Journal of Population Therapeutics & Clinical Pharmacology

RESEARCH ARTICLE
DOI: 10.53555/fy0ha784

"EVALUATION AND COMPARISON OF ANTIMICROBIAL EFFICACY OF STRAWBERRY, KIWI, AND POMEGRANATE EXTRACT AGAINST S. MUTANS AS MOUTH RINSE AND E. FAECALIS AS A ROOT CANAL IRRIGANT: AN IN VITRO STUDY"

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ABSTRACT:

Background: The study focuses on assessing their efficacy against Streptococcus mutans, a key contributor to dental caries when employed as a mouth rinse. Additionally, the antimicrobial impact of these extracts is evaluated against Enterococcus faecalis, a persistent pathogen associated with root canal infections, when used as a root canal irrigant.

Aim: This research aims to investigate the antimicrobial properties of natural fruit extracts, namely Strawberry, Kiwi and Pomegranate, as potential alternatives to conventional oral care agents.

Methods and Material: Extract of strawberry, kiwi and pomegranate was prepared as an experimental group. Well diffusion technique was used to inoculate bacteria in agar medium, followed by inoculation of microbial culture. Media was transferred to petri dishes and bacteria was spread over a dish with a sterile cotton swab. Plates were incubated in an upright position under aerobic condition at 37°C for 24 hours and zone of inhibition was measured.

Statistical analysis used: Statistical analysis was carried out using descriptive statistics in the Statistical Package for the Social Sciences (SPSS) software, version 25.0.

Results: Kiwi exhibited maximum antimicrobial efficacy followed by strawberry and pomegranate when compared with sodium hypochlorite (2.5%) as a root canal irrigants against E. faecalis. Whereas strawberry was effective as a mouth rinse when compared to chlorhexidine (0.2%) against S. mutans.

Conclusions: This research contributes valuable insights into the feasibility of incorporating natural fruit extracts into oral care practices, offering a potentially safer and more sustainable alternative to traditional chemical-based products.

Keywords: Mouthwash, Root canal irrigant, Streptococcus mutants, Enterococcus faecalis.

INTRODUCTION:

Dental caries, a prevalent infectious disease impacting the oral cavity, stands as a global health concern marked by the demineralization of tooth enamel. Among the diverse bacterial species associated with cariogenic processes, Streptococcus mutans takes center stage as the principal etiological agent. This acid-tolerant Gram-positive bacterium plays a pivotal role in metabolizing dietary carbohydrates, producing acids, particularly lactic acid, which exerts a profound demineralizing effect on ename.[1] The multifactorial etiology of tooth decay involves the interplay of host factors, environmental conditions, and bacterial activity.[2]

In recent years, the field of dentistry has witnessed a growing inclination towards the use of herbal and natural products for treating various oral conditions. The resurgence of interest in traditional remedies, often referred to as "grandmother's remedies," has gained substantial traction, reflecting a broader societal shift towards embracing natural alternatives in healthcare.[3] Mouthwashes, a common prescription in dentistry for preventing and treating oral conditions, are increasingly incorporating natural products due to their perceived benefits. This study addresses the evolving landscape of oral care, where traditional and herbal remedies are gaining prominence. A critical examination of the effectiveness of these new-age mouthwashes, in comparison to established gold standards, forms the core of this investigation.[4] The rising awareness of complementary and alternative medicine, coupled with the belief in fewer side effects associated with alternative therapies, has fueled the popularity of herbal mouthwashes.[5]

Root canal therapy, a fundamental aspect of endodontic treatment, aims to eliminate microorganisms from the root canal, creating a conducive environment for tissue healing. Enterococcus faecalis, commonly isolated from failed root canals, poses a challenge in endodontics.[6] Chemical root canal irrigants such as sodium hypochlorite and 2% chlorhexidine have proven effective against E. faecalis.

The success of endodontic treatment hinges on the meticulous disinfection of the root canal system, necessitating thorough mechanical preparation and copious irrigation.[7] Given the rise in antibiotic-resistant bacterial strains, researchers are exploring herbal alternatives. Notably, strawberries, pomegranates, and kiwis have demonstrated antibacterial and antioxidant activities, making them potential candidates for oral care applications. Strawberries boast a rich nutrient profile,[8] pomegranate extracts exhibit broad-spectrum antimicrobial activity,[9] and kiwis, abundant in flavonoids, offer diverse biological properties.[10]

Against this backdrop, the present research endeavors to systematically evaluate and compare the antimicrobial efficacy of pomegranate, kiwi, and strawberry extracts against S. mutans and E. faecalis, considering their application as a mouth rinse and root canal irrigant, respectively. This investigation contributes to the evolving discourse on herbal alternatives in oral care, with implications for advancing effective and sustainable practices in dentistry.

MATERIAL AND METHODS:

Fresh strawberry, kiwi and pomegranate were collected from the local market. The study and the lab procedures were carried out at the Laboratory.

