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Activity of Silver Nanoparticles Synthesized From Buttermilk Filtrate and Activity of Spices against Dental Cavity Causing Organisms

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Abstract— Bacterial oral pathogens can cause severe diseases with complications, severe soft tissue invasions, toxic syndromes and also cause infective endocarditis. This research paper provides comprehensive view on the silver nanoparticle synthesis from buttermilk filtrate, its characterization and its antimicrobial activity against dental pathogens. Also some spices was used to check their antimicrobial potential. The dental cavity causing organisms were isolated from patients having dental cavity problems. These organisms were identified as *streptococcus spp.* and *candida spp*. by performing morphological, cultural and biochemical characteristics referring to the bergey's manual of determinative bacteriology. Silver nanoparticles were synthesized using buttermilk filtrate and characterization were done by UV-Vis spectroscopy and scanning electron microscopy. Extract of different spices as well as biologically synthesized silver nanoparticles were checked against identified clinical isolates by well diffusion method. Nanoparticles synthesized using buttermilk filtrate were more effective as compared to crude extracts against pathogenic strains of bacteria.

Keywords— Buttermilk, Silver nanoparticles(AgNPs), *streptococcus spp*., *candida spp*., UV-Vis spectroscopy, SEM, *Cinnamomum verum*(cinnamon), *Curcuma longa*(turmeric).

I. INTRODUCTION

Oral pathogenic bacteria can cause severe human diseases with complications including cavity, sepsis, severe soft tissue invasions, toxic shock like syndrome and also causes the infective endocarditis. [1]
Many oral pathogenic microorganisms was involved in periodontal, vascular disease, tooth decay or dental caries. [2]
This study focuses on evaluation and antimicrobial activity of *Cinnamomum verum* (cinnamon), *Curcuma longa* (turmeric), and silver nanoparticles synthesized from buttermilk filtrate against cavity causing organisms. The dental cavity causing organisms was been isolated from infected patients.

There are reports of AgNPs being synthesized using bacteria, fungi, yeast and fruit, plant waste etc [3]. Silver nanoparticles synthesis using buttermilk filtrate is reported for the first time. There are reports stating activity of herbal extract and Silver nanoparticles against cavity causing organisms. [4]

Nanoparticles have vast range of applications as in biological research, antimicrobial compound, chemotherapy, food and agriculture. [5]
It is imperative to study their antimicrobial potential against the oral cavity causing pathogens so that spices and nanoparticles use together for determining their antimicrobial principle which further can be applied for developing effective treatment of patients.

II. MATERIALS AND METHODS

1. Preparation of buttermilk filtrate:

Commercial curd was procured from the local market and diluted as 3:2 using distilled water (sterile) and mixed thoroughly to make the buttermilk. This buttermilk was filtered by membrane filter (0.22µ) (Filtrate was stored in sterile storage bottle in refrigerator).

2. Preparation of silver Nitrate solution:

Analytical grade silver nitrate (AgNO3), a costly chemical was purchased from Hi media.
1 mM stock solution of $AgNO_3$ in chloride free distilled water was prepared according to calculations.

3. Silver nanoparticles synthesis from Filtered Buttermilk:

Same volume of 1mM silver nitrate solution and buttermilk filtrate (2.5 ml each) was added in sterile 6 each bumper tubes, and this mixture was incubated for 24 hrs. at room temperature. Buttermilk filtrate without addition of 1 mM $AgNo_3$ kept as control.

4. Collection of sample :

The sample was taken by swabbing oral cavity of a total 5 volunteers aged 28-30 years by sterile swab. All of the patients were informed about aim of the study and they were signed informed consent before entering this study, all of the dental condition and diet habits were recorded for each participant. [5]

sample collected using sterile swab before tooth brushing in the morning and before breakfast, and were placed separately into 1 ml of 0.1% buffered peptone water, further vortexed to dispersed and the suspension dilutions were plated on Nutrient agar(NA), Blood agar(5% sheep blood) and PDA (Potato dextrose agar) plates. Blood agar was used because most of the dental pathogens requires selective medium for isolation. PDA was used for the isolation of fungal pathogens. Single pure colonies were in a given sector of a plate, well isolated colonies were selected and sub cultured for isolation on a solid medium to ensure purity. [5]

5. Identification of isolates:

For identification, different parameters like cultural, morphological and biochemical tests was done by referring to Bergey's manual of determinative bacteriology.

