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ANTIBACTERIAL PROPERTIES OF HONEY AND GREEN TEA EXTRACTS AGAINST MULTI-DRUG RESISTANT *STREPTOCOCCUS MUTANS* ISOLATED FROM DENTAL PLAQUE SAMPLES

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ABSTRACT

Streptococcus mutans is the major etiological agent for the dental plaque formation. A study on the occurrence of multidrug resistant (MDR) *Streptococcus mutans* and control of *S. mutans* isolated from dental caries patients against green tea and honey extracts among the Bangalore urban population as in search of alternate therapy for dental caries. In this study, 118 dental plaque samples were collected and 87 were identified as MDR *S. mutans* isolates by using biochemical tests, Disc-diffusion test against commercial antibiotics and antibacterial property of green tea & honey against 10 MDR *S. mutans* isolates was done by using agar well diffusion method. The zone diameters (mm) of each isolate against selected antibiotics obtained were noted and compared with reference antibiotics namely ciprofloxacin, erythromycin and penicillin G. The zone diameters of Green tea organic and aqueous extracts were ranged from 14mm to 29mm. Similarly, for Honey extracts it ranged from 4-18mm. The antibiograms of green tea extracts has revealed that the MDR *S. mutans* is sensitive to these at 100mg/ml concentration. The antibiograms of honey with different dilutions has revealed that, with dilution the activity got reduced and found sensitive with no dilution. The MIC of green tea and honey used were found to be 70mg/ml and 100mg/ml respectively with 50µl loaded to the wells. The study revealed that the Green tea and Honey were equally effective to ciprofloxacin and erythromycin in controlling MDR *S. mutans.*

KEY-WORDS: **Dental caries, Multi-drug resistance, Well-diffusion, Alternate therapy, Green tea and Honey extracts.**

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INTRODUCTION

Dental caries is a most common dental problem for world-wide population.[1–4] About 36% of world population had dental caries at least once in their life time. The dental plaque formation is a major cause for dental caries.[5-6] The prevalence of major microbes in dental plaque were found to be *Streptococcus mutans* (40%), *Streptococcus sorbinus* (16%), *Lactobacillus sps* (15%) and other species of bacteria. *S. mutans* is a major etiological agent which promotes the dental plaque formation.[7] In this post-antibiotic era, there is a gradual increase in resistance to major antibiotic groups like macrolides, Beta-Lactams and fluoro-quinolones.[26] The increase in drug resistance in *S. mutans* leading to vigorous use of antibiotics resulting in several side effects. Hence, there is a requirement of alternate therapy with minimum side effects. Extensive research has been carried out on medicinal plants, traditional medicines and ayurveda against to multidrug resistant bacteria.[8] In the ancient times honey was used as household remedy for skin ailments. Honey is super saturated nectar collected by bees from a wide variety of plants. Though honey is known as food but not well recognized as medicines.[8] The medicinal and antimicrobial activities of honey for wound healing have been introduced for approximately 4500 years. Honey act as anti-allergic and improves immunity, tissue growth and regeneration. [9-11] It is well documented that honey is an effective agent against numerous pathogenic microorganisms including bacteria, fungi and viruses. A significant amount of earlier work focused on multi-floral or pasture honeys and their efficacy against gram positive organisms, especially *Staphylococcus aureus* and its antibiotic-resistant variants. Tea (*Camellia sinensis*) is one of the most widely consumed beverages in Asian countries.[12] Tea is known to be a rich source of polyphenol, but polyphenol type and levels vary depending on the processing method.[13] There are various ways a tea can be processed (green, black, oolong Green tea is unfermented and contains mostly monomeric polyphenols such as epicatechin, epicatechingallate, and epigallocatechin; black tea has been fermented and many of the simple catechins have been oxidized to larger molecules of higher molecular weight.

MATERIAL AND METHODS

In the present study, 118 dental plaque samples with caries were collected from patients over a period of 3 months (from January 2014 to March 2014) visiting MS Ramaiah Dental college with prior consent. Immediately transferred into the TH broth and transported to the M. S. Ramaiah Institute of Technology, Biotechnology Laboratory. There isolates were subjected to isolation and identification by routine biochemical tests and sub-cultured for further use. Drug resistance pattern was analyzed by Kirby-Bauer disc diffusion method.[15-16] 87 multi-drug resistant *S. mutans* isolates were determined, from those 10 isolates were subjected to Agar well diffusion test using Honey and Green tea extracts. Based on the results obtained, Honey and Green Tea antibiotic properties were compared with pure antibiotics used for reference plate as per the CLSI limits (see Figure 1A, B &C). Standard lyophilized cultures were obtained from the Microbial Type Culture Collection & Gene Bank (MTCC), Chandigarh, namely *Streptococcus mutans* -MTCC 497 and revived using Todd Hewitt (TH) broth.

