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RESEARCH ARTICLE

A Comparative Antimicrobial Activity of Terminalia Chebula Mediated Silver Nanoparticles Based Mouthwash

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ABSTRACT

Aim: The aim of this study is to compare the antimicrobial activity of Terminalia chebula mediated silver nanoparticles based mouthwash with a commercially available herbal mouthwash for its activity against 4 pathogens namely, Streptococcus mutans, Staphylococcus aureus, Enterococcus faecalis and Candida albicans at different concentrations (25 µL, 50 µL and 100 µL).

Background: Silver nanoparticles synthesised from plant sources are eco-friendly, cost-effective and have a variety of applications. The antimicrobial activity of silver nanoparticles produced by various herbal sources has been well studied. Terminalia chebula has been used in the study to produce silver nanoparticles due to their anti-inflammatory, antioxidant and antimicrobial properties.

Materials and methods: Terminalia chebula was used to prepare the plant extract, followed by the synthesis of silver nanoparticles. UV-vis spectroscopy was done for characterisation of the Ag-NPs synthesised. The NPs were then used to prepare a mouthwash. The Agar-Well Diffusion Method was used to determine the antimicrobial efficacy of Ag-NPs mouthwash in different concentrations, namely 25, 50 & 100 µL. A commercially available herbal mouthwash was used as a positive control. The zone of inhibition was recorded for each plate and the results were analysed using IBM SPSS software (Version 20.0).

Results: A colour change was observed after the synthesis of Ag-NPs. The prepared particles were then characterised by a peak seen at 440 nm in UV-vis-spectroscopy. The zone of inhibition increased in size with an increase in Ag-NP concentration.

Conclusion: Silver nanoparticles produced using terminalia chebula has good antimicrobial properties and can be used as a mouthwash as an alternative to other conventional herbal mouthwashes.

KEYWORDS:
Silver nanoparticles, terminalia chebula, mouthwash, antimicrobial, innovative, eco-friendly etc.

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INTRODUCTION

In recent years, silver nanoparticles have been vastly used in the medical field due to its antioxidant, anti-inflammatory, antimicrobial and antifungal properties. (1) They are highly efficient growth inhibitors against a variety of microorganisms and have been used in diverse medical equipment and antibacterial control systems. (2) The use of these silver nanoparticles is highly important as several pathogenic bacteria have developed resistance against the available antibiotics. (3) Silver nanoparticles are produced through physical and chemical methods which are expensive and less eco-friendly. Thus, herbal production of silver nanoparticles has seen a rise in recent years as an alternative. (4,5) In this study we have used Terminalia chebula to develop silver nanoparticles and further use it for our study.

T. chebula is a medicinal plant known as Kadukka in Tamil. (6,7) It has been in use as a traditional medicinal herb in the

Indian subcontinent. Studies have shown that it has antibacterial and antioxidant activities owing to its chief constituents which are gallic acid, chebulic acid, chebulagic acid and corilagin. (6)

Long since, various herbal products have been used for cleaning the oral cavity. Extracts of guava, neem, tulsi, green tea etc. have advantages of varying degrees over the chemical mouthwashes. These mouthwashes have lesser side effects and do not cause staining of teeth. They can be easily formulated and are available over the counter for the general public to use. (8) Indigenously prepared mouthwashes using neem and mango have shown a highly significant decrease in the gingival index which is comparable to the action of chlorhexidine. (9)

Our team has extensive knowledge and research experience that has translated into high quality publications (10–22),(23-27) (28) (29). The aim of this study is to test the antimicrobial activity of a mouthwash formulated using herbally synthesised silver nanoparticles using Terminalia Chebula and compare it with a commercially available herbal mouthwash for its activity against 4 pathogens namely, Streptococcus mutans, Staphylococcus aureus, Enterococcus faecalis and Candida albicans at different concentrations (25 µL, 50 µL and 100 µL).

MATERIALS AND METHODS

Preparation Of Plant Extract

Leaves of Terminalia chebula were collected from Chennai. The collected leaves were washed 3-4 times using distilled water and dried in shade for 7-14 days. The well dried leaves were then crushed into powder by using mortar and pestle. Later 1 gm of the powder was dissolved in distilled water and boiled for 5-10 minutes at 60-70°C. The solution was then filtered using whatman no.1 filter paper. Finally, the filtered plant extract was collected and stored at 4°C until further use.

