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Comparative evaluation of antimicrobial potential of herbal extracts on *Streptococcus mutans* and *Enterococcus faecalis* An in vitro study

Avaliação comparativa do potencial antimicrobiano de extratos de ervas em Streptococcus mutans e Enterococcus faecalis: estudo in vitro

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ABSTRACT

Though aloe vera extract, green tea extract and coriander oil are proven antimicrobial agents, very little information is available regarding its effects on oral bacteria, *Streptococcus mutans*, which is responsible for initiating caries and *Enterococcus faecalis*, responsible for failure of root canal treatment. **Objective:** To find the antimicrobial activity of aloe vera extract, black tea extract and coriander oil against *S. mutans* and *E. faecalis*. **Materials and Methods:** The agar well diffusion method was used to determine the antibacterial activity of Aloe vera extract, black tea extract and coriander oil. Different concentration of prepared plant extracts and coriander seed oil (50 & 100 μl) was incorporated into the wells and the plates containing *S. mutans* and *E. faecalis* were incubated at 37 °C for 24 h. The antibiotic (amoxicillin 30 μl) was used as positive control. Zone Of Inhibition (ZOI) was recorded in each plate. **Results:** For *S. mutans*, the maximum ZOI was created by coriander oil with a diameter of 25.00±0.58 mm at 50 μl and for *E. faecalis*, maximum ZOI was created by aloe vera extract 16.00±0.58 mm at 100 μl concentration which were far better than the control: amoxicillin 30 μl concentration. **Conclusion:** The extracts of Aloe vera, black tea and coriander oil, showed significant activity against the investigated microbial strains, *Streptococcus mutans* and *Enterococcus faecalis* which further helps in the development of new topical agents that help in reducing the numbers of these organisms present in the oral cavity.

KEYWORDS

Oral microbiota; Aloe vera; black tea; coriander oil; *E. faecalis*; *S.mutans*.

RESUMO

Embora o extrato de aloe vera, extrato de chá verde e óleo de coentro sejam agentes antimicrobianos comprovados, há pouca informação disponível sobre seus efeitos nas bactérias orais, *Streptococcus mutans*, que é responsável por iniciar cáries e *Enterococcus faecalis*, responsável pela falha do tratamento de canal radicular. **Objetivo:** Avaliar a atividade antimicrobiana do extrato de aloe vera, extrato de chá preto e óleo de coentro contra *S. mutans* e *E. faecalis*. **Materiais e Métodos:** O método de difusão em agar foi usado para determinar a atividade antibacteriana do extrato de Aloe vera, extrato de chá preto e óleo de coentro. Diferentes concentrações dos extratos de plantas e óleo de semente de coentro (50 e 100 μl) foram preparados e colocados nos poços e nas placas contendo *S. mutans* e *E. faecalis* e foram incubadas a 37°C por 24 h. O antibiótico (amoxicilina 30 μl) foi utilizado como controle positivo. A zona de inibição (ZOI) foi registrada em cada placa. **Resultados:** Para *S. mutans*, a ZOI máxima foi obtida com o óleo de coentro com um diâmetro de 25,00 ± 0,58 mm a 50 μl e para *E. faecalis*, a ZOI máxima foi obtiada pelo extrato de aloe vera 16,00 ± 0,58 mm na concentração de 100 μl, as quais foram melhores do que o controle: concentração de 30 μl de amoxicilina. **Conclusão:** Os extratos de Aloe vera, chá preto e óleo de coentro apresentaram atividade significativa contra as cepas microbianas investigadas, *Streptococcus mutans* e *Enterococcus faecalis* auxiliando no desenvolvimento de novos agentes tópicos visando a redução do número desses organismos presentes no cavidade oral.

PALAVRAS-CHAVE

Microbiota oral; Aloe vera; Chá preto; Oleo de coentro, *Streptococcus mutans*, *Enterococcus faecalis*.