Laboratory procedure

Description of specimen preparation:

Fresh fruits of Strawberries (Fragaria x ananassa), Kiwi (Actinidia chinensis), and Pomegranate (P. granatum L.) were procured from the local market in Bhopal city. Extracts were prepared at the laboratory. After thorough washing and peeling, the pulp was separated from the seeds. The extract was then prepared by diluting it in distilled water. Concentrations of 100 mg/ml for Pomegranate, Strawberry, and Kiwi were prepared for the well diffusion technique.

Well Diffusion Method:

The agar plate surface was inoculated by spreading a volume of microbial inoculums over the entire agar surface. A hole with a diameter of 6 to 8 mm was punched with a sterile cork borer. A volume (30µL) of the antimicrobial agent or extract solution at the desired concentration was introduced into the well. Agar plates were incubated under suitable conditions depending upon the test microorganism. The antimicrobial agent diffused in the agar medium, inhibiting the growth of the microbial strain tested.

Preparation of Media:

For bacterial culture growth, Nutrient agar medium was prepared by mixing Nutrient broth, agar, and distilled water. Nutrient broth provided peptone, Sodium chloride, yeast extract, vitamins, and carbohydrates essential for bacterial growth, while agar solidified the medium.

Inoculation of Microbial Culture:

Media was poured into Petridishes under aseptic conditions. Sterilized media was poured into 90mm sterilized petri plates, left to solidify. Microbial culture was spread over prepared plates with a sterile cotton swab.

Incubation:

Plates were incubated in an upright position under aerobic conditions at 37°C for 24 hrs for bacterial cultures. Wells containing the same volume of distilled water served as negative controls. After the incubation period, the zone of inhibition (figure 1) was measured using the Himedia zone scale in mm. The zone of inhibition increases with the concentration of the fruit extract. Media was autoclaved at 121°C for 15-20 minutes after the procedure.[11]

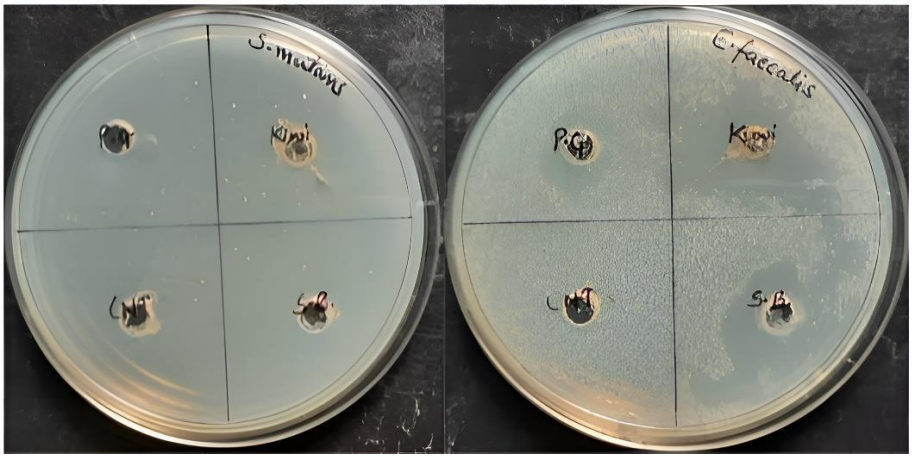

Figure 1: Image of Zone of Inhibition

P.G.- Pomegranate extract of 100mg/ml
Kiwi - Kiwi extract of 100mg/ml
S.B. - Strawberry extract of 100mg/ml

STATISTICAL ANALYSIS:

Statistical analysis was carried out using descriptive statistics in the Statistical Package for the Social Sciences (SPSS) software, version 25.0, (IBM SPSS, Inc. Chicago, Illinois). Mean Zone of inhibition was compared using unpaired student t-test and one way ANOVA test. A $P < 0.005$ was statistically significant

RESULTS:

The antimicrobial efficacy Pomegranate, Strawberry, Kiwi was systematically compared with standard root canal irrigant and mouth rinse, i.e 2.5% sodium hypochlorite, 0.2% chlorhexidine respectively, in the present study. The mean zone of inhibition values, expressed as mean ± standard deviation, were analyzed to discern the effectiveness of each agent in combating oral pathogens.

For Root Canal Irrigation: (Table 1)
Kiwi (22 ± 0.76) demonstrated the highest antimicrobial efficacy among the tested agents.
Strawberry (18 ± 0.47) exhibited a substantial antimicrobial effect, following closely behind Kiwi.
Pomegranate (12 ± 0.47) and sodium hypochlorite (12 ± 0.5) showed comparable results, indicating a similar level of antimicrobial activity.

Table 1: Comparison of the antimicrobial efficacy of Punica granatum (Pomegranate), Fragaria ananassa (Strawaberry), Actinidia deliciosa (Kiwi) and standard drug sodium hypochlorite (2.5%) as root canal irrigant

Organisms	Study Groups	Zone of Inhibition Mean + SD	F value	p-value
E. faecalis	Punica granatum (Pomegranate)	12± 0.47	232.2	0.000*
	Fragaria ananassa (Strawaberry)	18± 0.47		
	Actinidia deliciosa (Kiwi)	22± 0.76		
	Sodium hypochlorite (2.5%)	12±0.47		

For Mouth Rinse: (Table 2)

Strawberry (24±0.47) exhibited antimicrobial efficacy comparable to chlorhexidine (24±0.5), a standard antimicrobial agent.

