamonumtamala, Cinnamonum cassia* (A) tannins (B) glycosides (C) terpenoids (D) cardiac glycosides.

Synthesis of silver nanoparticles using plant extract

Till now many research have been published on green synthesis of nanoparticle using plant extract. ~~Biosynthess~~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 $AgNO_3$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 absorptionwas observed in 4ml extract added silver nitrate solution as shown in figure 2.

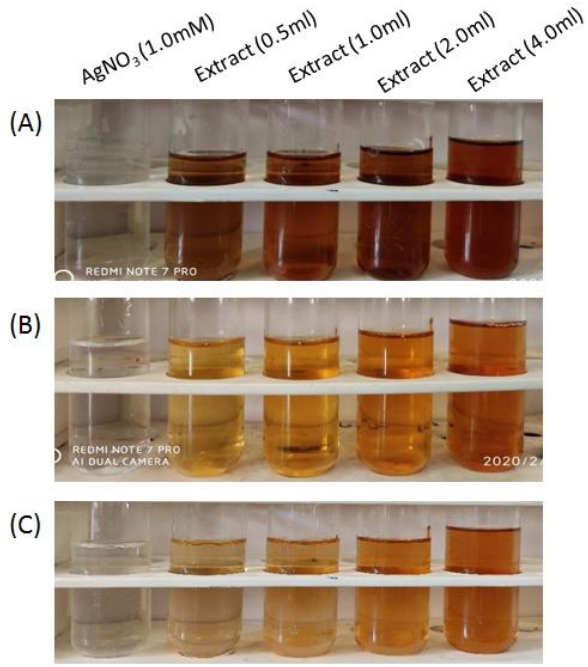


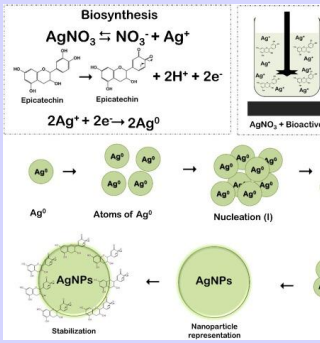
Figure 2. Ag Nanoparticles synthesis from aqueous extract of (A) *Syzygiumaromaticum*, (B) *Cinnamonumtamala*(C) *Cinnamonum cassia* under sunlight exposure.

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 ~~nanopartcle~~nanoparticle from other medicinal plant extract also have been reported and ~~tetsted~~tested for their antimicrobial activity [25-26].

Comment [CdM21]: The authors should include the mechanism to produce AgNPs. For this, I suggest the following paper:

https://doi.org/10.1016/j.colsurfa.2020.125125

In the section "3.1.3. Chemical mechanism to describe the synthesis of AgNPs" of the aforementioned paper you can observe in details a mechanism.



Please, include a short comment for the mechanism in your manuscript. This will help to understand the sysnthesis.

UV-Visible ~~Analysis~~ of AgNPs~~Synthesis~~

The primary characterization of ~~*S.aromaticum*(Clove), *C.tamala* (Bay leaf), *C. cassia* (Cinnamon bark)~~ AgNPs~~synthesis~~ bioreduced with ~~using the~~ plants aqueous extract was conducted~~done~~ by UV-visible spectroscopy.After amendment of the *S.aromaticum*(Clove), *C.Tamala* (Bay leaf), *C. cassia* (Cinnamon bark) aqueousextract to silver nitrate solution, the colour of silver nitrate solution changed to dark brown after 2 minexposure to sun light. The color change ~~This change in colour of solution indicates the~~indicate a possible formation of silver nanoparticles,and therefore, should be 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~~absorbance of maximum ~~color~~colour developed plant extract of all three plants are shown in figure 3.

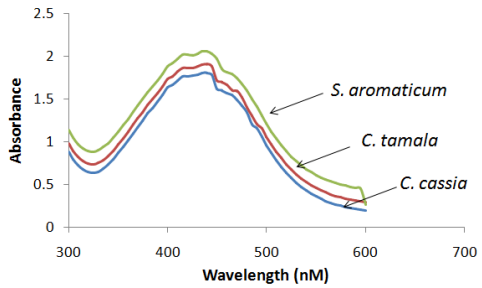


Figure 3. UV-Visible Spectrophotometer *of Syzygiumaromaticum*, *Cinnamonumtamala* and *Cinnamonum cassia*AgNPs

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*AgNPsin case of both pathogens(Figure 4c,d). Maximum inhibition zonefor*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.*

Comment [CdM22]: This sentence is not necessary. You are repeating information from the methodology.

Comment [CdM23]: In Fig 2 you are reporting that different concentration of plant extract was used for the synthesis of AgNPs. I believe that the UV-Vis of all test should be shown in Fig. 3. Additionally, the control (Just AgNO3) also be available. In better words, for plant should be one figure with the four concentration used.

Comment [CdM24]: If you are writing that plants extracts have antimicrobial activity, I would like to observe the results. Why to not shown here the data??

Comment [CdM25]: You are repeating information from the methodology. It is not necessary.

*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), *Azotobacterchroococcum* WR 9, and *Bacillus licheniformis* (MTCC 9555) [9,24,27].