Cultural Examination-

Morphological characteristics of the colonies was studied on Blood Agar and Potato Dextrose Agar (PDA). Colour, size and edge of colonies were recorded after 48 hrs of Incubation at 37ºC.

Microscopic Examination-

A single colony of isolate from Blood agar plate was fixed a clean slide to study gram stain, under light microscope [6].

Monochrome staining of the isolate from PDA plate was performed.

Biochemical test:

Sugar utilization-

Peptone water basal medium with 1% of each sugar maltose, Lactose, Sucrose, Glucose, Mannitol with phenol red indicator in individual tube inoculated with loopful culture of respective organism and incubated at 37ºC for 24 hrs. Production of acid changes medium colour red to yellow. [7]

Enzyme Production-

Catalase test- culture of organism streaked on Sterile Nutrient agar slant and after incubation at 37ºC for 24 hrs 3% H_2O_2 reagent was added to see effervences. [7]

Germ tube test- (to confirm *Candida albicans*)-

Culture of the organism was incubated with Human serum at 37ºC for 2 hrs and observed under microscope for germ tube.

6. Spices extract preparation:

Cinnamomum verum (cinnamon), *Curcuma longa* (turmeric) were procured from the local market. All spices was dried at room temperature and grounded in fine powder using mixer grinder. Aqueous extract were prepared. Infusion method for extraction is used, where 1 gm powder of cinnamon and turmeric were mixed in 10 ml distilled water separately, mixture was kept on water bath at boiling for 30 min. Mixture was filtered through muslin cloth and kept in an oven at 40º C for drying, Further dried extract was mixed with distilled water 20% (w/v) and used to check antimicrobial activity. [8]

7. Characterization of AgNPs:

Silver nanoparticles was characterized with the help of UV-Vis spectroscopy and Electron Microscopy (SEM). Synthesized AgNPs solution used periodically to observe the absorbance of sample between 190 to 800 nm with UV- vis spectrophotometer at room temperature. Further characterization of AgNPs was done by using Scanning Electron Microscopy (SEM)

The particle size and surface morphology of synthesized AgNPs was determined by using SEM- Scanning Electron Microscopy (SEM) [9]. Analysis of nanoparticles size was done at CIF, Savitribai Phule Pune University.

8. Method for performing antimicrobial activity:

Well diffusion method was used for performing antimicrobial activity of synthesized AgNPs and spices extract. [10] In brief a pure colony of isolate *streptococcus spp*. and *candida spp.* each was grown in Muller Hinton Broth (MHB) medium for overnight at 37ºC (180 rpm), and then sub cultured in the same medium to adjust optical density of 0.13 at 600 nm. The prepared inocula was uniformly spread on plates of Muller Hinton Agar (MHA) and wells (6 mm) was made by using sterile cork borer. Then 20 µl of AgNPs solution and Cinnamon extract and turmeric extract was added in separate wells on each separate culture loaded MHA plates. At the same time streptomycin (10 µg/ml) was used in separate well as control. Further these plates were incubated at 37ºC for 24 hrs., As the activity performed in duplicates, then ZOI was measured in mm and mean of the ZOI was recorded [11].

III. RESULTS

Characterization of silver Nanoparticles:

Change in the solution after incubation from colorless to brown primarily indicates silver nanoparticles were synthesized. (Fig-1) UV- vis spectroscopy were used to confirm the formation of silver nanoparticles in reaction solution. Fig-2 displayed the UV-vis spectrum of AgNPs colloidal solution. A broad SPR band arose at 404 nm, indicating formation of AgNPs.

