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Original Article

Dentistry Section

Efficacy of Extract Derived from Desiccated Carrot (*Daucus carota* subsp.*sativus*) against Oral Microflora: An In-vitro Study

PREETHI RAJAMANICKAM[1], SUMATHI FELICITA[2]

ABSTRACT

Introduction: Fixed orthodontic appliances with its various components cause inaccessible areas for plaque and make tooth cleaning difficult. Several chemical formulations have been used for mechanical cleaning, but due to their unpleasant side-effects researchers now concentrate on herbal drugs.

Aim: To determine the antibacterial effect of carrot extract on *Lactobacillus*, *Aggregatibacter actinomycetemcomitans* and *Streptococcus mutans*.

Materials and Methods: This was an in-vitro study conducted to determine the antibacterial activity of *Daucus carota* subsp. sativus (carrot) extract against *Lactobacillus*, *Aggregatibacter actinomycetemcomitans* and *Streptococcus mutans*. A 500 grams of healthy and mature carrots were cleaned, washed and peeled then dried in a hot air oven at 40°C for 5 days and then ground. Ground powder was mixed with distilled water and ethanol to obtain the aqueous and ethanolic extract respectively. The antimicrobial activity of these extracts was studied using well diffusion methods in culture plates under three different concentrations. Antimicrobial activity was studied by measuring the area of inhibition. Cytotoxic activity of the samples was also assessed. Kruskal-Wallis test was performed to compare antimicrobial activity of aqueous and ethanolic extract mouthwash.

Results: Aqueous mouthwash showed moderate antimicrobial activity without statistically significant difference against *S.mutans* (p-value=0.06), *Lactobacillus* sp. (p-value=0.7), or *A. Actinomycetemcomitans* (p-value=0.16) microbes, at three different concentrations. Ethanolic extract had moderate antimicrobial activity against all the three microorganisms, but more significant at 100 microlitre concentrations with a p-value of 0.03 against S. mutans. The cytotoxic effects of the ethanolic and aqueous mouthwashes were less cytotoxic at minimal concentrations.

Conclusion: Extract derived from *Daucus carota* was proven to possess antimicrobial activity against *S.mutans*, *A.actinomycetemcomitans* and *Lactobacillus*. Further research is required to advocate its efficacy at lower concentrations.

Keywords: Antimicrobial activity, Caffeic acid, Carotenoids

INTRODUCTION

White Spot Lesions (WSL) are areas of decalcification on the enamel surfaces adjacent to fixed appliances. These are nothing but subsurface enamel porosity from carious demineralisation seen as a result of prolonged undisturbed plaque accumulation. Under these situations, acids diffuse into the enamel and the demineralisation continues in the subsurface enamel, then the intact enamel surface collapses and becomes cavitated [1,2]. Fixed orthodontic appliances with its various components cause inaccessible areas for plaque and make tooth cleaning difficult [3].

Dental plaque has long been thought to be the most important factor in the development of caries, gingivitis, and periodontal disease [4]. To avoid this, it is critical to maintain adequate plaque control. Plaque control can be performed using mechanical, chemical, or a combination of the two methods. The first line of treatment for WSL is maintaining proper oral hygiene which can be achieved by educating the patients. Along with mechanical hygiene, mouthwash, which is a chemical plaque control approach, should be employed [4,5]. Mouthwashes have been suggested for the prevention and treatment of oral illnesses, particularly oral microorganisms [6]. Water and active ingredients such as antibiotics, anti-fungals, and anti-inflammatory compounds are commonly found in them. Mouthwashes have been discovered to help with the removal and eradication of germs [7]. Chlorhexidine (CHX) is regarded, the gold standard among mouthwashes and was used as part of a periodontal treatment routine [8-10]. CHX, on the other hand, was known to produce a variety of adverse effects, ranging from mild ones like a change in the patient's taste sense and tooth staining to less prevalent ones like mucosal erosion and parotid oedema [11,12]. Because of the negative side-effects of CHX, its use for long-term therapy has been restricted or discouraged [13]. Several mouthwashes have been explored for long-term therapy without the same side-effects as CHX, but none have been successful in giving a similar antiplaque and antigingivitis impact as the latter.