Synthesis Of Nanoparticles

1 millimolar of silver nitrate was dissolved in 90 mL of double distilled water to make a metallic solution. To this, the plant extract of Terminalia chebula was added and this solution was made into a 100 mL solution. The colour change was observed visually and photographs were taken for the record. The formulation was later kept in a magnetic stirrer for the synthesis of its nanoparticles.

Characterisation of the Synthesised Silver Nanoparticles

The synthesis of silver nanoparticles was initially characterised by using UV-vis-spectroscopy, where 3 ml of the solution was taken in a cuvette and scanned in a UV-vis-spectrometer within a wavelength field limit of 250 nm to 750 nm. The results were recorded for its graphical analysis.

Preparation of the Silver Nanoparticle Powder and mouthwash

The silver nanoparticle formulation was centrifuged in a Lark refrigerated centrifuge. The centrifugation was done at 8000 rpm for a time duration of 10 minutes and the resultant pellet was collected and then washed twice with distilled water. It was later purified and dried at 60°C. Finally, the silver nanoparticle powder that was obtained was collected and stored in an air-tight Eppendorf tube. The silver nanoparticles solution was mixed with foaming agent, preservative, sweetener, flavouring agent and deionised water to a mouthwash.

Study Of Antimicrobial Activity Of Nanoparticles Against Streptococcus Mutans, Staphylococcus Aureus, Enterococcus Faecalis And Candida Albicans

The Agar-Well Diffusion Method was used to assess the antimicrobial efficacy of Ag-NPs. Different dilutions of Ag-NPs mouthwash were tested against 4 organisms - Streptococcus mutans, Staphylococcus aureus, Enterococcus faecalis and Candida albicans. Their fresh suspensions were dispersed on the surface of Muller-Hinton Agar containing plates (for Streptococcus mutans, Staphylococcus aureus, Enterococcus faecalis) and Rose Bengal Agar containing plate (for Candida albicans). Ag-NP concentrations of (25, 50 & 100) µL were incorporated into the wells and the plates were then incubated at 37°C for 24 hours. A commercially available herbal mouthwash was used as a positive control and the zones of inhibition were recorded for each well.

Statistical Analysis

The data was recorded in a Microsoft Excel 2016 (Microsoft Office 10) spreadsheet and was later exported to the Statistical Package for the Social Sciences for Windows (Version 20.0, SPSS, Inc., Chicago, USA) where it was subjected to statistical analysis. A one-way ANOVA test was used with the level of significance set at $p<0.05$.

RESULTS AND DISCUSSION

Visual Observation

As the terminalia chebula was mixed with the aqueous solution of silver nanoparticles, it changed colour from white to dark brown due to the conversion of silver ions to silver nanoparticles. This is due to the excitation of silver plasmon resonance of synthesised silver nanoparticles.

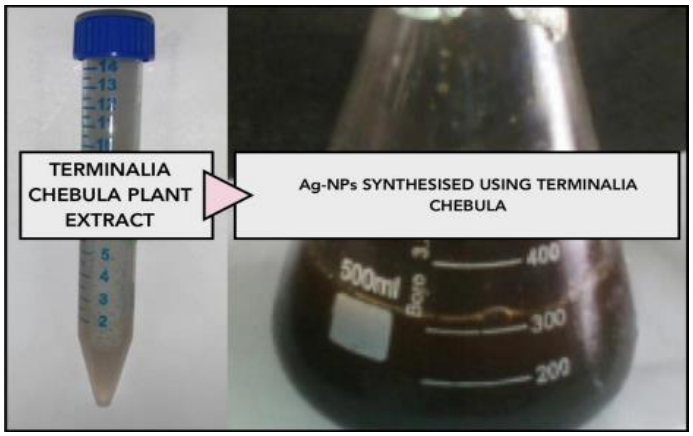

Fig.1: Showcasing a visually observable colour change from white to dark brown after the synthesis of nanoparticles from a metallic formulation of silver nitrate using Terminalia chebula.

UV-vis SPECTROSCOPY

The bioreduction of pure silver nitrate to silver nanoparticles was characterised by employing UV-vis-spectroscopy. This was carried out to assess the successful formation and stability of the Ag-NPs. It was done using a UV-vis-spectrometer within a wavelength field limit of (250 – 750) nm. Its peak (Surface Plasmon Resonance) was noted at 440 nm.