Identification of Multidrug resistant Streptococcus mutans by antibiotic disc-diffusion test

penicillin G (10 units), ciprofloxacin (05 µg), erythromycin (15 µg) discs and bacitracin were procured from Hi-media. Drug resistant patterns of samples were assayed by Kirby-Bauer Disc-Diffusion method. The MHA plates were inoculated with the *S. mutans* sub-cultures along with the standard using separate sterile cotton swab for each bacterium.[26] The results were interpreted based on Clinical and Laboratory Standards Institute (CLSI) guidelines.[17,26] The MDR *S. mutans* from isolates were determined and used for well diffusion method against the honey and Green tea extracts.[15-16]

Preparation of Honey and Green tea extract

Commercially available Honey (Purity claimed 100%) and Green tea samples were purchased from provisional store in Bangalore city, Karnataka, India.

Aqueous Green tea extract

The dry Green tea was blended into a fine powder using a blender. About 5g powder was mixed with 50ml milli-Q water (100mg/ml) and heated at 80°C for 30 minutes with continuous stirring. The slurry was centrifuged at 10,000rpm for 10 min at 8°C. The decanted supernatant was filtered twice through Whatmann no-1 filter paper. The filtrate was stored at -20°C until further use.[18]

Organic Green tea extract

Organic extract was prepared by taking 50ml of ethanol (100%) and 5g of the dry powdered tea sample. A pestle & mortar was used and macerated for proper extraction of contents to the solvent and this mixture was filtered through Whatmann filter paper no-1 and obtained a crude aqueous extract. The extract obtained was reduced to approximately 5 ml over a water bath kept at 50°C to get a 100% (w/v) concentrated organic extract of green tea and stored at -20°C until further use.

Honey sample

Different concentrations of honey (0, 2:1, 4:1 & 6:1) were prepared by diluting honey with milli-Q water.

Determination of antibacterial activity of Green tea and Honey by Agar-Well Diffusion

About 10 MDR *S. mutans* isolates were inoculated individually into Muller- Hinton agar plates. Wells were made and filled with 50µl of the Honey and Green tea extracts in different wells. The plates were then incubated for 24 hours at 35±2˚C. The diameter of the zone of inhibition around the well was measured to the nearest millimeter. The extracts were diffused through the agar. The inhibition zone diameter that was produced indicates the susceptibility or resistance of a bacterium to the extracts. These antibiotic susceptibility patterns were compared with the reference plate in which the antibiotics ciprofloxacin, penicillin G and erythromycin disc were used. Based on the zone diameter observed and measured, the antimicrobial activities of the extracts were estimated.

RESULTS

In the present study, out of 118 isolates collected, 87 isolates were found to be MDR *S. mutans* based on the biochemical tests and Kirby-Bauer Disc diffusion method. From these isolates 10 isolates were selected for the Agar well diffusion method. The Green tea and Honey extracts showed significant results. The antimicrobial sensitivity test was conducted using the aqueous and organic extracts of Green tea and different concentrations of Honey on the selected isolates. The inhibition zones were noted and tabulated (Table 2 &3, Figure 1A, B & C). In well diffusion the results were noted and found that S12 (29mm), S6 (28mm) and S3 (26mm) as higher sensitivity to Green tea organic extracts at 100mg/ml concentration. The Isolates S5 and S35 (20mm) showed less sensitivity to both aqueous and organic extracts of Green tea extracts. The isolates S22 (22mm), S24 (24mm), S28 (24mm) and S30 (22mm) showed lesser sensitivity (See table 2). These values were compared with CLSI limits for sensitivity or resistance (See table 1). It was found that Green tea ethanol extract showed antimicrobial activity equivalent to that of penicillin, aqueous extract showed antimicrobial activity equivalent to that of ciprofloxacin when tested against standard and clinical isolates (Figure 2). Different dilutions of Honey were used but effective results were observed with undiluted honey against *S. mutans.* The maximum zone of inhibition for honey of different dilutions was found to be 17mm. It was found that with increase in concentration, the zone of inhibition was seen to increase only for few isolates (Figure 2). Honey with high sugar content may control the growth of MDR *S. mutans.* In this case decrease in sugar content with dilution was unable to control the *MDR S. mutans.* For this the ability of the *S. mutans* also depended on the patient's diet. The antibiotic sensitivity test results for the selected antibiotics used were interpreted as per the CLSI guidelines (Table-1). The results were compared with the results of Green tea and Honey extracts. The zone of Green tea extracts were showed similar zone diameter with antibiotics used against MDR *S. mutans* (>20mm) (Table-2). Similarly, honey extracts showed comparatively less zone of inhibition (Table-3). The origin and season of the Honey collection in commercially available Honey is unkown. However the raw honey whose origin and season of collection is known could help to yield better results.[8]