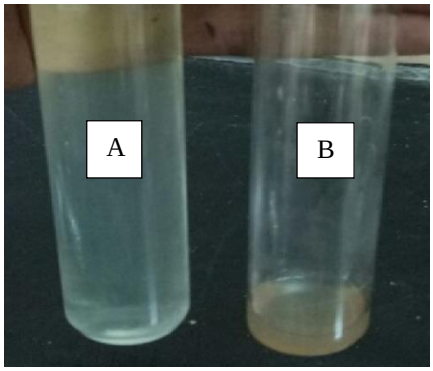


Fig-1 Synthesis of AgNPs
A- Before Incubation
B- After 24 hrs. of Incubation

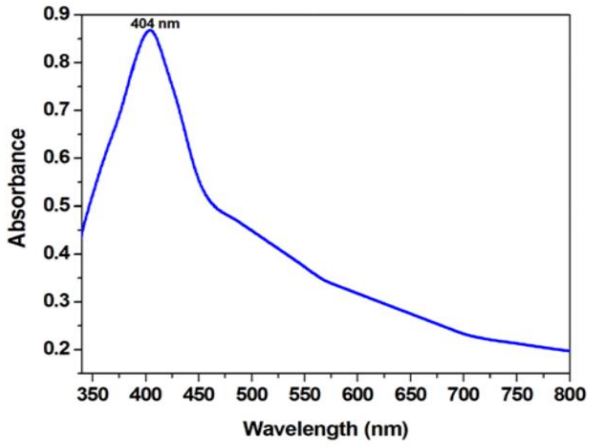


Fig-2 UV-Visible Spectrum of solution formed after 12 hrs. of incubation

Scanning Electron Microscopy:

Figure shows the SEM image of the synthesized silver nanoparticles, SEM image confirms the existence of small and spherical nanoparticles, it also shows aggregation of nanoparticles which may be occur at sample preparation [12]. The average particle size was found around 60 nm.

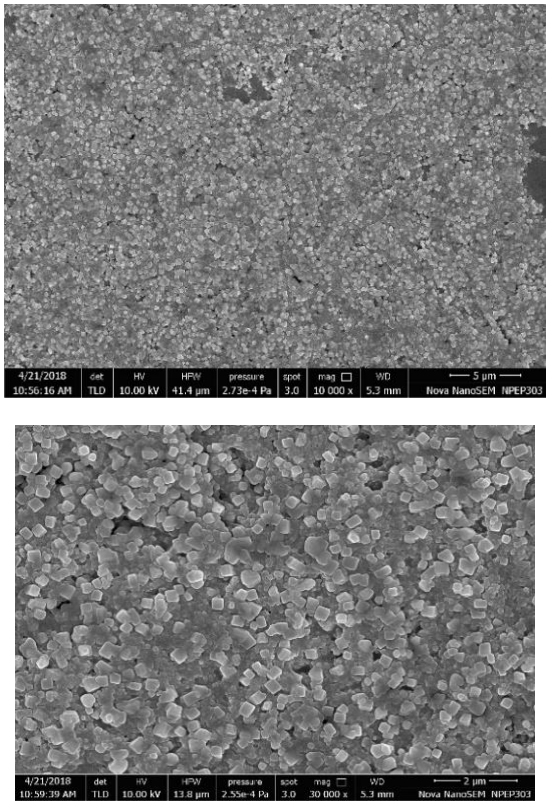


Fig 3- SEM image of synthesized AgNPs by using Buttermilk.

Identification of Isolates:

Identification of the isolates was done on the basis of morphological, cultural and biochemical characteristics referring to the bergey's manual of determinative bacteriology.