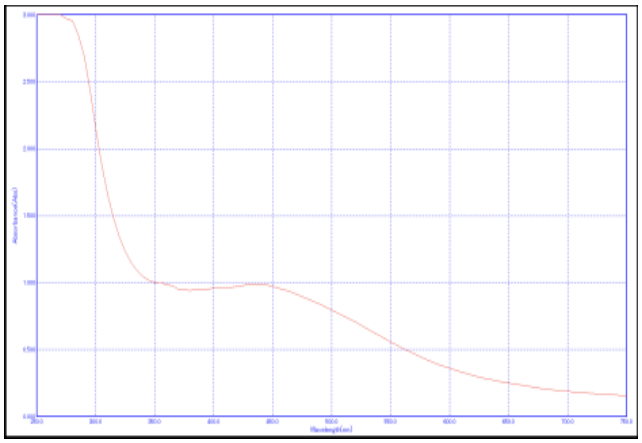

Fig.2: Showing the characterisation of Ag-NPs post bioreduction using UV-vis-spectroscopy, showing a peak at 440 nm with wavelength given in nanometer units in the 'X' axis and absorbance in the 'Y' axis.

Antimicrobial Activity

The zone of inhibition was seen to increase with an increase in the concentration of the silver nanoparticles in the agar well. It was also registered that the zone of inhibition was the largest for the bacteria S. mutans and smallest for C. albicans. All measurements were done in triplicate.

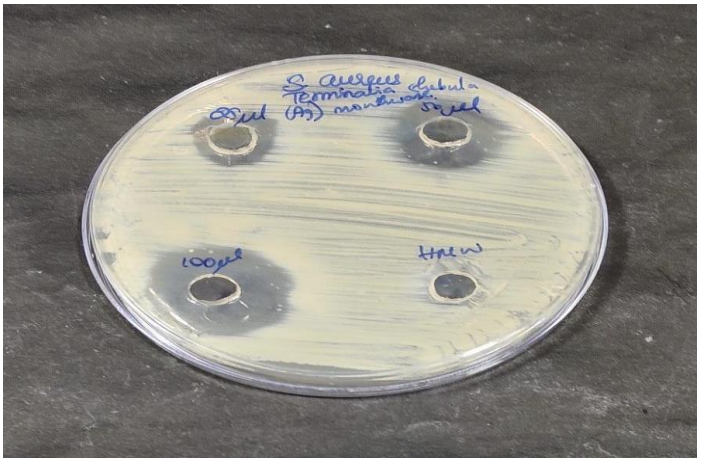

Fig.3: Showcasing the effect of various concentrations of Ag-NPs on Staphylococcus aureus with their zones of inhibition:
1. Effect of 25 µL of Ag-NPs on Staphylococcus aureus with its moderately sized zone of inhibition
2. Effect of 50 µL of Ag-NPs on Staphylococcus aureus with its large sized zone of inhibition
3. Effect of 100 µL of Ag-NPs on Staphylococcus aureus with its large zone of inhibition
4. Effect of herbal mouthwash on Staphylococcus aureus with its less apparent zone of inhibition

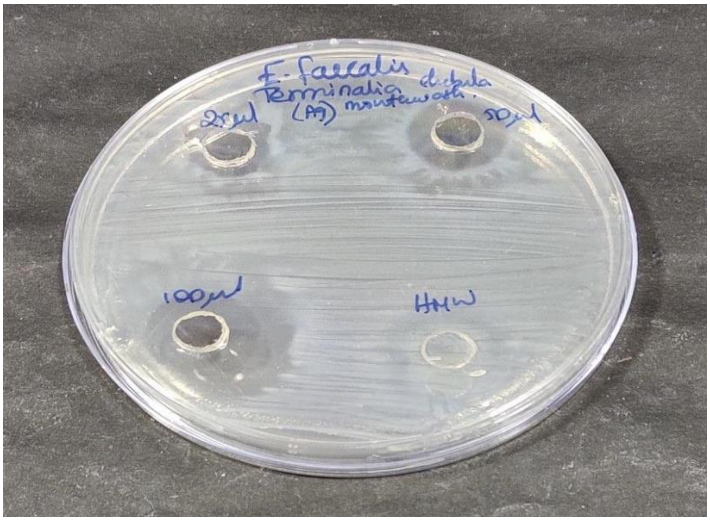

Fig.4 - Showcasing the effect of various concentrations of Ag-NPs on Enterococcus faecalis with their zones of inhibition:
1. Effect of 25 µL of Ag-NPs on Enterococcus faecalis with its moderately sized zone of inhibition
2. Effect of 50 µL of Ag-NPs on Enterococcus faecalis with its large sized zone of inhibition
3. Effect of 100 µL of Ag-NPs on Enterococcus faecalis with its large sized zone of inhibition
4. Effect of herbal mouthwash on Enterococcus faecalis with its less apparent zone of inhibition