INTRODUCTION

Dental caries is the localised destruction of susceptible dental hard tissues by acidic by-products from bacterial fermentation of dietary carbohydrates [1-3]. Dental caries results from an ecological imbalance in the physiological equilibrium between tooth minerals and oral microbial biofilms [4,5]. Endogenous bacteria, mainly *Streptococcus mutans*, in the biofilm produce weak organic acids as a by-product of metabolism of fermentable carbohydrates. These weak organic acids reduce the local pH and demineralises the tooth [4-8]. Caries can be arrested and potentially reversed in its early stages, but is often not self-limiting and without proper care, caries can progress until the tooth is destroyed [1]. Although the primary focus of *S. mutans* virulence is in the oral cavity, it can enter into systemic circulation causing bacteraemia leading to infective endocarditis [9,10].

Enterococcus faecalis, is a normal inhabitant of the oral cavity. It's Prevalence is increased in oral rinse samples from patients receiving initial endodontic treatment, those midway through treatment and patients receiving endodontic retreatment when compared to patients with no endodontic history [11]. *E. faecalis* is associated with different forms of periradicular diseases including primary endodontic infections and persistent infections [12]. According to the literature, *E. faecalis* in dentinal tubules has been shown to resist intracanal dressings of calcium hydroxide for over 10 days [13,14]. *E. faecalis* has the potential to form biofilm that helps it resist destruction as the bacteria becomes 1000 times more resistant to phagocytosis, antibodies, and antimicrobials than nonbiofilm producing organisms [15].

Use of plants is still being explored and is considered to be a promising treatment option for various diseases [16]. Aloe vera has been used therapeutically for many centuries because of its lengthy historic reputation as a potential curative agent and its widespread use in complementary therapies [17,18]. Although a lot of works have been carried out on the medicinal uses of Aloe vera, there is still little information on its anti-microbial effect against carious bacteria.

Tea is a widely consumed beverage and is popularised throughout the world. The growing interest in the potential health benefits of tea, together with its popularity as a beverage, have lead to numerous investigations on the chemical constituents of tea and their biological properties [19], such as antimutagenic [20], anticarcinogenic and antioxidant [21,22], antibacterial [23,24] and antiallergic activities [25].

Coriander sativum (coriander) is a well-known herb widely used as a spice and in folk medicine [26]. Coriander oil is one among the 20 major essential oils in the world market [27]. It is a known antimicrobial agent but it's mechanism of action is unclear [26,28].

Though the above mentioned plant extracts and essential oil are proven antimicrobial agents, very little information is available regarding its effects on oral bacteria. This study is to determine the antimicrobial activity of aloe vera, black tea and coriander oil against *Streptococcus mutans* and *Enterococcus faecalis* using agar gel diffusion method. Different concentrations of the plant materials are used and MIC is determined.

MATERIALS AND METHODS:

Preparation of plant extract:

Aloe vera extract preparation: Dried and powdered *Aloe vera* leaves was purchased. Aqueous extract of Aloe vera was prepared by mixing 5 g of *Aloe vera* powder with 100 ml distilled water and was heated at 70 °C for 5 minutes (Figure 1). The extract was then filtered using Whitman's filter paper. The extract was again heated for 10 minutes and used for further experiments.

Black tea extract preparation: Black tea was purchased from local market and the extract was prepared by mixing 10 g of black tea powder into 100 ml distilled water. The mixture was then heated at 70 oC for 5 minutes (Figure 2). The solution was filtered using Whitman's paper and is again heated for 10 minutes. The concentrated extract was used for further experiments.

Coriander oil: coriander oil prepared by steam distillation method was purchased from Allin Enterprises, India.

Figure 1 - Mixture of 5 g of dried Aloe vera leaf powder in 100 ml distilled water.

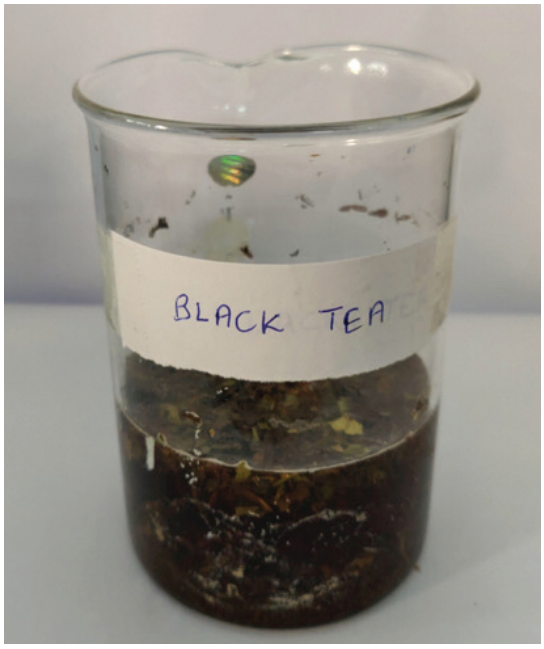

Figure 2 - Mixture of 10 g of black tea powder in 100 ml distilled water.

