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Evaluation of Antioxidant and Antimicrobial Efficacy of Camellia Sinensis and Alstonia Scholaris Extracts on Streptococcus Mutans and Lactobacillus Acidophilus - An in Vitro Study

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Context Dental caries is showing an upward trend in India and there is a need to explore innovative strategies to prevent the disease. Literature evaluating antibacterial activity of Camellia sinensis and Alstonia scholaris plant extracts on Streptococcus mutans and Lactobacillus acidophilus is practically non-existent. To assess the minimum inhibitory concentration (MIC) and antimicrobial efficacy of Camellia sinensis and Alstonia scholaris on S. mutans and L. acidophilus. This was an in vitro study carried over a period of three months. The leaves of Camellia sinensis and Alstonia scholaris were collected, and crushed to obtain coarse powder. Plant extraction was performed using Soxhelet appartus. Anti- oxidant assay was performed for both the plant extracts against DPPH radical using Spectrophotometer at 517nm. Inhibition percentage was calculated through absorbance value measured from spectrophotometer. Anti- microbial activity of both the plant extracts against Microbial Type Culture Collection strains of Streptococcus mutans and Lactobacillus acidophilus was assessed using Agar well diffusion method. 0.2% Chlorhexidine was used as positive control and ethanol as negative control. The experiment was performed in triplicates. Mean inhibition zone in each set of experiment was computed using three readings after accounting for well diameter. One Way Analysis of Variance (ANOVA), Tukey's post hoc test and independent sample't' test were performed to compare the mean inhibition zone. The plant extracts were effective against Streptococcus mutans and Lactobacillus acidophilus. Camellia sinensis at 4% concentration produced a mean inhibition zone of 30.3± 3.9 mm against Streptococcus mutans and 23.8± 2.2 mm against Lactobacillus acidophilus. Alstonia scholaris at 10% concentration produced a mean inhibition zone of 21.6± 2.8 mm against Streptococcus mutans and 24.1± 1.6 mm against Lactobacillus acidophilus. Camellia sinensis and Alstonia scholaris have significant anti- oxidant and anti- microbial property against Streptococcus mutans and Lactobacillus acidophilus.

Keywords: Anti- Oxidant; Anti- Microbial; Alstonia Scholaris; Camellia Sinensis; Dental Caries; Dental Plaque; Lactobacillus Acidophilus; Streptococcus Mutans.

Initially originated from China, tea has ruled heart of people as one of the most popular beverage.[1] Tea is known to be produced from *Camellia sinensis*, a shrub of *Theaceae* family.[2] Since ancient era, herbs have been used in nutrition, fragrance, flavoring, and beverages and as an

essential medicine.[3] In Japan, there is a tradition to drink green tea after every meal to cleanse their mouth.[4] Green tea is enriched with proteins, phenolic compounds, flavonoids and minerals.[3] Many studies have reported its antibiotic, anti inflammatory, anti oxidative, antifungal, anti diabetic, anti viral, anti mutagenic properties.[5-11] The limited literature suggests that green tea interferes with caries formation at every step and inhibit the process.[11]

Similarly, *Alstonia scholaris* has also reported potent anti microbial activity in ancient era.[12,13,14,15] *Alstonia scholaris* belongs to the family of *Apocynaceae.* The plant is called as *Datyuni* and *Chatiun* in Hindi, *Devil tree* in English, *Doddapala* in kannada and *Saptaparna* in Sanskrit. Traditionally, the plant is used as analgesic, immunomodulant in liver disorders, kidney problems, skin disorders, respiratory disorders, urinogenital disorders, Central Nervous System disorder, cardiac disorders and gastro- intestinal disorders. The plant possesses antimicrobial, antioxidant, anticancer, analgesic, anti-inflammatory, anti fertility, and anti inflammatory activity. It contains various phytochemicals like alkaloids, phlobatanins, phenolics, steroids, saponins, flavonoids and tannins.[16]

Streptococcus mutans (S. mutans) is one among the leading micro organisms responsible for dental caries.[1] It initiates the dental caries and *Lactobacillus acidophilus* (*L. acidophilus*) is further responsible for its progression.[17] The literature available on the antimicrobial efficacy of *Camellia sinensis* and *Alstonia scholaris* plant extracts on these oral microorganisms is scanty. Hence, this research was undertaken to systematically assess the minimum inhibitory concentration and antimicrobial efficacy of *Camellia sinensis* extract and *Alstonia scholaris* on *S. mutans* and *L. acidophilus*.

MATERIALS AND METHOD

Study design and setting

This was an *in vitro* study conducted at Division of Biotechnology and Bioinformtics, Department of Water and Science, Faculty of Life Science, JSS Academy of Higher Education and Research over a period of three months. Study protocol was approved by the Institutions ethics committee (IEC).

Plant material

The leaves of *Camellia sinensis* and *Alstonia scholaris* plants were collected from in and around Mysuru after authentication by a taxonomist. The leaves were rinsed with water and shade dried over a period of three-four weeks at room temperature. The dried leaves were powdered using domestic blender and mixer grinder to obtain fine powder. Thereafter, the powder was filled in airtight plastic bottles and stored in refrigerator at 4^0C until further use.