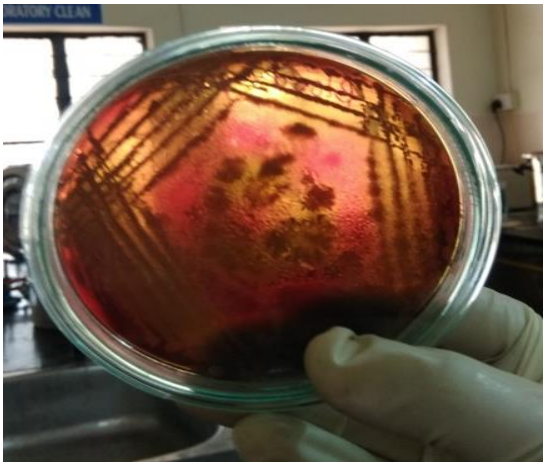


Fig 4: Isolate 1 on Blood agar showing alpha Hemolysis

Fig-5: Isolate 2 on Potato Dextrose agar White, Large and sticky colonies

Table 1: Morphological and cultural characteristics of Isolate1 and Isolate 2

Isolate 1	Isolate 2
Growth on Blood Agar	Growth on Potato Dextrose Agar
Alpha hemolysis with greenish zone was observed on Blood Agar plate.	White, Large and sticky colonies was observed on PDA.
Gram Staining- Gram Positive	Monochrome staining- Purple coloured spherical organism observed
Motility- Non-motile	Motility- NA

Biochemical tests was performed by referring to bergye's manual of determinative bacteriology.

Table 2: Biochemical Test of Isolate 1 (From Blood Agar)

Biochemical Test (Sugar Utilization)	Results
Maltose	Positive
Lactose	Positive
Sucrose	Positive
Glucose	Positive
Mannitol	Positive

Enzyme Production	Result
Catalase Test	Positive

Table 3: Biochemical Test of Isolate 2 (From PDA)

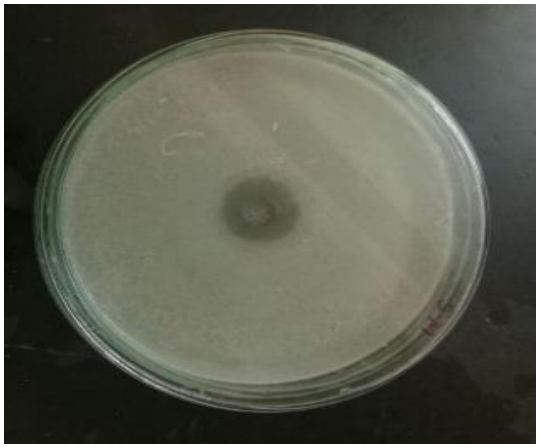
Biochemical Test (Sugar Utilization)	Results
Maltose	Positive
Glucose	Positive
Lactose	Negative

Germ tube test	Result
Formation of germ tube	Negative

On the basis of results obtained from Morphological, Cultural and biochemical tests by referring to Bergey's manual the isolated organisms may be *Streptococcus spp*. and *Candida spp*.

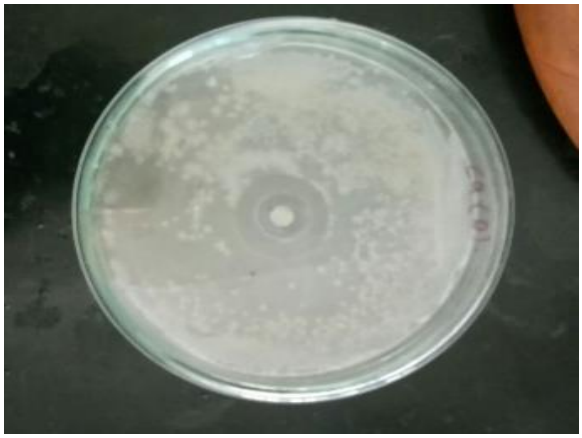
Antimicrobial activity of *Cinnamomum verum* (cinnamon), *Curcuma longa* (turmeric) and synthesized AgNPs against isolated organisms:

Out of the selected spices *Cinnamomum verum* showed maximum zone of inhibition in diameter against these isolates. It showed 15 mm zone against *Streptococcus* and around 12 mm zone against *Candida* spp. Whereas, AgNPs showed around 25 mm zone of inhibition against *Streptococcus* spp. and 20 mm against *Candida* spp.