Antibacterial activity of plant extracts against oral pathogens:

The agar well diffusion method was used to determine the antibacterial activity of Aloe vera extract, black tea extract and coriander oil. Different concentrations of the plant extracts and oil was tested against *S. mutans* and *E. faecalis*. The fresh bacterial suspension was dispersed on the surface of Muller Hinton agar plates. Different concentration of prepared plant extracts and coriander seed oil (50 & 100 μl) was incorporated into the wells and the plates were incubated at 37 °C for 24 h. The antibiotic (amoxicillin 30 μl) was used as positive control. Zone of inhibition was recorded in each plate.

Statistical analysis:

The ZOI test of each plant extract at 50 and 100 μl concentrations was repeated thrice to obtain the triplicate values. The mean, standard deviation and standard error was calculated using the triplicate values.

RESULTS

Antimicrobial activity of *Aloe vera* extract, black tea extract and coriander oil at 50 and 100 µl concentration against S. mutans: Figure 3 and Figure 4

From Table I it is appreciable that the mean ZOI of S. mutans with Aloe vera extract at 50 μl concentration was found to be 17 mm with a standard error of ±0.58 mm. And at 100 μl it was found to be 20 mm with standard error of 0.58 mm. Similarly for black tea and coriander oil, the final ZOI was found to be 21.67±0.88 mm and 25.00±0.58 mm at 50 μl concentration and 24.33±0.88 mm and 23.33±0.58 mm at 100 μl concentration respectively.

Table I - ZOI of aloe vera extract, black tea extract and coriander seed oil against S. mutans at 50 and 100 µl respectively obtained by repeating the test 3 different times, the mean ZOI, standard deviation, standard error and the final ZOI (mean ± standard error) are indicated the table

Aloe Vera							
	I	II	III	MEAN	STD DEV	STD ERR	FINAL ZOI
50 µl	16	17	18	17.00	1	0.58	17.00±0.58
100 µl	19	20	21	20.00	1	0.58	20.00±0.58
Black tea							
	I	II	III	MEAN	STD DEV	STD ERR	FINAL ZOI
50 µl	20	22	23	21.67	1.527525232	0.88	21.67±0.88
100 µl	23	24	26	24.33	1.527525232	0.88	24.33±0.88
Coriander oil							
	I	II	III	MEAN	STD DEV	STD ERR	FINAL ZOI
50 µl	24	25	26	25.00	1	0.58	25.00±0.58
100 µl	22	23	25	23.33	1.527525232	0.88	23.33±0.58

Antimicrobial activity of *Aloe vera* extract, black tea extract and coriander oil at 50 and 100 µl concentration against *E. faecalis*: Figure 5 and Figure 6

From Table II it is evident that the mean ZOI of *E. faecalis* with *Aloe vera* extract at 50 μl concentration was found to be 15.33 mm with a standard error of ±0.33 mm. And at 100 μl it was found to be 16 mm with standard error of 0.58 mm. Similarly for black tea and coriander oil, the final ZOI was found to be 15.00±0.58 mm and 13.00±0.58 mm at 50 μl concentration and 18.00 ±0.58 mm and 13.33±0.58 mm at 100 μl concentration respectively.