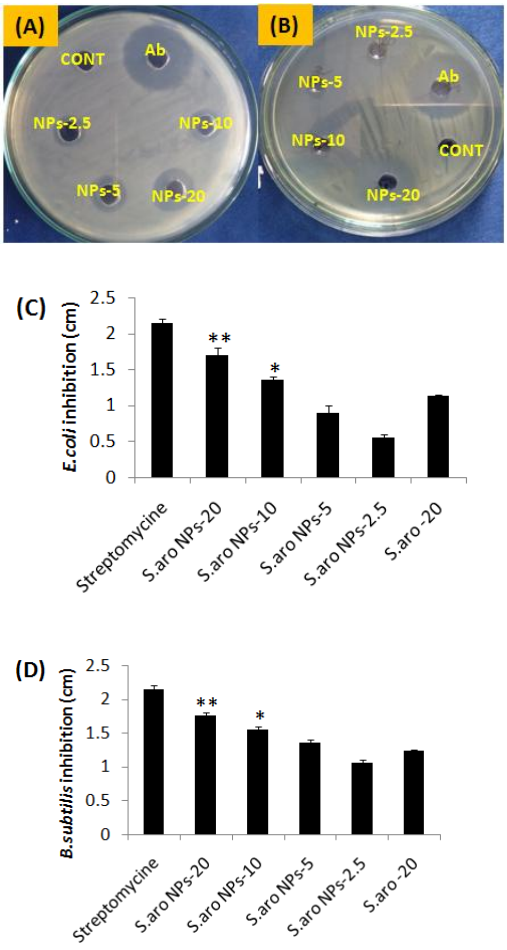
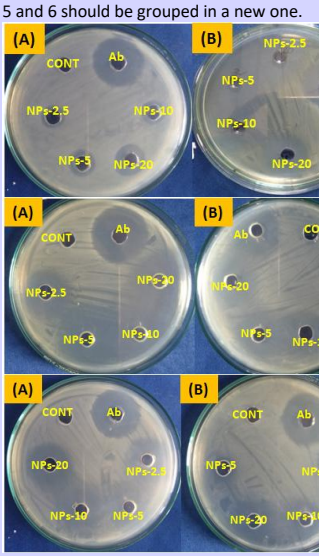


Figure 4. Antimicrobial activity of *Syzygiumaromaticum*AgNPs at different concentration on (A) *E. coli*, (B) *B. subtilis* (C) Inhibition zone of *E. coli* compared with antibiotic & water extrat (D) Inhibition zone of *B. subtilis* compared with antibiotic & water extract. NPs-20 (Ag-Nanoparticles-20mg/ml),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

Antimicrobial activity of *C. cassia* AgNPs

Comment [CdM26]: The discussion of the antimicrobial activity are poor. The authors should create one section of antimicrobial activity, and the, discuss the results. IN the current form, the authors are just speculating the results.

The Figures 4 to 6, should be remake. For this, I suggest that authors create a new figure with the pictures of the disks. In better words, the "a" and "b" of the Fig 4, 5 and 6 should be grouped in a new one.



Then, the bars graph should be grouped in the second figure.

*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*AgNPswere substantially inhibited higher (2.25cm) than streptomycinantibiotics.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 antibactrial activity of *C. cassia*AgNPs against *E.coli*[24]. In another experiments, substantially inhibitedthe growth of *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Bacillus cereus*by 19 mm, 13 mm, 20 mm and 11 mm respectively after applicationof*C. cassia*AgNPs[28].