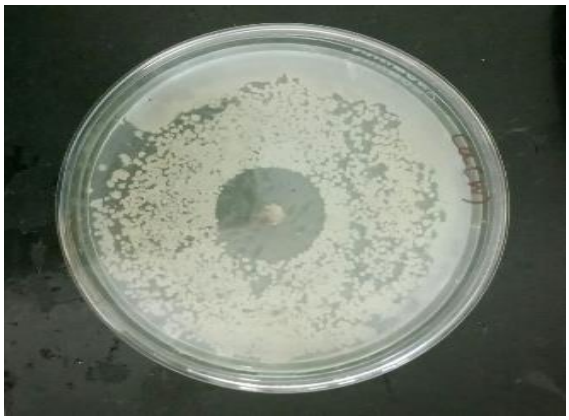


Zone of Cinnamon Against *Streptococcus spp.*

Zone of Cinnamon against *Candida spp.*



Zone of AgNPs against *Candida spp.*



Zone of AgNPs against *Streptococcus spp*

Zone of Turmeric Against *Streptococcus spp.*

Zone of Turmeric Against *candida spp.*

Fig 6: Antimicrobial activity of Cinnamon, AgNPs and turmeric extract against *Streptococcus spp.* and *Candida spp.*

Table 4: Antimicrobial activity of spices and AgNPs against dental pathogens

Name of organism	Zone of Inhibition (mm)		
	Cinnamomum verum	*Curcuma longa*	Silver Nanoparticles
Streptococcus spp.	15	1	25
Candida spp.	12	2	20

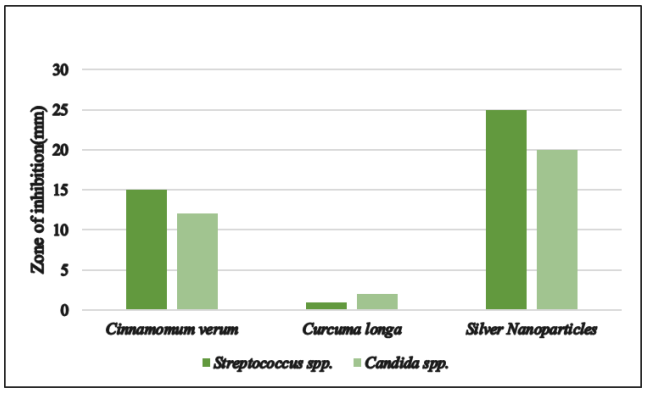


Fig 7- Activity of spices and AgNPs against Dental pathogens

DISCUSSION

Silver nanoparticles was synthesized by using culture supernatant *Lactobacillus delbruckii by* sani et. al (2017) Their result support this hypothesis that buttermilk and dairy waste may also be used for the silver nanoparticle synthesis.

Antimicrobial activity of Indian spices was checked against pathogenic bacteria by Rawat et.al.(2015), Their result support this hypothesis that spices extract can be used to check antimicrobial activity against Dental pathogens.

Antimicrobial activity of silver nanoparticles was checked against oral microbiomes by Francisco et al (2019), Potential inhibitory effect was seen.

In current study AgNPs showed good inhibitory effect against dental pathogens (zone of inhibition- 25 mm and 20 mm against *Streptococcus spp.* and *Candida spp.* respectively), where experiment is performed in duplicates.

IV. CONCLUSION AND FUTURE SCOPE

The method was optimized first time for silver nanoparticle synthesis using buttermilk filtrate.
The current study is a preliminary research work focusing on the investigation of antimicrobial potential of silver nanoparticles synthesized from buttermilk filtrate and also some Indian spices against dental cavity causing organisms.
Silver nanoparticles synthesized from buttermilk filtrate was found most effective against dental pathogens followed by cinnamon and turmeric extract.
After detailed study on MIC and MBC of silver nanoparticles against Clinical pathogens, further it may be used for the treatment of Dental caries.
Also Indian spices like cinnamon, turmeric in addition with silver nanoparticles may also be used for designing the new herbal toothpaste which can show great effect against cavity causing organisms.

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