Plant extraction

The extraction process of finely ground *Camellia sinensis* and *Alstonia scholaris* were carried out using Soxhlet apparatus. "Thimble" was filled with 50 g of ground powder and loaded into the Soxhlet extractor. Subsequently, distillation flask was filled with solvent (ethanol). This cycle was repeated many times to get desired concentrated compound into the distillation flask. A rotary evaporator at 30°C–60°C was used to dry and concentrate the solvent extract under reduced pressure (30 ± 10 mbar) to a syrupy consistency. Finally, extract was dried at room temperature. The weight of the dried mass was recorded for further experimental studies.

Anti oxidant Assays

The Anti oxidant activity of *Camellia sinensis* and *Alstonia scholaris* was measured against 1, 1-diphenyl-2-picrylhydrazyl (DPPH) free radical activity. DPPH is a nitrogen centered free radical which gets reduced to diphenyl picryl hydrazine upon reduction. The color changes from purple to yellow/ colorless upon reduction.[15]

DPPH assay

Standard 1ml of ascorbic acid solution (control) was mixed with 3 ml of 0.002% DPPH solution and checked for absorbance using Ultra Violet-Visible Spectrophotometer at 517nm. Similarly, 1ml of *Camellia sinensis* and *Alstonia scholaris* was mixed with 0.002% DPPH solution at different concentrations (25, 50, 75, 100, 125 and 150 ìg/ml in methanol) and checked for absorbance at 517 nm. The mixtures were kept in dark for 30 min to stabilize them before measuring the absorbance. High free radical-scavenging was indicated by low absorbance of the mixture which in turn reflects high anti oxidant activity.

The percent inhibition was calculated for *Camellia sinensis* and *Alstonia scholaris* as:

Inhibition (%) of DPPH activity =A-B/A * 100

Where A is Absorbance of control and B is Absorbance of Test.

Experiments were conducted in triplicates.

Bacteria

MTCC (Microbial Type Culture Collection) strains of *S. mutans* (MTCC 890) and *L. acidophilus* (MTCC 10307) were collected. Bacteria were enlivened at chemical laboratory for further microbiological assay. Bacterial cultures were maintained on Brain Heart Infusion (BHI) agar slants with periodic sub culturing and stored at 4° C.

Antimicrobial efficacy testing

Agar well/ disc diffusion method was used to assess the antimicrobial efficacy of plant extracts (50 µl volume). 0.2% Chlorhexidine as positive control and ethanol as negative control was used. Initially antimicrobial efficacy was checked at varying concentration (1%, 2%, 3%, 4%, 6%, and 10%) for both the plant extracts. A transparent scale was used to measure the diameter of the inhibition zone at three different planes on the undersurface of agar plate. The experiment was further triplicated using the most effective single concentration based on the initial experimentation. Diameter of inhibition zone was computed at three different planes on the undersurface of agar plate. Mean inhibition zone was computed based on results of these experiments. Minimum inhibitory concentration was that minimum concentration of plant extract that inhibited the growth of these microorganisms.

Statistical Analysis

Data analysis was done using SPSS version 22. Mean diameter of inhibition zone was compared using One Way Analysis of Variance (ANOVA) and Tukey's post hoc test between test, positive and negative control for both the bacteria. Anti microbial efficacy between 4% *Camellia sinensis* and 10% *Alstonia scholaris* extracts against *S. mutans* and *L. acidophilus* was compared using independent sample't' test. Anti- oxidant capacity of Ascorbic acid (standard), *Camellia sinensis* and *Alstonia scholaris* was compared at different concentration using One- Way Analysis of Variance (ANOVA) and Tukey's post hoc test. The statistical significance was fixed at 0.05.

RESULTS

The details of plant extracts and bacteria used in the study are denoted in Table 1 and 2 respectively. The result showed potent antibacterial and anti- oxidant activity of *Camellia sinensis* and *Alstonia scholaris* extracts against *S. mutans* and *L. acidophilus*. Anti microbial activity was based upon the assessment of mean zone of inhibition. Larger mean zone of inhibition indicated higher antimicrobial activity. Similarly, anti- oxidant activity was assessed by recording absorbance value through spectrophotometer. Percentage inhibition of DPPH free radical was calculated through absorbance value. High inhibition percentage indicated high anti oxidant activity.

MIC of *Camellia sinensis* and *Alstonia scholaris* extracts against *S. mutans* and *L. acidophilus*

Camellia sinensis did not demonstrate any inhibitory activity against *S. mutans* and *L. acidophilus* at concentrations 1%, 2% and

Table 1. Details of plant extracts used in the experiment

Plant (common name)	Botanical name	Family	Weight of dried extract	Yield (%)
Green tea	*Camellia sinensis*	Theaceae	9.075 gm	18.15
Blackboard tree, Devil tree, Ditabark, Milkwood-pine, White cheesewood Chatian/Chitvan. (Hindi), Maddale (Kannada)	*Alstonia scholaris*	*Apocynaceae*	6.791	13.58

3%. *Alstonia scholaris* did not demonstrate any inhibition against *S. mutans* and *L. acidophilus* at 1%, 2% 3%, 4% and 6%. Hence, Minimum Inhibitory Concentration for *Camellia sinensis* against *S. mutans* and *L. acidophilus* was considered to be 4% while for *Alstonia scholaris*, it was 10%.