Herbal mouthwashes have recently acquired popularity for their antibacterial capabilities, however none have been able to equal CHX's [13,14]. Since, then a variety of herbal mouthwashes are made available from horsetail herb, plantain leaf, aloe vera, organic echinacea angustifolia root, jyestiamadh, neem, clove oil, pudina, ajwain, white oak bark, organic lobelia herb and seed, organic peppermint leaf, tea tree essential oil, myrrh gum, triphala, tulsipatra, wildcrafted goldenseal root, clove essential oil, peppermint essential oil [15,16].

Daucus Carota

This species serves as a natural source of food dye, carotene. Carrots are members of the Umbelliferae family, an extensive order of herbaceous plants that is very important to humans [17,18]. Carrots are an important root vegetable that are high in bioactive substances such as carotenoids and dietary fibre, as well as a variety of other functional components with health promoting effects [17].

Caffeic acid is the predominant phenolic acid in carrots with appreciable amounts of thiamin, riboflavin, niacin, folic acid and vitamin C in carrot roots [19,20]. Caffeic acid also showed a beneficial effect on the healing of oral surgical wounds like it decreased inflammation and accelerated granulation tissue formation and epithelialisation [21].

It was found that the alcoholic extract has more effect on the growth of bacteria which explain the efficiency of carrot to reduce the contamination by *Pseudomonas aeruginosa* [22]. Thus, considering the antimicrobial effect of caffeic acid and presence of considerable amounts of ascorbic acid in carrots, the aim of this study was to determine the antibacterial effect of carrot mouthwash on *Lactobacillus*, *Aggregator actinomycetemcomitans* and *Streptococcus mutans* which are the most important oral microbial flora.

MATERIALS AND METHODS

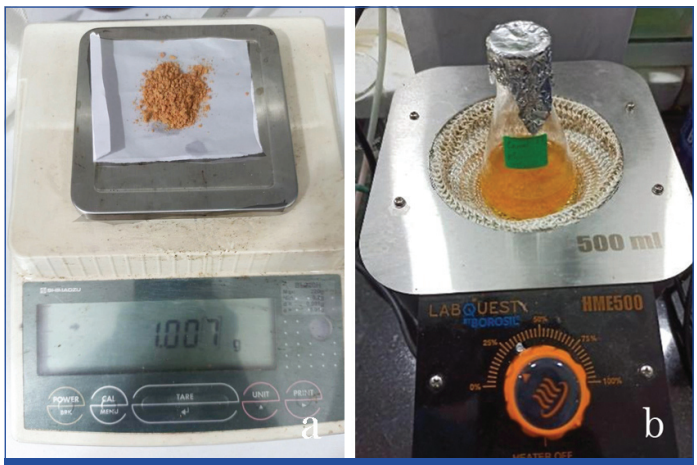
This study was an in-vitro study conducted at Saveetha Institute of Medical and Technical Sciences, Chennai, Tamil Nadu, India, during the period of October 2020 to February 2021.

Procedure

Five hundred grams of healthy and mature carrots were purchased from the local vegetable market. Unhealthy, diseased and dried carrots were excluded. Cleaned and washed carrots were manually peeled using a sterilised peeler and the peels were then dried in a hot air oven (Infra hot air oven TCK 41 dolphin automation) at 40°C for 3 days. The dried carrot peels were powdered in a laboratory electric blender and were kept in airtight bottles until further use [23]. This powder was further used to make the following extracts and mouthwashes [Table/Fig-1a].