Table 1
CLSI zone diameters for the identification of Sensitive/Resistant pattern of S. mutans

Antibiotic	Symbol	Disc Content	Resistant mm or less	Sensitive mm or More
Penicillin G	PG	10 Units	19	28
Erythromycin	ER	15 mcg	15	21
Ciprofloxacin	RC	5 mcg	15	21

CLSI limits for the reference antibiotic discs penicillin G, erythromycin and ciprofloxacin

Table 2
Zone diameters for Green tea extracts by Agar well diffusion

S No	Isolate No.	Organic extract 100mg/ml (50µl)	Aqueous extract 100mg/ml (50µl)
1	**MTCC**	**23**	**20**
2	**S3**	26	20
3	**S5**	20	18
4	**S6**	28	17
5	**S12**	29	16
6	**S22**	22	19
7	**S24**	24	20
8	**S28**	24	23
9	**S30**	22	14
10	**S35**	20	19
11	**S38**	21	18

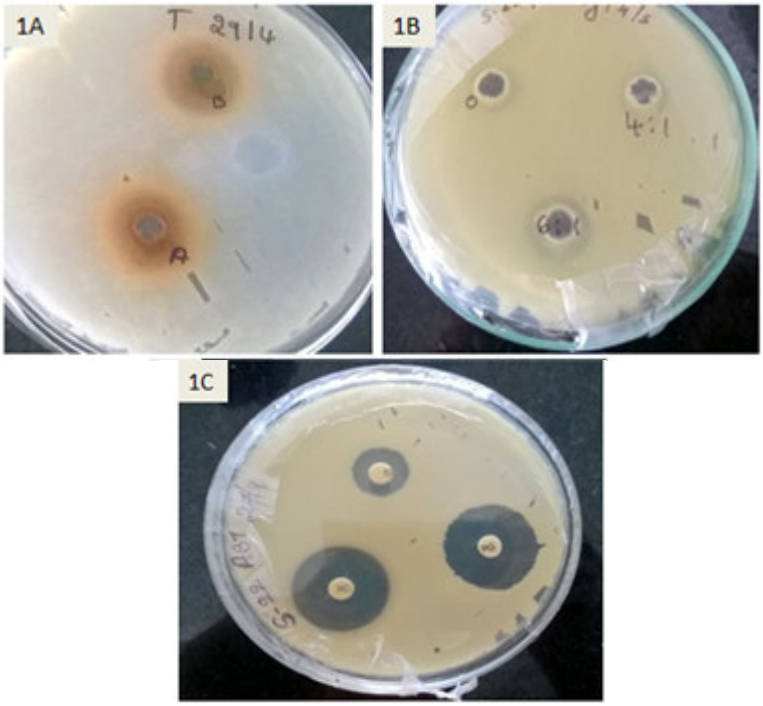
Organic and Aqueous Green tea extracts showing zone of inhibition against MDR S. mutans isolates.

Table 3
Zone diameters for Honey extracts by Agar well diffusion

Honey extracts					
S No	**Isolate No.**	**Without dilution**	**2:1**	**4:1**	**6:1**
1	**MTCC**	**18**	**9**	**8**	**10**
2	**S3**	17	-	4	6
3	**S5**	15	-	5	7
4	**S6**	10	4	6	9
5	**S12**	8	-	5	6
6	**S22**	12	-	4	8
7	**S24**	15	6	5	7
8	**S28**	9	-	4	8
9	**S30**	13	5	4	6
10	**S35**	14	6	5	6
11	**S38**	11	4	5	7

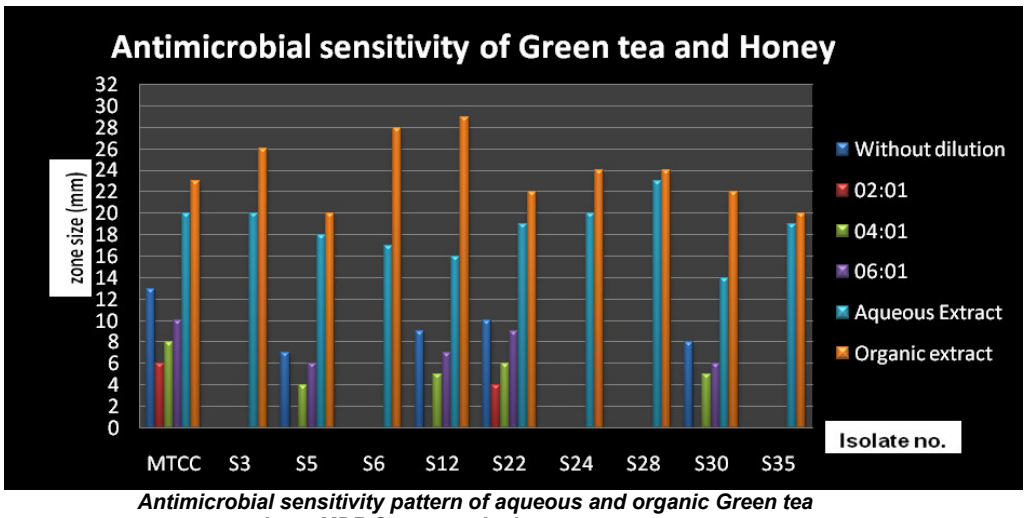
Honey extracts (Without dilution, 2:1, 4:1 and 6:1 dilutions) showing zone of inhibition (in mm) against MDR S. mutans isolates.