Fig.5: Showcasing the effect of various concentrations of Ag-NPs on Streptococcus mutans with their zones of inhibition:
1. Effect of 25 µL of Ag-NPs on Streptococcus mutans with its moderately sized zone of inhibition
2. Effect of 50 µL of Ag-NPs on Streptococcus mutans with its large sized zone of inhibition
3. Effect of 100 µL of Ag-NPs on Streptococcus mutans with its large zone of inhibition
4. Effect of herbal mouthwash on Streptococcus mutans with its less apparent zone of inhibition

Fig.6: Showcasing the effect of various concentrations of Ag-NPs on Candida albicans with their zones of inhibition:
1. Effect of 25 µL of Ag-NPs on Candida albicans with its moderately sized zone of inhibition
2. Effect of 50 µL of Ag-NPs on Candida albicans with its large sized zone of inhibition
3. Effect of 100 µL of Ag-NPs on Candida albicans with its large zone of inhibition
4. Effect of herbal mouthwash on Candida albicans with its less apparent zone of inhibition

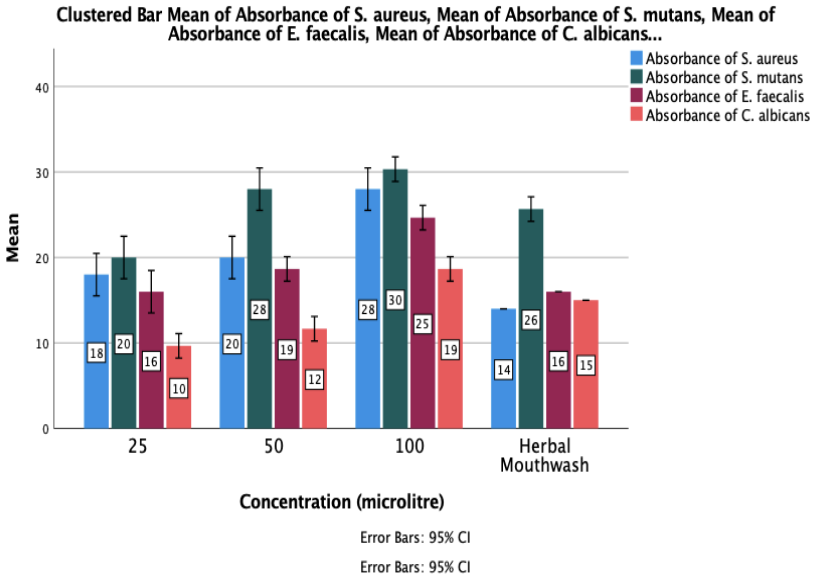

Fig.7: Bar chart showcasing the zones of inhibition for all three dilutions of Ag-NPs mouthwash along with the positive control (commercial herbal mouthwash) the dilutions of the silver nanoparticles in microliter units and the positive control are denoted by the 'X' axis and the measurements of the zones of inhibition in millimetre units are denoted by the 'Y' axis.The blue bars depict the zones of inhibition for Staphylococcus aureus, the green bars for Streptococcus mutans, the pink bars for Enterococcus faecalis and the orange bars for Candida albicans. There is a significant increase in the zone of inhibition with an increase in concentration ($p<0.05$) (One Way anova followed by post hoc). At 100 microlitre concentration the zone of inhibition against all the tested organisms were significantly more for Ag-NP mouthwash than the commercially available herbal mouthwash ($p<0.05$) (One Way anova followed by post hoc).

The zone of inhibition for S. aureus (18 mm at 25 microlitre concentration) is higher than that of the commercially available mouthwash (14 mm) ($p=0.002$; $p<0.05$; statistically significant). The zone of inhibition for S. mutans (28 mm at 50 microlitre concentration) is higher than that of the commercially available mouthwash (26 mm) ($p=0.033$; $p<0.05$; statistically significant). The zone of inhibition for E. faecalis (19 mm at 50 microlitre concentration) is higher than that of the commercially available mouthwash (16 mm) ($p=0.004$; $p<0.05$; statistically significant). The zone of inhibition for C. albicans (19 mm at 100 microlitre concentration) is higher than that of the commercially available mouthwash (15 mm) ($p<0.001$; $p<0.05$; statistically significant).