Prepration of the Extract

Plain ethanolic carrot extract: Two grams of the obtained powder was mixed with 50 mL of ethanol. The grounded carrot powder was weighed in a weighing balance {Shimadzu BL-220H High-Precision Electronic Balance (Measuring Capacity 0.22 Kg (220 g)}. The solution was prepared in a conical flask with its mouth closed with an aluminium foil. The obtained ethanolic extract was then kept in an orbital shaker (Grocel Lark OS shaker) for 5 days (89 rpm) and then heated in a heating mantle (Labquest HME 500) at 25°C for 85 minutes [Table/Fig-1b].



[Table/Fig-1]: a) Ground carrot powder in a weighing machine; b) Ethanolic extract heated in an electric mantle.

Plain aqueous carrot extract: One gram of obtained powder was mixed with 50 mL of distilled water. The aqueous extract was directly heated without subjecting it to the shaking. A 50 mL of aqueous extract was reduced to 40 mL while ethanolic extract was reduced to 10 mL. Both the solutions were then filtered with Whatman filter paper [Table/Fig-2a] [23].

Ethanolic carrot mouthwash preparation: For mouthwash preparation, 2 mL of stevia solution (sweetening agent) was mixed with 1 mL of ethanolic carrot extract, 100 microlitre of coriander extract (flavouring agent), sodium benzoate 0.1 g, and sodium lauryl sulphate 0.01 g. Then this solution was mixed with 7 mL of distilled water to make it 10 mL [24].

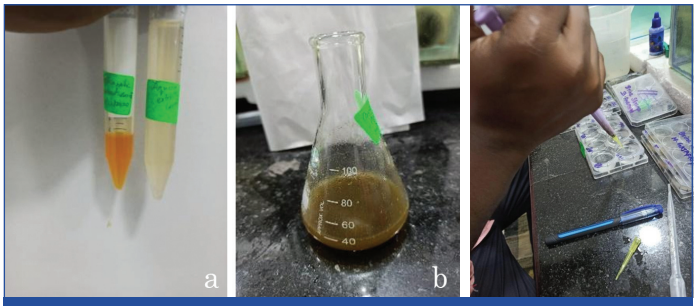
Aqueous carrot mouthwash preparation: For aqueous mouthwash preparation, 2 mL of stevia solution [Table/Fig-2b] was mixed with 1 mL of aqueous carrot extract, 100 microlitre of coriander extract (flavouring agent), sodium benzoate 0.1 g, and sodium lauryl sulphate 0.01 g. Then this solution was mixed with 7 mL of distilled water to make it 10 mL [24].

Stevia extract (sweetening agent): 0.5 g of stevia powder was mixed with 25 mL of distilled water and boiled for 15 minutes and filtered [25].

Cytotoxic Activity

A transparent container was 3/4th filled with water, with saline (25 g iodine free NaCl dissolved in 800 mL of water) and the shrimp egg capsule was dispensed in the tank and allowed to hatch for a day. Adequate aeration with an air pump was provided to the tank for survival of the eggs. After 24 hours, the hatched artemia nauplii were transferred to a 6 well plate at the count of 10 per well. In the 6 well plate [Table/Fig-3] half of the well was filled with saline and the sample solution to be tested was added at concentrations of 5, 10, 20, 40 and 80. This method of cytotoxicity assessment was performed separately for both the aqueous and ethanolic mouthwash solutions. A control well was also added for which a commercial mouth (Zerosense) was used at concentration of 80 for comparing with the ethanolic carrot extract and 40 for comparing with the aqueous extract [26]. The number of live nauplii was counted after 24 hours. The cytotoxic activity of the solution was indicated by the percentage of live nauplii which was calculated by the formula below.

%Death={(Number of dead nauplii)/(Number of dead nauplii+Number of live nauplii)}×100 [27].