Table II - ZOI of aloe vera extract, black tea extract and coriander seed oil against E. faecalis at 50 and 100 µl respectively obtained by repeating the test 3 different times, the mean ZOI, standard deviation, standard error and the final ZOI (mean ± standard error) are indicated in the table

Aloe Vera							
	I	II	III	MEAN	STD DEV	STD ERR	FINAL ZOI
50 µl	15	15	16	15.33	0.577350269	0.33	15.33±0.33
100 µl	15	16	17	16.00	1	0.58	16.00±0.58
Black tea							
	I	II	III	MEAN	STD DEV	STD ERR	FINAL ZOI
50 µl	14	15	16	15.00	1	0.58	15.00±0.58
100 µl	17	18	19	18.00	1	0.58	18.00±0.58
Coriander oil							
	I	II	III	MEAN	STD DEV	STD ERR	FINAL ZOI
50 µl	12	13	14	13.00	1	0.58	13.00±0.58
100 µl	12	13	15	13.33	1.527525232	0.88	13.33±0.58

Antimicrobial activity of amoxicillin at 30 µl concentration against *S. mutans* and *E. faecalis:*

Commercially available concentration of amoxicillin (30 μl) used as an antibacterial agent was tested against *S. mutans* and *E. faecalis*. This was used as a positive control. The Mean ZOI and standard error of amoxicillin against *S. mutans* and *E. faecalis* was found to be 14.67±0.88 mm and 13.33±0.58 mm respectively.

Table III - ZOI of 30 µl of amoxicillin against S. mutans and E. faecalis respectively obtained by repeating the test 3 different times, the mean ZOI, standard deviation, standard error and the final ZOI (mean ± standard error) are indicated in the table

Amoxicillin (30 µL)							
	I	II	III	MEAN	STD DEV	STD ERR	FINAL ZOI
S. mutans	13	15	16	14.67	1.527525232	0.88	14.67±0.88
E. faecalis	12	13	15	13.33	1.527525232	0.88	13.33±0.58

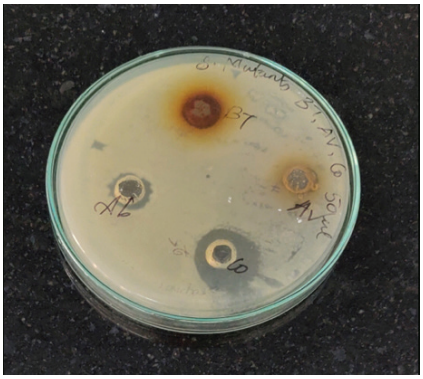

Figure 3 - Muller Hinton agar plate with S. mutans - ZOI of 50 µl of AV (Aloe vera extract), BT (Black tea extract), CO (Coriander oil) and 30 µl Ab (amoxicillin).

Figure 4 - Muller Hinton agar plate with S. mutans - ZOI of 100 µl of AV (Aloe vera extract), BT (Black tea extract), CO (Coriander oil) and 30 µl Ab (amoxicillin).

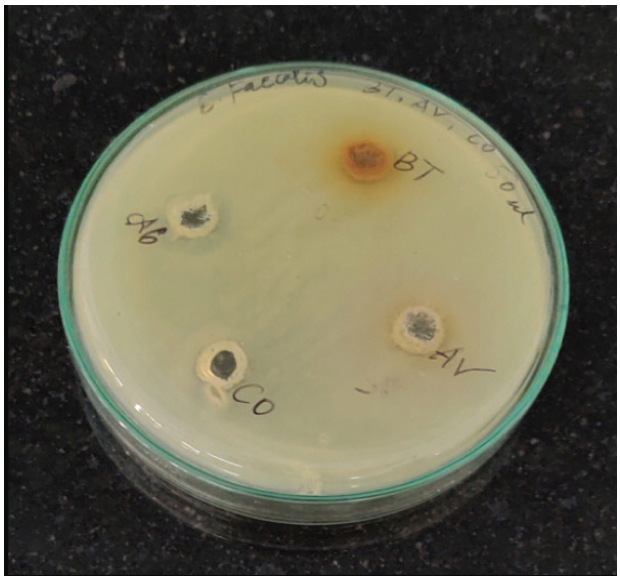

Figure 5 - Muller Hinton agar plate with E. faecalis - ZOI of 50 µl of AV (Aloe vera extract), BT (Black tea extract), CO (Coriander oil) and 30 µl Ab (amoxicillin).