In the initial experiment, *Camellia sinensis* produced a mean inhibition zone of 27.5± 0.7mm against *S. mutans* and 26.0± 1.4mm against *L. acidophilus* at 4% concentration which was significantly lower when compared with mean Zone of Inhibition produced by 0.2% Chlorhexidine against *S. mutans* (32.5±0.7mm) and *L. acidophilus* (34.0±1.4mm) ($p < 0.05$, Table 3). Mean zone of inhibition significantly increased with increasing concentration of *Camellia sinensis* against *S. mutans* ($p < 0.05$, Table 3). However, there was no significant difference in the mean zone of inhibition produced by *Camellia sinensis* at 4% and 6% concentrations on these bacteria ($p = 0.38$, Table 3). Mean zone of inhibition significantly decreased with increasing concentration of *Camellia sinensis* against *L. acidophilus* ($p < 0.05$, Table 3).Here also, the difference between 4% and 6% was not statistically significant ($p > 0.41$, Table 3).

Alstonia scholaris at 10% concentration produced a mean inhibition zone of 23.0± 2.8mm and 25.5± 2.1mm against *S. mutans* and *L. acidophilus* respectively which was significantly lower than that produced by 0.2% chlorhexidine ($p < 0.05$, Table 3).

Mean inhibition zone of *Camellia sinensis* at 10% concentration against *S. mutans* was significantly higher (37.50± 3.54 mm) than that produced by 10% *Alstonia scholaris (*23.00± 2.83 mm). However, the difference in mean inhibition zone between 10% *Camellia sinensis* and 0.2% chlorhexidine was not statistically significant.

Antimicrobial efficacy of 4% *Camellia sinensis* and 10% *Alstonia scholaris* against *S. mutans* and *L. acidophilus* in subsequent experiments conducted in triplicates

Camellia sinensis produced a mean zone of inhibition of 30.3± 3.3mm, 23.8± 2.2mm against *S. mutans* and *L. acidophilus* respectively at 4 % concentration which was significantly less than that produced by 0.2% Chlorexidine against these bacteria (34.1± 2.1mm, 33.7± 1.7mm ($p < 0.05$, Table 4). *Alstonia scholaris* produced a mean zone of inhibition of 21.6± 2.8mm, 24.1± 1.6mm against *S. mutans* and *L. acidophilus* respectively at 10 % concentration which was significantly less than that produced by 0.2% Chlorexidine against these bacteria (24.0±2.3mm, 26.1± 1.9mm) ($p < 0.05$, Table 5). Negative control failed to inhibit the growth of these bacteria.

Mean inhibition zone produced by 4% *Camellia sinensis* against *S. mutans* (30.3± 3.3mm) was significantly higher than that produced by 10% *Alstonia scholaris* (21.6± 2.8mm) ($p < 0.001$, Table 6). However, the difference in the mean inhibition zone produced by 4% *Camellia sinensis* against *L.acidophilus* (23.8± 2.2mm) and 10% *Alstonia scholaris* (24.1± 1.6mm) was not statistically significant ($p > 0.05$, Table 6).

Antioxidant activity of *Camellia sinensis* and *Alstonia scholaris* in comparison with standard Ascorbic acid

The mean Inhibition percentage was found to be significant significantly higher for *Camellia sinensis* and *Alstonia scholaris* in comparison with Ascorbic acid at all the levels of concentration (25% 50%, 75%, 100%, 125% and 150%) ($p < 0.05$, Table 7). The mean Inhibition percentage increased with increasing concentrations of plant extracts. Multiple pair wise comparison between different concentrations of each plant extracts

Table 2. Details of bacteria used for anti microbial efficacy testing

Bacteria	MTCC number	Selective media used for revival	Types of hemolysis on blood agar	Media for anti microbial efficacy testing
S mutans	890	Brain Heart Infusion with 5% sheep blood	Gamma hemolysis	Brain Heart Infusion agar
L acidophilus	10307	Brain Heart Infusion with 5% sheep blood	Alpha hemolysis	Brain Heart Infusion agar

Table 3. Anti microbal efficacy of *Camellia sinensis* and *Alstonia scholaris* extracts against *S. mutans* and *L. acidophilus* at different concentrations in the initial experiment