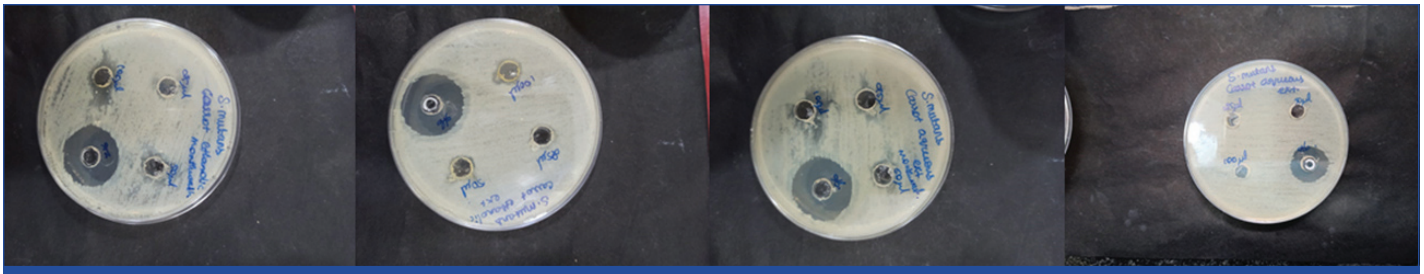
[Table/Fig-2]: a) Ethanolic and aqueous carrot extract. b) Stevia extract.
[Table/Fig-3]: Cytotoxic activity assessment in a 6 well plate. (Images from left to right)

Antimicrobial Activity

Agar well diffusion method: The antibiotic sensitivity was studied using well diffusion methods in culture plates under three different concentrations (25%, 50%, 100%) for both aqueous and ethanolic extract and also for the mouthwashes prepared from both the extracts. Apart from the 4 test solutions, amoxycillin was used as a control. Freshly isolated colonies of *Aggregatibacter actinomycetemcomitans*, *Lactobacillus* species and *Streptococcus mutans* were allowed to grow in the culture media and allowed to solidify. After solidification, wells were made using a sterile cork borer (6 mm in diameter) into agar plates containing inoculums (well diffusion method). Freshly prepared test solutions were poured into the wells. After an incubation period of 24 hours, antimicrobial activity was studied by measuring the diameter of inhibition zone [Table/Fig-4-6] [28].

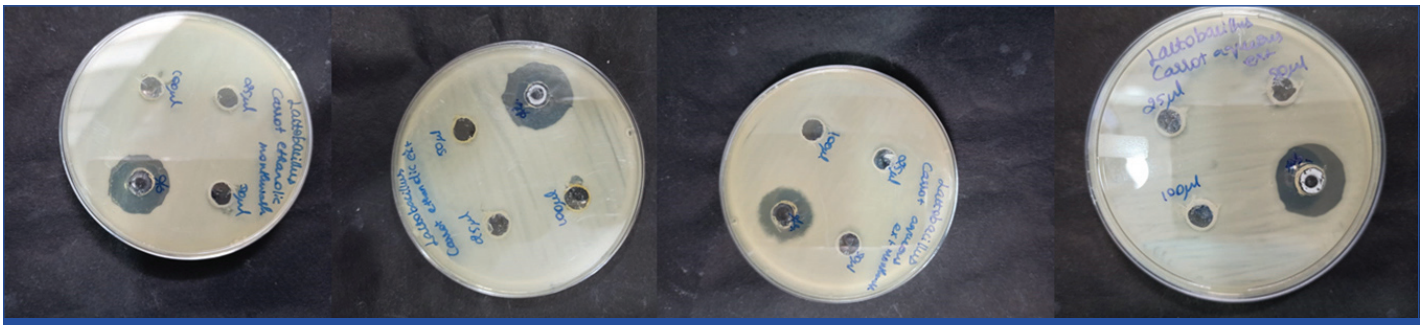
STATISTICAL ANALYSIS

Statistical analysis was performed in Statistical Package for the Social Sciences (SPSS) software version 23.0. Kruskal Wallis test was performed to compare antimicrobial activity of aqueous and ethanolic extract mouthwash. This was done to assess whether there was any significant difference in the antimicrobial activity exhibited at different concentrations against the three microorganisms.