For S. aureus, the mean zone of inhibition increases with increase in concentration from 18 mm at 25 microlitre to 20 mm at 50 microlitre ($p=0.085$; $p>0.05$; not statistically significant), from 18 mm at 25 microlitre to 28 mm at 100 microliter ($p<0.001$; $p<0.05$; statistically significant) and from 20 mm at 50 microlitre to 28 mm at 100 microliter ($p<0.001$; $p<0.05$; statistically significant).

For S. mutans, the mean zone of inhibition increases with increase in concentration from 20 mm at 25 microlitre to 28 mm at 50 microlitre ($p<0.001$; $p<0.05$; statistically significant), from 20 mm at 25 microlitre to 30 mm at 100 microliter ($p<0.001$; $p<0.05$; statistically significant) and from 28 mm at 50 microlitre to 30 mm at 100 microliter ($p=0.033$; $p>0.05$; not statistically significant).

For E. faecalis, the mean zone of inhibition increases with increase in concentration from 16 mm at 25 microlitre to 19 mm at 50 microlitre ($p<0.004$; $p<0.05$; statistically significant), from 16 mm at 25 microlitre to 25 mm at 100 microliter ($p<0.001$; $p<0.05$; statistically significant) and from 19 mm at 50 microlitre to 25 mm at 100 microliter ($p<0.001$; $p<0.05$; statistically significant).

For C. albicans, the mean zone of inhibition increases with increase in concentration from 10 mm at 25 microlitre to 12 mm at 50 microlitre ($p=0.005$; $p<0.05$; statistically significant), from 10 mm at 25 microlitre to 19 mm at 100 microliter ($p<0.001$; $p<0.05$; statistically significant) and from 12 mm at 50 microlitre to 19 mm at 100 microliter ($p<0.001$; $p<0.05$; statistically significant).

Table 1: Table depicts the descriptive data of the zone of inhibition at various concentrations of the Ag-NP mouthwash against the four species as observed in this study

Organism	Concentration	N	Mean	Std. deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
Absorbance of S. aureus	25	3	18.00	1.000	0.577	15.52	20.48	17	19
	50	3	20.00	1.000	0.577	17.52	22.48	19	21
	100	3	28.00	1.000	0.577	25.52	30.48	27	29
	Herbal Mouthwash	3	14.00	0.000	0.000	14.00	14.00	14	14
	Total	12	20.00	5.377	1.552	16.58	23.42	14	29
Absorbance of E. faecalis	25	3	16.000	1.0000	0.5774	13.516	18.484	15.0	17.0
	50	3	18.667	0.5774	0.3333	17.232	20.101	18.0	19.0
	100	3	24.667	0.5774	0.3333	23.232	26.101	24.0	25.0
	Herbal Mouthwash	3	16.000	0.0000	0.0000	16.000	16.000	16.0	16.0
	Total	12	18.833	3.7376	1.0790	16.459	21.208	15.0	25.0
Absorbance of S. mutans	25	3	20.00	1.000	0.577	17.52	22.48	19	21
	50	3	28.00	1.000	0.577	25.52	30.48	27	29
	100	3	30.33	0.577	0.333	28.90	31.77	30	31
	Herbal Mouthwash	3	25.67	0.577	0.333	24.23	27.10	25	26
	Total	12	26.00	4.068	1.174	23.42	28.58	19	31
Absorbance of C. albicans	25	3	9.67	0.557	0.333	8.23	11.10	9	10
	50	3	11.67	0.557	0.333	10.23	13.10	11	12
	100	3	18.67	0.557	0.333	17.23	20.10	18	19
	Herbal Mouthwash	3	15.00	0.000	0.000	15.00	15.00	15	15
	Total	12	13.75	3.596	1.038	11.47	16.03	9	19

Table 2: Table depicts the one way ANOVA analysis performed for the various concentrations of the Ag-NP mouthwash's antimicrobial activity on the four species as observed in this study.

		Sum of Squares	df	Mean Square	F	Sig.
Absorbance of S. aureus	Between Groups	312.000	3	104.000	138.667	<0.001
	Within Groups	6.000	8	0.750		
	Total	318.000	11			
Absorbance of S. mutans	Between Groups	176.667	3	58.889	88.333	<0.001
	Within Groups	5.333	8	0.667		
	Total	182.000	11			
Absorbance of E. faecalis	Between Groups	150.333	3	50.111	120.267	<0.001
	Within Groups	3.333	8	0.417		
	Total	153.667	11			
Absorbance of C. albicans	Between Groups	140.250	3	46.750	187.000	<0.001
	Within Groups	2.000	8	0.250		
	Total	142.250	11			

Table 3: Table showing pairwise comparison between groups using Post Hoc (Tukey HSD) test results, with the confidence interval set to 95%.