	Concentration of plant extracts	*Camellia sinensis* (CS) (Mean ± SD)	*Alstonia scholaris* (AS)(Mean ± SD)	Chlorhexidine (C) (0.2%) #(Mean ± SD)	Statistical inference	Post hoc
S. mutans	4% (1)	27.50± 0.71	No activity	32.50±0.71	t value: -7.07 df: 2 p value: 0.02	
	6% (2)	31.00± 1.41	No activity	34.00± 2.83	t value:-1.34 df: 2 p value: 0.31	
	10% (3)	37.50± 3.54	23.00± 2.83	37.00± 1.41	F value: 18.07	CS vs AS:0.03
Statistical inference:	F value: 10.30 df: 2 p value: 0.04	No statistic computed	F value: 3.00 df: 2 p value: 0.19		df: 2 p value: 0.02	CS vs C: 0.98 AS vs C: 0.03
Post hoc:	1 vs 2: 0.381 vs 3: 0.042 vs 3: 0.12		1 vs 2: 0.731 vs 3: 0.182 vs 3: 0.37			
L. acidophilus	4% (1)	26.00± 1.41	No activity	34.00±1.41	t value: -5.66 df: 2 p value: 0.03	
	6% (2)	22.50± 3.54	No activity	32.50± 3.54	t value: 2.83 df: 2 p value: 0.11	
	10% (3)	11.00± 1.41	25.50± 2.12	27.50± 3.54	F value: 25.60	CS vs AS: 0.02
Statistical inference:	F value: 22.39 df: 2 p value: 0.01	No statistic computed	F value: 2.57 df: 2 p value: 0.22		df: 2 p value: 0.01	CS vs C: 0.01 AS vs C: 0.73
Post hoc:	1 vs 2: 0.411 vs 3: 0.022 vs 3: 0.03		1 vs 2: 0.881 vs 3: 0.222 vs 3: 0.35			

only 0.2% Chlorhexidine was used as positive control for comparison with different concentrations of plant extracts.

Table 4. Antimicrobial efficacy of 4% *Camellia sinensis* against *S. mutans* and *L. acidophilus*

Bacteria	*Camellia sinensis* (Mean± SD) (1)	Positive control (Mean± SD) (2)	Negative control (Mean± SD) (3)	Total (Mean± SD)	Statistical inference*	Post hoc test#
Streptococcus mutans	30.33± 3.28	34.11± 2.15	00	21.48± 15.71	F value: 614.59 df: 2 P value: 0.00	1 vs 2: 0.005 1vs 3: 0.00 2 vs 3: 0.00
Lactobacilli acidophilus	23.78± 2.22	33.67± 1.73	00	19.15± 14.48	F value: 1017.66 df: 2 P value: 0.00	1 vs 2: 0.00 1vs 3: 0.00 2 vs 3: 0.00

*One Way Analysis of Vaiance # tukey's post hoc

Table 5. Antimicrobial efficacy of 10% *Alstonia scholaris* against *S. mutans* and *L. acidophilus*

	Alstonia scholaris (Mean± SD)	Positive control (Mean± SD)	Negative control (Mean± SD)	Statistical inference*	Post hoc test#
Streptococcus mutans	21.56± 2.79	24.00±2.29	00	F value: 361.52 df: 2 P value: 0.00	1 vs 2: 0.051 1vs 3: 0.00 2 vs 3: 0.00
Lactobacilli acidophilus	24.11± 1.62	26.11± 1.97	00	F value: 1900.70 df: 2 P value: 0.00	1 vs 2: 0.021 1vs 3: 0.00 2 vs 3: 0.00

*One Way Analysis of Vaiance # tukey's post hoc

demonstrated a significant difference between most of different concentrations (25% 50%, 75%, 100%, 125% and 150%) against DPPH free radical ($p<0.05$) except *Alstonia scholaris* at 100% Vs 125% ($p = 0.13$, Table 7).

DISCUSSION

Dental caries is demonstrating an upward trend in India and other developing countries. Treatment of dental caries is quite expensive and not a realistic option for developing countries such as India. Although, chlorhexidine is considered as a gold standard in preventing dental plaque formation, it causes some minor side effects on long term use like discoloration of teeth, taste alteration, oral/ mucosal ulceration and parotid swelling.[1, 2, 5] The need to evolve innovative strategies to prevent dental caries has resulted in multiple studies which have evaluated the antimicrobial efficacy of plant extracts on dental caries microorganisms in the recent past.[18-21]

Camellia sinensis and *Alstonia scholaris* plants were known for their medicinal properties since centuries.[10] Lack of sufficient literature evaluating antimicrobial efficacy of these plant extracts on dental caries bacteria led us to undertake this *in vitro* study which assessed anti oxidant and antimicrobial activity of *Camellia sinensis* and *Alstonia scholaris* extracts on *Streptococcus mutans* and *Lactobacillus acidophilus*.