[Table/Fig-4]: Zone of inhibition exhibited by ethanolic extract mouthwash, ethanolic extract, aqueous extract mouthwash and aqueous extract on *Streptococcus mutans*.

[Table/Fig-5]: Zone of inhibition exhibited by ethanolic extract mouthwash, pure ethanolic extract, aqueous extract mouthwash and pure aqueous extract on A. Actinomycetemcomitans.



[Table/Fig-6]: Zone of inhibition exhibited by ethanolic extract mouthwash, pure ethanolic extract, aqueous extract mouthwash and pure aqueous extract on *Lactobacillus* species.

RESULTS

Antimicrobial Activity

Among the four test solutions like plain ethanolic carrot extract, plain aqueous carrot extract, ethanolic carrot mouthwash, aqueous carrot mouthwash, ethanolic extract and ethanolic carrot mouthwash showed the highest antimicrobial activity against all the microorganisms at 100 microlitre concentrations. The diameter of the inhibition zones are presented in [Table/Fig-7,8]. The growth inhibition zone measured for ethanolic extracts for various bacteria ranged between 9±0.7mm each at 25 µL, and 50 µL and 12±0.3 at 100 µL concentrations against the *Lactobacillus* species, 10±0.3 at 25 µL, 12±0.2 at 50 µL and 15±0.7 at 100 µL concentrations against the S.mutans, 10±0.7 at 25 µL, 12±0.1 at 50 µL and 15±0.7 at 100 µL concentrations against *Actinomyces* species. The growth inhibition zone measured for aqueous extracts was about 9 mm against all the three microorganisms at all concentrations. For the mouthwashes prepared from both the extracts, with aqueous mouthwash, zone of inhibition of 9.5±0.5mm at both 25 µL and 50 µL concentrations, 9.1±0.7 mm at 100 µL concentrations were exhibited against *Lactobacillus*, 11.5±0.5 mm each at 25 µL, 12±1 at 50 µL and 13.5±0.5 at 100 µL concentrations against S.mutans and 11.5±0.5 mm at 25 µL and 50 µL and 12.1±0.7 at 100 µL concentrations against *A.actinomycetemcomitans* were exhibited. With ethanolic mouthwash, zone of inhibition of 9.1±0.7 mm against *Lactobacillus* species at all 3 concentrations, 15±1 mm at 25 µL, 18±1 at 50 µL and 20±1 at 100 µL concentrations against *S.mutans* and 9.1±0.7 mm at 25 µL, 13±1 at 50 µL and 13.6±0.7 at 100 µL concentrations against *A.actinomycetemcomitans* were exhibited.

	Aqueous extract concentrations (in microlitres)				Ethanolic extract concentrations (in microlitres)			
Organism	25 µL	50 µL	100 µL	Control	25 µL	50 µL	100 µL	Control
Lactobacillus	9	9	9	18±0.7	9±0.7	9±0.7	12±0.3	21±0.7
S.mutans	9	9	9	20±0.6	10±0.3	12±0.2	15±0.7	25±0.4
A. actinomy-cetemcomitans	9	9	9	20±0.4	10±0.7	12±0.1	15±0.7	23±0.3

[Table/Fig-7]: Antimicrobial activity-zone of inhibition (in mm) exhibited by the 2 extracts and the control, against the three microorganisms.

	Aqueous mouthwash concentrations (in microlitres)				Ethanolic mouthwash concentrations (in microlitres)			
Organism	25 µL	50 µL	100 µL	Control	25 µL	50 µL	100 µL	Control
Lactobacillus	9.5±0.5	9.5±0.5	9.1±0.7	18.1±0.7	9.1±0.7	9.1±0.7	9.1±0.7	20±1
S.mutans	11.5±0.5	12±1	13.5±0.5	22±1	15±1	18±1	20±1	26±1
A. actinomycetemcomitans	11.5±0.5	11.5±0.5	12.1±0.7	18±1	9.1±0.7	13±1	13.6±0.7	17±.05

[Table/Fig-8]: Antimicrobial activity-zone of inhibition exhibited by the 2 mouthwashes and the control, against the three microorganisms.