Pomegranate (22±0.5) demonstrated significant antimicrobial activity, approaching the effectiveness of the standard.

Kiwi (19 ± 0.47) displayed promising antimicrobial efficacy as a mouth rinse.

Table 2: Comparison of the antimicrobial efficacy of Punica granatum (Pomegranate), Fragaria ananassa (Strawaberry), Actinidia deliciosa (Kiwi) and standard drug chlorhexidine (0.2%) as a mouth rinse

Organisms	Study Groups	Zone of Inhibition Mean+ SD	F-value	p-value
S. mutans	Punica granatum (Pomegranate)	22±0.5	71.140	0.000*
	Fragaria ananassa (Strawaberry)	24±0.47		
	Actinidia deliciosa (Kiwi)	19±0.47		
	Chlorhexidine (0.2%)	24±0.5		

For Mouth Rinse and root canal irrigant: (Table 3)

Strawberry and kiwi exhibited maximum antimicrobial efficacy against S. mutans and E. faecalis respectively**.**

Strawberry demonstrated least antimicrobial activity amongst all the three fruits against S. mutans as a mouth rinse.

While pomegranate, as a root canal irrigant, showed least antimicrobial activity amongst all the three fruits against E. faecalis.

Table 3: Comparison of the antimicrobial efficacy of Punica granatum (Pomegranate), Fragaria ananassa (Strawaberry), Actinidia deliciosa (Kiwi) as mouth rinse and as root canal irrigant against E. faecalis and S mutans

Organisms	**Punica granatum (Pomegranate)**	**Fragaria ananassa (Strawaberry)**	**Actinidia deliciosa (Kiwi)**
	Mean+ SD	**Mean+ SD**	**Mean+ SD**
E. faecalis	12± 0.47	18± 0.47	22± 0.76
S. mutans	22±0.5	24±0.47	19±0.47
t-value	25.24	15.63	5.81
p-value	0.001*	0.001*	0.004*

Discussion:

The presented study delves into the global health concern of dental caries, an exceedingly prevalent oral infection affecting populations worldwide. Recognizing the pivotal role of Streptococcus mutans in dental caries initiation through biofilm formation on tooth surfaces, this research explores the antimicrobial potential of natural fruit extracts, specifically strawberry, kiwi, and pomegranate, against S. mutans as a mouth rinse and Enterococcus faecalis as a root canal irrigant.[12]

The comprehensive analysis of these fruits reveals promising attributes. In the present research we observed that for a root canal irrigant: Kiwi (22± 0.76) showed the best anitimicrobial efficacy followed by strawberry (18±0.47) whereas pomegranate (12±0.47) and sodium hypochlorite (12±0.5) showed the similar results. For a mouth rinse, strawberry (24±0.47) showed the similar result as a chlorhexidine (24±0.5) followed by pomegranate (22±0.5) and kiwi (19±0.47). Henceforth, we can conclude that strawberry shows promising results against S. Mutans as a mouth rinse whereas, kiwi showed the best antimicrobial efficacy against E. Faecalis as a root canal irrigant.

Strawberries, rich in essential nutrients like potassium, magnesium, phosphorus, calcium, iron, and vitamins A, C, E and K, exhibit properties that prevent dental plaque formation, bacterial attachment, and enamel demineralization by reducing acid production.[13] According to S. Mariem et al the berry fraction of strawberries demonstrates strong anti-biofilm activity on S. mutans, inhibiting biofilm adhesion to tooth structures without affecting bacterial viability, a crucial aspect in minimizing the emergence of resistant strains.[14]

According to Barairo et al (2019), Kiwi, known for its flavonoid content, displays antibacterial properties.[15] Previous studies corroborate the antimicrobial efficacy of flavonoids against various microorganisms, aligning with the findings of this research where kiwi emerges as the most effective root canal irrigant against E. faecalis.[16]

Pomegranate, recognized for its diverse phytochemical compounds, including ellagic acid and larger hydrolyzable tannins like punicalagin, showcases antimicrobial activity.[17] While previous research supports the notion of pomegranate's antibacterial properties, the current study identifies its potential as a mouth rinse, with promising results against S. mutans.[18]

The comparative assessment of these fruits as root canal irrigants and mouth rinses provides intriguing insights. Kiwi demonstrates the highest antimicrobial efficacy as a root canal irrigant, surpassing both strawberry and pomegranate. Conversely, strawberry exhibits comparable results to chlorhexidine as a mouth rinse, indicating its potential as an effective and natural alternative in combating S. mutans.

Henceforth, this research underscores the significant antimicrobial potential of strawberry, kiwi, and pomegranate extracts against key oral pathogens. The observed efficacy positions strawberry as a

promising candidate for mouth rinse applications against S. mutans, while kiwi emerges as the most effective root canal irrigant against E. faecalis. These findings hold promise for the development of natural and sustainable alternatives in oral care, contributing to both dental health and environmental well-being.

Conflicting Interest: NIL

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