Camellia sinensis did not demonstrate any inhibitory activity against *S. mutans* and *L. acidophilus* at initial concentration (1%, 2% and 3%). *Alstonia scholaris* did not demonstrate any inhibiotry activity against *S. mutans* and *L. acidophilus* at initial concentration of 1%, 2%, 3%, 4% and 6%. This may be due to lack of sufficient concentration of phytochemical constituents in plant extracts for inhibiting the bacteria. Based on these results, MIC of *Camellia sinensis and Alstonia scholaris against S. mutans* and *L. acidophilus* was considered to be 4% and 10% respectively. Inhibitory activity of *Camellia sinensis* is attributed to the presence of various phytochemicals. It has been reported in the literature that *Camellia sinensis* has the potential to inhibit dextran and levan produced from sucrose by acting upon *S. mutans*.[7] It has been found to even interfere with the process of bacterial attachment to tooth enamel.[11] Plant is said to contain anti- microbial and anti- oxidant activity due to the presence of polyphenols and catechins.[10] Similarly, some studies have reported anti- microbial, phyto chemical and antioxidative property of different parts viz. leaves flowers, bark, root and latex of *Alstonia scholaris*.[15, 16, 22, 23] The available literature over assessment of anti- microbial activity of *Alstonia scholaris* against *S. mutans* and *L. acidophilus* is limited. *Araghizadeh A et al* assessed the antimicrobial activity of *Camellia sinensis* against *S. mutans* at a concentration starting from 1.56mg/ ml to 50 mg/ml. There was no inhibition of bacteria at a concentration of 1.56mg/ ml. However, a mean zone of inhibition of 7.5±2.76 mm was formed at a concentration of 3.12 mg/ml which increased to 36.3±1.08 mm at a concentration of 50mg/ ml.[5] A review study by Reygaert W C reported that MIC of *Camellia sinensis* against *S. mutans* to vary from 2.58- 3.98 mg/ ml.[24]

The mean zone of inhibition for *S. mutans* increased with increasing concentration of *Camellia sinensis*. On the other hand, the

Table 6. Comparison of antimicrobial efficacy of 4% *Camellia sinensis* and 10% *Alstonia scholaris* against *S. mutans* and *L. acidophilus*

	Camellia sinensis (Mean± SD)	*Alstonia scholaris* (Mean± SD)	Statistical inference*
Streptococcus mutans	30.33± 3.28	21.56± 2.79	t value: 6.118 df: 16 P value: 0.00
Lactobacilli acidophilus	23.78± 2.22	24.11± 1.62	t value: -0.364 df: 52 P value:0.721

* independent sample 't' test

Table 7. Details of antioxidant capacity of Ascorbic acid (standard), 4% *Camellia sinensis* and 10% *Alstonia scholaris*

Concentration	Ascorbic acid Mean% inhibition ± SD (1)	*Camellia sinensis*_ Mean% inhibition ± SD (2)	*Alstonia scholaris*_ Mean% inhibition ± SD (3)	Statistical inference*	Post hoc test#
25%	3.47± 0.76	17.00± 2.00	14.33± 4.04	F value: 22.11 df: 2 P value: 0.002	1 vs 2: 0.002 1vs 3: 0.006 2 vs 3: 0.48
50%	10.21± 0.26	29.00± 1.00	35.76± 0.88	F value: 853.12 df: 2 P value: 0.00	1 vs 2: 0.00 1vs 3: 0.00 2 vs 3: 0.00
75%	17.41± 0.52	41.22± 1.55	46.86± 1.22	F value: 528.73 df: 2 P value:0.00	1 vs 2: 0.00 1vs 3: 0.00 2 vs 3: 0.003
100%	20.25± 0.27	60.67± 2.52	70.39± 1.96	F value: 621.01 df: 2 P value: 0.00	1 vs 2: 0.00 1vs 3: 0.00 2 vs 3: 0.002
125%	28.17± 0.14	71.33± 1.53	75.28± 2.11	F value: 903.67 df: 2 P value: 0.00	1 vs 2: 0.00 1vs 3: 0.00 2 vs 3: 0.042
150%	31.53± 0.18	89.66± 0.57	90.33± 1.15	F value: 6108.66 df: 2 P value: 0.00	1 vs 2: 0.00 1vs 3: 0.00 2 vs 3: 0.550
Total	18.50± 9.97	51.48± 25.71	55.49± 26.58		
Statistical inference*	F value: 1920.24 df: 5 P value:0.00	F value: 820.77 df: 5 P value: 0.00	F value: 508.43 df: 5 P value: 0.00		
Post hoc test	25% vs 50%: 0.00 25% vs 75%: 0.00 25% vs 100%: 0.00 25% vs 125%: 0.00 25% vs 150%: 0.00 50% vs 75%: 0.00 50% vs 100%: 0.00 50% vs 125%: 0.00 50% vs 150%: 0.00 75% vs 100%: 0.00 75% vs 125%: 0.00 75% vs 150%: 0.00 100% vs 125%: 0.00 100% vs 150%: 0.00 125% vs 150%: 0.00	25% vs 50%: 0.00 25% vs 75%: 0.00 25% vs 100%: 0.00 25% vs 125%: 0.00 25% vs 150%: 0.00 50% vs 75%: 0.00 50% vs 100%: 0.00 50% vs 125%: 0.00 50% vs 150%: 0.00 75% vs 100%: 0.00 75% vs 125%: 0.00 75% vs 150%: 0.00 100% vs 125%: 0.00 100% vs 150%: 0.00 125% vs 150%: 0.00	25% vs 50%: 0.00 25% vs 75%: 0.00 25% vs 100%: 0.00 25% vs 125%: 0.00 25% vs 150%: 0.00 50% vs 75%: 0.00 50% vs 100%: 0.00 50% vs 125%: 0.00 50% vs 150%: 0.00 75% vs 100%: 0.00 75% vs 125%: 0.00 75% vs 150%: 0.00 100% vs 125%: 0.13 100% vs 150%: 0.00 125% vs 150%: 0.00		