Ethanolic extract showed moderate antimicrobial activity against all the 3 microorganisms, but more significant at 100 microlitre concentrations with greater zones of inhibition.

Further Kruskal-Wallis test was performed to find the significant difference in the antimicrobial activity of the two mouthwashes at different concentrations however, it was not performed between the two extracts. This comparison was done to analyse the minimum effective concentration of the aqueous and ethanolic mouthwashes to cause the antimicrobial activity. Results showed there were no significant differences in the antimicrobial activity between different concentrations of aqueous mouthwash against the *Lactobacillus* species (p-value=0.7), *Actinomycetemcomitans* (p-value=0.16) or *S.mutans* (p-value=0.06). Also, there were no significant differences in the antimicrobial activity between different concentrations of ethanolic mouthwash against *Lactobacillus* (p-value=1) and *Actinomyces* sp. (p-value=0.5), but against *Streptococcus mutans* there was a statistically significant difference (p-value=0.03) [Table/Fig-9].

Mouthwash	Organism	p-value
Ethanolic mouthwash	*S.mutans*	0.03
	Lactobacillus species	1.00
	Actinomyces species	0.055
Aqueous mouthwash	*S.mutans*	0.06
	Lactobacillus species	0.7
	Actinomyces species	0.16

[Table/Fig-9]: The difference in the dose dependent antimicrobial activity of the 2 mouthwashes against the 3 microorganisms.
*Kruskal Wallis test

Cytotoxicity: A 100% nauplii survived at 5 microlitre concentration of ethanolic or aqueous mouthwashes, at 40 microlitre concentrations, the nauplii showed 70% and 80% survival rates in the aqueous and ethanolic mouthwashes, respectively and at 80 microlitre concentrations the nauplii showed 50% and 30% survival rates in the aqueous and ethanolic mouthwashes, respectively. The cytotoxic effects of the ethanolic and aqueous mouthwashes were less cytotoxic at minimal concentrations [Table/Fig-10].

	Number of live nauplii			
	Ethanolic mouthwash		Aqueous mouthwash	
Concentration in microlitres	Day 1	Day 2	Day 1	Day 2
5	10	10	10	10
10	10	9	10	9
20	10	5	10	10
40	10	7	10	8
80	10	3	10	5
Control (Zerosense mouthwash)	10	7	10	9

[Table/Fig-10]: Table showing the cytotoxic activity indicating the number of live nauplii.

Cytotoxicity test was not performed for the plain aqueous and ethanol carrot extracts and it was carried out only with the two mouthwashes prepared to determine whether the newly formulated mouthwash will cause any cell death due to leaching of toxic substances or from direct contact.

DISCUSSION

In the present study, antimicrobial activity of carrot peel extracts against three pathogens was assessed in terms of zone of inhibition of bacterial growth. Carrot is rich in β-carotene, ascorbic acid and tocopherol and it is also classified as a vitaminised food [29] and due to the significant levels of variety of different compounds present, carrots are also mentioned as a food with significant health promoting properties [30]. Carotenoids are important micronutrients for human health [31]. The total carotenoids content in the edible portion of carrot roots range from 6,000-54,800 µg/100 g. The main physiological function of carotenoids is as a precursor of vitamin A [32]. In the past decade, carotenoids, because of their protective impact against some types of malignancies have gained a lot of attention of researchers [31,33,34] and because of their physiological effects, such as antioxidant, antimutagenic, and anticancer properties, phenolics or polyphenols have gotten a lot of attention [31].