Figure 1
Agar well diffusion of Green tea and Honey extracts



1A. MHA plates showing antibiograms of Green Tea (aqueous & organic extracts) against to MDR *S.mutans*, 1B. Honey extracts (without dilution, 4:1, 6:1), 1C. Antibiograms of erythromycin, ciprofloxacin and penicillin-G.

Figure 2
Pattern of Green tea and Honey extracts against MDR S. mutans isolates



Antimicrobial sensitivity pattern of aqueous and organic Green tea extracts against MDR S. mutans isolates.

DISCUSSION

Streptcoccus mutans is the major causative agent of dental caries. This has a major prevalence percentage in most of the dental plaque samples. Over a few decades it has got increased in resistance to various drugs like erythromycin, penicillin G, methicillin and chloramphenicol.[19-20,26] This increase in resistance is leading to difficulty for the treatment against several oral infections. Several studies reveal that increase in resistance to drugs like ciprofloxacin, erythromycin and penicillin G are at a faster rate. So, to treat these MDR *S. mutans,* alternate therapy is required. Earlier studies found that active chemicals present in Tea and Honey have antimicrobial properties.[9,11,18,22] A recent study revealed that the aqueous extract of Green tea has good antibacterial activity against *S. aureus, P. aeruginosa* and *E. coli* (ATCC cultures) at the concentration of 100mg/ml and 200mg/ml respectively.[23] According to Abdelmediz[21] mouth rinsing with Honey and Green tea reduced the growth of salivary *S. mutans*. The tea plant's (*Camellia sinensis*) leaves contain polyphenolic components which are effective against a wide spectrum of microbes. In the past two decades, various studies have shown that the Green tea polyphenolic catechins, in particular (−)-epigallo

catechingallate and (−)-epi catechingallate, can inhibit the growth of a wide range of Gram-positive and Gram-negative bacteria species with moderate potency. Evidence is emerging that these molecules may be useful in the control of common oral infections, such as dental caries and periodontal disease.[14] Even though, few drugs are still effective against drug resistant *S. mutans* adverse reactions of systemic drugs during last seven years (2010-2016) for various following organ groups received: skin, gastro-intestinal disorders, hepato-biliary disorders, general disorders, blood disorders, neurological reactions, respiratory disorders, immune system disorders, musculo-skeletal disorders, psychiatric disorders, renal and urinary disorders.[24-25] Honey with high sugar content may control the growth of MDR *S. mutans.* In this case decrease in sugar content with dilution was unable to control the *MDR S. mutans.* For this the ability of the *S. mutans* also depended on the patient's diet. A recent study with different types of honey was showed that it has antimicrobial properties against throat infection causing microbes like *Staphylococcus*, *Pseudomonas* and *Klebsiella* species.[8] The gradual increase in drug resistance to common antibiotics like penicillin-G, ciprofloxacin and erythromycin leads to difficulty in treatment. Hence, alternative therapy for these MDR bacteria has to be improved. If the Green tea is included in the regular diet that might control the growth of these MDR *S. mutans* at initial stages itself. In this study it was observed that Green tea at low concentrations (100mg/ml) found effective against MDR *S. mutans* but in case of Honey the effective concentration was found to be comparatively at higher side.

CONCLUSION

The present study revealed the activity of Green tea and Honey extracts in the control of MDR *S. mutans*. The Green tea extracts showed greater efficacy against *S. mutans,* similar to that of the commonly used antibiotics. Between the aqueous and organic extracts of Green tea, there is not much significant difference in the activity. Comparatively, Honey extract was found to be less effective. The regular consumption of green tea in the daily diet may reduce the dental plaque formation. Further phytochemical study on the Green tea and Honey for finding active compounds which are controlling the MDR *S. mutans* can be done. However the activity of other commercially available green tea and honey samples may vary based on the content of phytochemicals based on it origin and hence the extract needs to be tested based on its origin.

CONFLICT OF INTEREST

Conflict of interest declared none.

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