*One- Way Analysis of Variance # tukey's post hoc

mean zone of inhibition for *L. acidophilus* decreased with increasing concentration of *Camellia sinensis*. This can be due to fact that higher concentration of the extract could have led to extract oversaturation which might have adversely influenced antimicrobial action against *L. acidophilus*.[25,26,27,28]

Anita P et al to assessed anti- microbial efficacy of *Camellia sinensis* against *S. mutans* and *L. acidophilus*. Study reported significant increase in mean zone of inhibition on increasing concentration of *Camellia sinensis* against *S. mutans* from 10.00 ± 0.01mm at 100 µg concentration to 12.66 ± 0.58mm at 200µg and finally 18.33± 0.58mm at 300µg concentration. They found a statistically significant increase in mean zone of inhibition with increasing concentration of *Camellia sinensis* against *L. acidophilus* from 8.33± 0.58mm at 100µg concentration to 10.00± 0.01mm at 200µg concentration and finally 12.67± 0.58mm at 300µg concentration.[2] These results were similar to the results of present study with regard to *S. mutans* while contradictory with respect to *L. acidophilus*. Tahir A and Moeen R also found anti- microbial efficacy of *Camellia sinensis* against *S. mutans* and *L. acidophilus* to increase with increasing concentration. [11] *Khan M R et al* reported *Alstonia scholaris* to inhibit the growth of *S. mutans similar to the results of our study*.[12,] Although, literature indicating antibacterial activity of *Alstonia scholaris* against gram positive and gram negative bacteria are available,[19,25,26] studies reporting antimicrobial activity of *Alstonia scholaris* against *S. mutans* and *L. acidophilus* are practically non-existent. Hence, We could not compare the results with other studies.

The subsequent experiment in triplicate sets was undertaken with 4% *Camellia sinensis and 10% Alstonia scholaris* with 0.2% Chlorhexidine as positive control to confirm our findings of initial experiment. 0.2% Chlorhexidine (34.11± 2.15mm, 33.67± 1.73mm) produced a significantly higher mean zone of inhibition against *S. mutans* and *L.acidophilus* compared to 4% *Camellia sinensis* (30.33± 3.28mm, 23.78± 2.22mm).

Anita P et al. reported 0.2% Chlorhexidine to exhibit a higher mean zone of inhibition against *S. mutans* and *L. acidophilus* in comparison with *Camellia sinensis* at 300µg concentration.[2] our findings were similar to the results of this study and others.[1,2, 6]

Chlorhexidine is considered the gold standard antiplaque agent and it was found in our study as well that it has the potential to inhibit these microorganisms. However, in view of its side effects on long term use, mouth rinses made of these extracts could be considered as potential alternates with further research.

Camellia sinensis at 4% demonstrated higher mean zone of inhibition against *S. mutans* than against *L. acidophilus* similar to the findings of a study by *Tahir A and Moeen R* who found *Camellia sinensis* at concentrations of 5000, 10000, 15000 and 20000 mg/ml to have greater mean zone of inhibition against *S. mutans* (19, 24, 34 and 35 mm) than against *L.* acidophilus (15, 20, 29 and 33mm).[11] This finding was also similar to study conducted by *Anita P et al*.[2]

Alstonia scholaris at 10% demonstrated a higher mean zone of inhibition against *L. acidophilus* than *S. mutans*. Although, the extract was found to be very effective against *L. acidophilus,* we could not compare this finding vowing to non-availability of studies on the efficacy of this extract on dental caries bacteria.

Mean inhibition percentage of DPPH radical increased with increasing concentration of Ascorbic acid, *Camellia sinensis* and *Alstonia scholaris*. The increase in mean inhibition percentage was found to be significant for all three samples. However, *Alstonia scholaris* showed a significant high inhibition percentage than standard Ascorbic acid and *Camellia sinensis* at all concentrations except at 25% where *Camellia sinensis* showed a higher mean inhibition percentage. Higher mean inhibition percentage indicated high antioxidant activity. This illustrates that *Camellia sinensis* and *Alstonia scholaris* have enough potent antioxidant activity to scavenge free radicals. Various studies have reported antioxidant and antibacterial property of *Alstonia scholaris*. [15, 16, 25, 26] Similar results were reported by *James J et al*, *Ramachandra Y L et al* and *Jain D P et al*. [18,27,28] *Ramachandra Y et al* found an increase in mean inhibition percentage from 26.37% to 72.82% with increase in *Alstonia scholaris* concentration of 200µg/ml to 1000 µg/ml. [18] *Jain D P et al* reported a significant increase in inhibition percentage of

DPPH radical from 10.82 ± 2.2 to 81.13 ± 2.6 with increase in concentration of *Camellia sinensis* from 10 mg/ ml to 180 mg/ ml.[27] Our results were similar to the results of all these studies.