Studies conducted earlier concluded that ethanol and methanol extracts of seeds of *Daucus carota* were effective against Escherichia coli, *Klebsiella pneumoniae, Salmonella typhi, Staphylococcus aureus, Candida albicans* [22,35]) Flavonoids, phenols, and a variety of glycosides have been discovered in the seeds of *Daucus carota*. These phytochemicals have all been shown to exhibit pharmacological characteristics, indicating that they could be used as antimicrobials [22,35]. Studies also showed that the alcoholic extracts of carrot had an inhibitory effect on the growth of *Pseudomonas aeruginosa* [22,35]. This antibacterial effect of carrot extract was attributable to phytochemicals, which are secondary metabolites found in the extract. The therapeutic properties of a plant are determined by its phytoconstituents. Carbohydrates, alkaloids, flavonoids, phenols, proteins, saponins, and triterphenoids were found in a phytochemical screening of *Daucus carota*, which are bioactive chemicals known to possess antioxidant and antibacterial properties, as well as significant sources of dietary fibre [36,37].

In this study, antimicrobial activity was tested both with the plain ethanolic and aqueous extracts derived from *Daucus carota* and also with the mouthwashes prepared from both the extracts. The antimicrobial activity of the extracts, increased linearly with increase in concentration of the extracts (µg/ mL) with differences in diameter of zone of inhibition. Carrot peel inhibited the growth of three periodontal pathogens responsible for WSL.

Also, in the present study, the cytotoxic effects of the ethanolic and aqueous mouthwashes were less cytotoxic at minimal concentrations. Not much evidence was presented in terms of the cytotoxicity of carrots. It was previously studied that phenylpropanoids (Epilaserine and 2-epilaserine) were compounds present in carrots were responsible for cytotoxic effects against HL-60 (Human Leukaemic) cells [36].

Previous study conducted by Anibijuwon II et al., showed that addition of carrot extract with Hyphothiocyanite enhanced its antimicrobial activity against Staphylococcus aureus and E coli. These phytochemicals have all been confirmed to possess pharmacological properties which support their potential use as antimicrobial agents [35].

Also, a study carried out with alcoholic extracts of carrot had an inhibitory effect on the growth of *Pseudomonas aeruginosa* [22]. The results are in agreement with our present study in terms of the antimicrobial activity. In addition to utilising the antimicrobial activity of *Daucus carota*, further stevia was used as a sweetening agent in an effort to make the formulation as herbal as possible. The herbal mix used in the present study was unique and new and to the best of our knowledge was the first of its kind in combination because neither carrot extract nor stevia was used in the formulation of mouthwashes previously. The study was conducted also with plain aqueous and ethanolic carrot extracts to confirm whether plain carrot extract has an antimicrobial action without any additives such as stevia or flavouring agents, since stevia has also proved to possess an antimicrobial effect against certain cariogenic bacteria [38].

Limitation(s)

The limitations of the present study were that of an herbal mouthwash was not used as a control. The chemical composition and the component in the *Daucus carota* responsible for its antimicrobial activity were not studied. Further, the effect of *Daucus carota* on supragingival and subgingival plaque was not studied separately. Thus, studies need to be conducted in future in detail about the

chemical composition and the minimum inhibitory concentrations needed to produce an antimicrobial activity. Further commercial herbal mouthwash should have been used as a control and more in-vivo studies should be conducted.

CONCLUSION(S)

Extract derived from *Daucus carota* has proven to possess antimicrobial activity against *S.mutans*, *A.actinomycetemcomitans* and *Lactobacillus*. The cytotoxic effects of the ethanolic and aqueous mouthwashes were less cytotoxic at minimal concentrations. Further research is required to advocate its efficacy at lower concentrations.

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PARTICULARS OF CONTRIBUTORS:
1. Postgraduate Student, Department of Orthodontics, Saveetha Dental College, Chennai, Tamil Nadu, India.
2. Reader, Department of Orthodontics, Saveetha Dental College, Chennai, Tamil Nadu, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:
Dr. Preethi Rajamanickam,
4/399, Balaji Nagar, 2nd Street, Santhosapuram, Chennai, Tamil Nadu, India.
E-mail: preethirajamanickams@gmail.com

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