Dependent Variable (Tukey HSD)	(I) Concentration	(J) Concentration	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Absorbance of S. mutans	25 µl	50 µl	-8.000*	0.667	<0.001	-10.13	-5.87
		100 µl	-10.333*	0.667	<0.001	-12.47	-8.20
		Herbal Mouthwash	-5.667*	0.667	<0.001	-7.80	-3.53
	50 µl	100 µl	-2.333	0.667	0.033	-4.47	-0.20
		Herbal Mouthwash	2.333	0.667	0.033	0.20	4.47
	100 µl	Herbal Mouthwash	4.667	0.667	<0.001	2.53	6.80
Absorbance of S. aureus	25 µl	50 µl	-2.000*	0.707	0.085	-4.26	-0.26
		100 µl	-10.000*	0.707	<0.001	-12.26	-7.74
		Herbal Mouthwash	4.000*	0.707	0.002	1.74	6.26

	50 µl	100 µl	-8.000*	0.707	<0.001	-10.26	-5.74
		Herbal Mouthwash	6.000*	0.707	<0.001	3.74	8.26
	100 µl	Herbal Mouthwash	14.000*	0.707	<0.001	11.74	16.26
Absorbance of E. faecalis	25 µl	50 µl	-2.6667*	0.527	0.004	-4.354	-0.979
		100 µl	-8.6667*	0.527	<0.001	-10.354	-6.979
		Herbal Mouthwash	0.000*	0.527	1.000	-1.688	1.688
	50 µl	100 µl	-6.000*	0.527	<0.001	-7.688	-4.312
		Herbal Mouthwash	2.6667*	0.527	0.004	0.979	4.354
	100 µl	Herbal Mouthwash	8.6667*	0.527	<0.001	6.979	10.354

Absorbance of C. albicans	25 µl	50 µl	-2.000*	0.408	0.005	-3.31	-0.69
		100 µl	-9.000*	0.408	<0.001	-10.31	-7.69
		Herbal Mouthwash	-5.333*	0.408	<0.001	-6.64	-4.03
	50 µl	100 µl	-7.000*	0.408	<0.001	-8.31	-5.69
		Herbal Mouthwash	-3.333*	0.408	<0.001	-4.64	-2.03
	100 µl	Herbal Mouthwash	-3.667*	0.408	<0.001	2.36	4.97

Silver nanoparticles exhibit strong antimicrobial activity against bacteria, viruses and fungi. This can be attributed to its membrane damage causing potential which has a highly toxic effect on the bacteria. Because of this property it can be used in controlling infections in areas of mild to severe burns. (30) Herbally mediated silver nanoparticles have shown good antifungal activity which is comparable to other antifungal drugs commercially available. (31) T. chebula extract contains 30% tannins, which is highly appreciated for its anticaries and antimicrobial activity. Tannic acid is bacteriostatic and bactericidal to some gram positive and gram negative bacteria. (32) It also possesses the property of substantivity which is added to its effectiveness as an anticaries property. (33) The extract of T. chebula shows broad spectrum antibacterial activity. (34) A study by Bhate et al. stated that the efficacy of Terminalia chebula mouth rinse was as good as that of chlorhexidine in terms of removal of dental plaque and reduction of gingival bleeding. (35) The present study reveals that the mouthwash prepared with Ag-NPs with T. chebula is superior to commercially available herbal mouthwash in its antimicrobial properties at 100 microlitre concentration. Even at a lesser concentration of 50 microlitre it had superior anti microbial effect for all the tested microbes except for Candida albicans(36) (37) (38) (39) (40) (41) (42) (43) (44) (45) (46) ((46,47) (48) (49). In the present study we have used a commercially available herbal mouthwash as the positive control. Further research wherein comparison with the gold standard mouthwash, chlorhexidine needs to be done to see whether it can be a substitute for the chlorhexidine mouthwash.

CONCLUSION

Through the outcome of our study we have speculated that silver nanoparticles synthesized using Terminalia chebula possess an evident antimicrobial potential against Staphylococcus aureus, Streptococcus mutans, Enterococcus faecalis and Candida albicans.

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CONFLICT OF INTEREST

None declared.

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