Novelty

The study evaluated the MIC, antibacterial activity of two potential plant extracts against two most disease causing pathogens in oral environment besides evaluating their anti-oxidant capacity. Evaluation of antimicrobial activity of *Alstonia scholaris* against *Streptococcus mutans* and *Lactobacilli acidophilus* was the first of its kind study.

Limitation

Phytochemical constituents of plant extracts vary depending upon their geographical location, brewing time, fermentation, etc. A qualitative and quantitative assay of phytochemical constituents could have validated the results of this study which could not be undertaken. We assessed antimicrobial activity of these extracts against *Streptococcus mutans* and *Lactobacilli acidophilus* while oral cavity has other pathogenic microorganisms as well.

Hence, more such researches can be performed to confirm anti- oxidant and anti-microbial property of *Camellia sinensis* and *Alstonia scholaris* against other oral bacteria.

CONCLUSION

• MIC of *Camellia sinensis* on *S. mutans and L. acidophilus was 4%.*

• MIC of *Alstonia scholaris* on *S. mutans and L. acidophilus was 10%.*

• The plant extracts were significant effective against bacteria, *S. mutans* and *L. acidophilus*. *Camellia sinensis* produced a mean inhibition zone of 30.33± 3.28 against *S. mutans* and 23.78± 2.22 against *L. acidophilus*. *Alstonia scholaris* produced a mean inhibition zone of 21.56± 2.79 against *S. mutans* and 24.11± 1.62 against *L. acidophilus*.

• Mean zone of inhibition of both the bacteria namely *S. mutans* & *L. acidophilus* and inhibition percentage of DPPH radical significantly increases with increase in concentration of plant extracts, *Camellia sinensis* and *Alstonia scholaris*

• Both the plant extract *Camellia sinensis* and *Alstonia scholaris* contains effective anti oxidative and anti- microbial activity.

REFERENCES

1. Subramaniam P, Eswara U, Maheshwar Reddy K R. Effect of different types of tea on Streptococcus mutans: an in vitro study. *Ind J Dent Res*; **23**(1): 43-8 (2012).
2. Anita P, Sivasamy S, Madan Kumar P D, Balan I N, Ethiraj S. In vitro antibacterial activity of Camellia sinensis extract against cariogenic microorganisms. *J Basic Clin Pharma*; **6**(1):35-9 (2015).
3. Grover H S, Deswal H, Hans V M, Devi L S. Effect of concentration and brewing time on the antimicrobial activity of Camellia sinensis (green tea) against oral bacteria. *Saudi J Oral Sci;* **4**(1):18-21 (2017).
4. Sakanaka S, Kim M, Taniguchi M, Yamamoto T. Antibacterial substances in japanese green tea extract against Streptococcus mutans, a cariogenic bacterium. *Agric Biol Chem;* **53**(9): 2307-2311 (1989).
5. Araghizadeh A, Kohanteb J, Fani M M. Inhibitory activity of green tea (Camellia sinensis) extract on some clinically isolated cariogenic and periodontopathic bacteria. *Med Princ Pract;* **22**: 368–372 (2013).
6. Wang Y F, Shao S H, Xu P, Yang X Q, Qian L S. Catechin-enriched green tea extract as a safe and effective agent for antimicrobial and anti-inflammatory treatment. *Afr J Pharm Pharmacol*; **5**(12): 1452-1461.
7. Jenabian N, Moghadamnia A A, Karami E, Mir A B P. The effect of Camellia sinensis (green tea) mouthwash on plaque-induced gingivitis: a single-blinded randomized controlled clinical trial. *DARU J Pharmaceutical Sciences;* **20**(39): 1-6 (2012).
8. Hirasawa M, Takada K. Multiple effects of green tea catechin on the antifungal activity of antimycotics against Candida albican. *J Antimicrobial Chemotherapy*; **53**(2): 225–229 (2004).
9. Tsao A S, Liu D, Martin J, Tang X, Lee J J, El-Naggar A K *et al*. Phase II randomized, placebo-controlled trial of green tea extract in patients with high-risk oral premalignant lesions. *Cancer Prev Res*; **2**(11): 931-941 (2009).
10. Archana S, Abraham J. Comparative analysis of antimicrobial activity of leaf extracts from fresh green tea, commercial green tea and black tea on pathogens. *J Applied Pharmaceutical Science,* **01**(08): 149-152 (2011).
11. Tahir A, Moeen R. Comparison of antibacterial activity of water and ethanol extracts of Camellia sinensis (L.) kuntze against dental caries and detection of antibacterial components. *J Med*