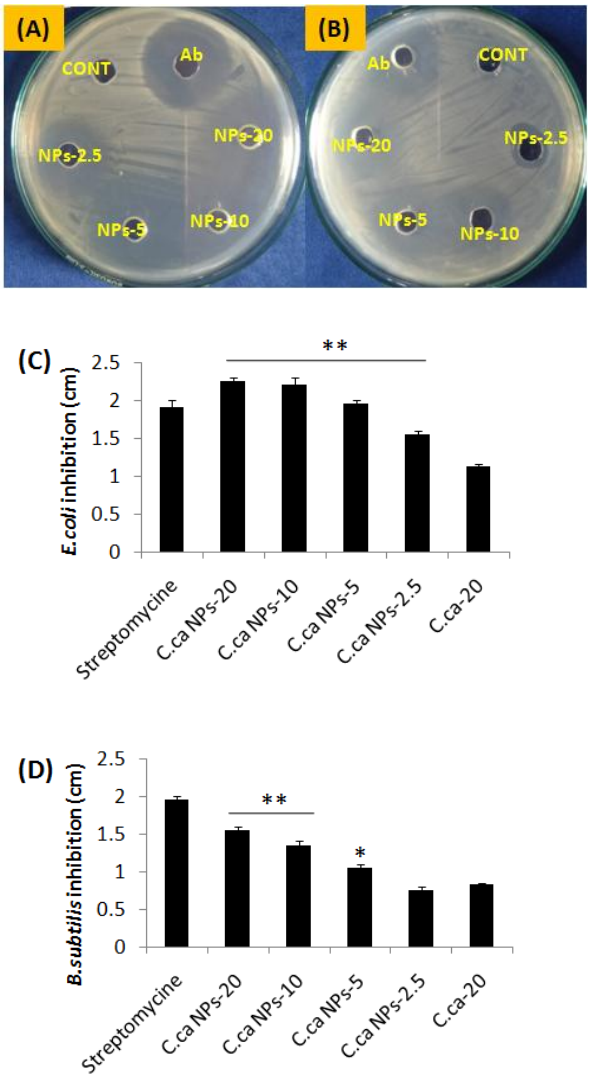


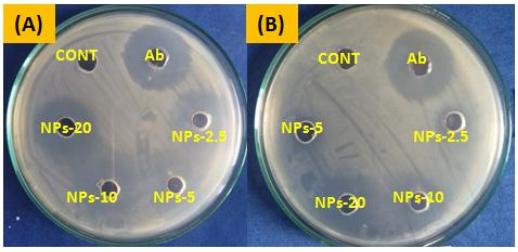
Figure 5. Antimicrobial activity of *Cinnamonum cassia* AgNPs at different concentration on (A) *E. coli* and (B) *B. subtilis* (C) Inhibition zone of *E. coli* compared with antibiotic & water extract (D)

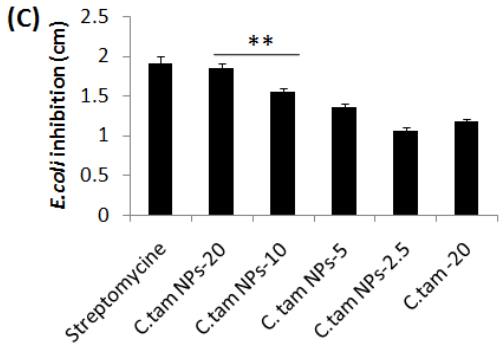
Inhibition zone of *B. subtilis* compared with antibiotic & water extract. NPs-20 (Ag-Nanoparticles- 20mg/ml), NPs-10 (Ag-Nanoparticles- 10mg/ml), NPs-5 (Ag-Nanoparticles- 5mg/ml),NPs-2.5 (Ag-Nanoparticles- 2.5mg/ml) (C.ca-20; *C. cassia* water extract 20mg/ml) (C.ca; *Cinnamonum cassia*). Asterisks 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 antimicrobialactivity, while formation of nanoparticle is another area to be explored[11]. Dash and group, recently reported the antimicribial 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*AgNPsagainst*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 higherthan their respective plant aqueous extract (1.1cm) (Figure 6c). In case of *B.subtlis*, 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.





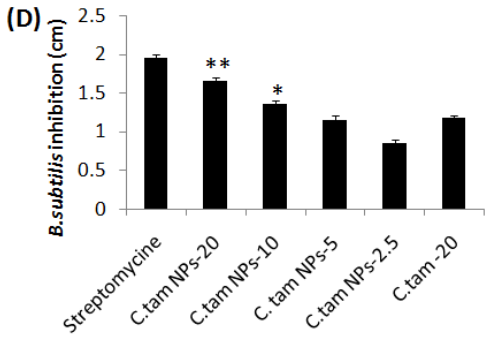


Figure 6. Antimicrobial activity of *Cinnamomumtamala*AgNPs at different concentration on (A) *E. coli* and (B) *B. subtilis* (C) Inhibition zone of *E. coli* compared with antibiotic & water extrat (D) Inhibition zone of *B. subtilis* compared with antibiotic & water extract. NPs-20 (Ag-Nanoparticles- 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; *Cinnamomumtamala*). 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 silvernanoparticle showed better inhibition zone than their respective aqueous extract.

Comment [CdM27]: You are not concluding the results. Please, re-write this section based on main findings. Additionally, you can add a paragraph of perspectives futures of AgNPs.

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, MalekiDizaj S. Application of nanoparticles in percutaneous delivery of active ingredients in cosmetic preparations. Biomed Pharmacother2018;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, MalekzadehAM.Biosynthesisof 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 J2016; 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, AkasitS,Chadchawan S. Effect of silver nanoparticles on rice (*Oryzasativa* L. cv. KDML 105) seed germination and seedling growth. EcotoxicolEnvironSaf2014;104:302-309.

8. Cortes-Rojas DF, de Souza CR Oliveira WP. Clove (*Syzygiumaromaticum*): 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 *Syzygiumaromaticum* (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 *Syzygiumaromaticum* (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 *Cinnamomumtamala* Leaves. J Nutr Disorders Ther 2016;6:190. doi:10.4172/2161-0509.1000190

12. Nahar K, Aziz S, Bashar MS, HaqueMdA, Al-Reza SMd. Synthesis and characterization of Silver nanoparticles from *Cinnamomumtamala* 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 Medicine2014:642942.

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, Alsalme A, Ahmad I. Antibacterial Effect of Silver Nanoparticles Synthesized Using *Murrayakoenigii* (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 *Withaniasomnifera* (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. Syzygiumaromaticum 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 *Sytiumaromaticum* 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 *Cinnamomumtamala* 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, TripathySK,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 *Syzygiumcumini*. 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.