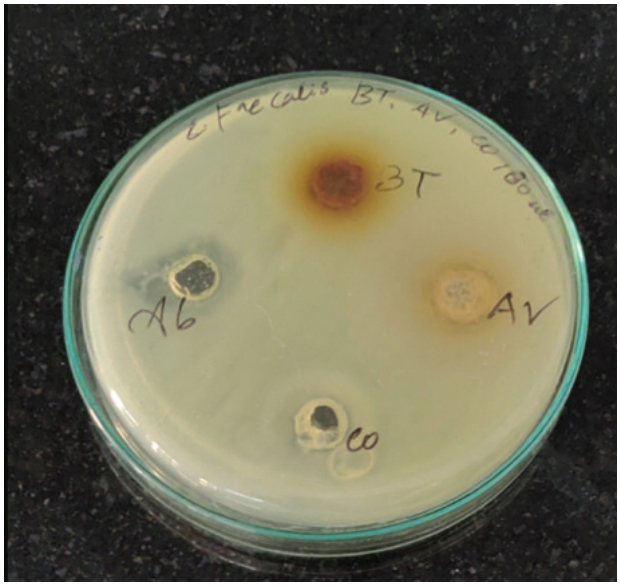

Figure 6 - Muller Hinton agar plate with E. faecalis - ZOI of 100 µl of AV(Aloe vera extract), BT (Black tea extract), CO (Coriander oil) and 30 µl Ab (amoxicillin).

DISCUSSION

Antimicrobial agents of plant origin have tremendous therapeutic potential. They are effective in the treatment for infectious diseases, and simultaneously they also mitigate many of the side effects that are often associated with synthetic antimicrobials [29]. Hence, the purpose of the present study was to evaluate the role of antimicrobial agents of plant origin in inhibition of the growth of *Streptococcus mutans* and *Enterococcus faecalis*.

Studies done by Muangsan et.al., [30] with *P. granatum* and Rani et.al., [31] with 54 medicinal herbs, demonstrated that the antimicrobial activity of the aqueous extracts were as strong as their alcoholic extract. Hence in this study the aqueous extracts of aloe vera and green tea was prepared and used.

In the present study both the plant extracts and essential oil showed good inhibition zones against *S. mutans* ranging from 16-26 mm and a moderate zone of inhibition against *E. faecalis* ranging from 15-19 mm. This shows the resistance acquired by *E. faecalis* against anti microbial agents. Among the 2 plant extracts and an essential oil, coriander oil was more effective against *S. mutans* with a maximum inhibition zone of 26 mm at 50 μl. For *E. faecalis*, black tea extract was more effective compared to others with maximise inhibitory zone of 19 mm at 100 μl.

The anti-microbial activity of these plant extracts and essential oil was compared with commercially available antibacterial agent – amoxicillin. This was used as a positive control. Powdered form of the drug was obtained and 30 μl solution was tested against *S. mutans* and *E. faecalis*. The effect of amoxicillin was more in *S. mutans* compared to *E. faecalis*. Both the plant extracts and essential oil had similar antibacterial effect as that of amoxicillin.

The proposed trial of extracts shall result in their clinical validation for the prevention of

dental caries and secondary infection in post endodontic treated root canals. Clinical proof of efficacy can then be used in the marketing of the plant extracts as therapeutic agent. This shall lead to the commercialisation of these plant extracts, in the form of extracts or oils or even by including them as a key ingredient in toothpastes and root canal medicaments.

CONCLUSION

The extracts of Aloe vera, black tea and coriander oil, showed significant activity against the investigated microbial strains, *Streptococcus mutans* and *Enterococcus faecalis*, which is promising. These extracts are not pure compounds and in spite of it, antimicrobial results were obtained. This recommends the potency of these extracts. The fragment of the derivation of antimicrobial compounds from plants seems lucrative as it will lead to the development of a phytomedicine to act against microbes. Isolation and purification of these phytoconstituents from plants may yield significant novel antimicrobials, as plant based antimicrobials have enormous therapeutic potential and they can serve the purpose without any adverse effects that are frequently associated with synthetic compounds.

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Conflict of interest

The authors have no proprietary, financial, or other personal interest of any nature or kind in any product, service, and/or company that is presented in this article.

Regulatory Statement

This study was conducted in accordance with all the provisions of the local human subjects oversight committee guidelines and policies of: Saveetha Institute of Medical and Technical Sciences. The approval code for this study is: IHEC/SDC/BDS/003/01.

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