Plant Res; **5**(18): 4504-4510 (2011).
12. Khan M R, Omoloso A D, Kihara M. Antibacterial activity of Alstonia scholaris and Leea tetramer. *Fitoterapia;* **74**: 736–740 (2003).
13. Misra C S, Pratyush K, Dev M.S L, James J, Veettil A K T, Thankamani V. A comparative study on phytochemical screening and antibacterial activity of roots of Alstonia scholaris with the roots leaves and stem bark. *Int. J. Res. Phytochem. Pharmacol.* **1**(2): 77-82 (2011).
14. Varshney A, Goyal M M. Phytochemical study on the leaves of Alstonia Scholaris and their effects on pathogenic organisms. *Ancient Science of Life;* **15**(1): 30-34 (1995).
15. Ganjewala D, Gupta A K. Study on phytochemical composition, antibacterial and antioxidant properties of different parts of Alstonia scholaris Linn. *Advanced Pharmaceutical Bulletin*; **3**(2): 379-384 (2013).
16. Khyade M S, Kasote D M, Vaikos N P. Alstoniascholaris (L.) R.Br. and Alstoniamacrophylla Wall.exG.Don: A comparative review on traditional uses, phytochemistry and pharmacology. *Journal of Ethnopharmacology*; **153**: 1–18 (2014).
17. Caufield P W, Schön C N, Saraithong P, Li Y, Argimón S. Oral lactobacilli and dental caries: a model for niche adaptation in humans. *JDR Clinical Research*; **94**(9) :2 (2015)
18. Chandrashekar BR, Nagarajappa R, Suma S, Thakur R. Herbal extracts in oral health care – A review on the current scenario and the need for future – *Pharmacognosy reviews*; **9**(18): 87 – 92 (2015).
19. Chandra shekar BR, Nagarajappa R, Jain R, Suma S, Singh R, Thakur R. Minimum Inhibitory Concentration of the plant extract combinations against dental caries and plaque microorganisms – An in vitro study. *Journal of Indian Association of Public Health Dentistry;* **14**: 456 – 62 (2016).
20. Chandra shekar BR, Nagarajappa R, Singh R, Suma S, Thakur R. Antimicrobial efficacy of the combinations of Acacia nilotica, Murraya koenigii (Linn.) Sprengel, Eucalyptus, and Psidium guajava on primary plaque colonizers: An in vitro study. *Indian Journal of Dental Research;* **27**: 415 – 20 (2016).
21. Chandra shekar BR, Nagarajappa R, Jain R, Singh R, Thakur R, Suma S. Antimicrobial efficacy of Acacia nilotica, Murraya koenigii (L.) Sprengel, Eucalyptus hybrid, Psidium guajava extracts and their combination on Streptococcus mutans and Lactobacillus acidophilus. *Dental Research Journal*; **13**(2): 168 – 73 (2016).
22. Ramachandra Y L, AshajyothI C, Padmalatha RAI S. Antioxidant activity of Alstonia Scholarsis extracts containing flavonoid and phenolic compounds. *Int J Pharm Pharm Sci;* **4**(3): 424-426 (2012).
23. Varshney A, Goyal M M. Phytochemical study on the leaves of Alstonia Scholaris and their effects on pathogenic organisms. *Ancient Science Life;* **15**(1): 30- 34 (1995).
24. Reygaert W C. The Antimicrobial possibilities of green tea. *Frontier Microbiolog*; **5**(434): 1- 8 (2014).
25. Yourassowsky E, Vanderlinden M P, Lismont M J, Schoutens E. Paradoxical action of penicillin g on staphylococcus aureus: a time study of the effect of a zonal antibiotic concentration gradient on bacterial growth. *Antimicrob Agent Chemotherap;* **8**(3): 262-265 (1975).
26. Miyazaki S *et al*. In- vitro activities of eleven antibiotics against vancomycin resistant enterococci isolated in Japan. *J Antimicrob Chemother;* **44**: 415- 416 (1999).
27. Prasetyoputri A, Jarrad M A, Cooper M A, Blaskovich M A T. The Eagle Effect and Antibiotic-Induced Persistence: Two Sides of the Same Coin?. *Trends microbiology*; 1-16 (2018).
28. Tsuji BT, Landersdorfer CB, Lenhard JR, Cheah S-E, Thamlikitkul V, Rao GG, Holden PN, Forrest A, Bulitta JB, Nation RL, Li J. Paradoxical effect of polymyxin B: high drug exposure amplifies resistance in Acinetobacter baumannii. *Antimicrob Agents Chemother;* **60**(7):3913–3920 (2016).
29. Patel J P, Gami B, Patel K, Solanki R. Antibacterial activity of methanolic and acetone extract of some medicinal plants used in indian folklore. *Int J Phytomedicine;* **3**: 261-269 (2011).
30. Khyade M S, Vaikos N P. Phytochemical and antibacterial properties of leaves of Alstonia scholaris R. *Br. Afr J Biotechnol;* **8**(22): 6434-6436 (2009).
31. James J *et al*. In vitro antioxidant activity of flowers and fruits of Alstonia scholaris. *Int J Phytomedicine;* **3**: 475-479 (2011).
32. Jain DP, Pancholi SS, Patel R. Synergistic antioxidant activity of green tea with some herbs. *J Adv Pharm Tech Res;* **2**: 177-83 (2011).