Academia Open
Vol. 11 No. 1 (2026): June
DOI: 10.21070/acopen.11.2026.13849

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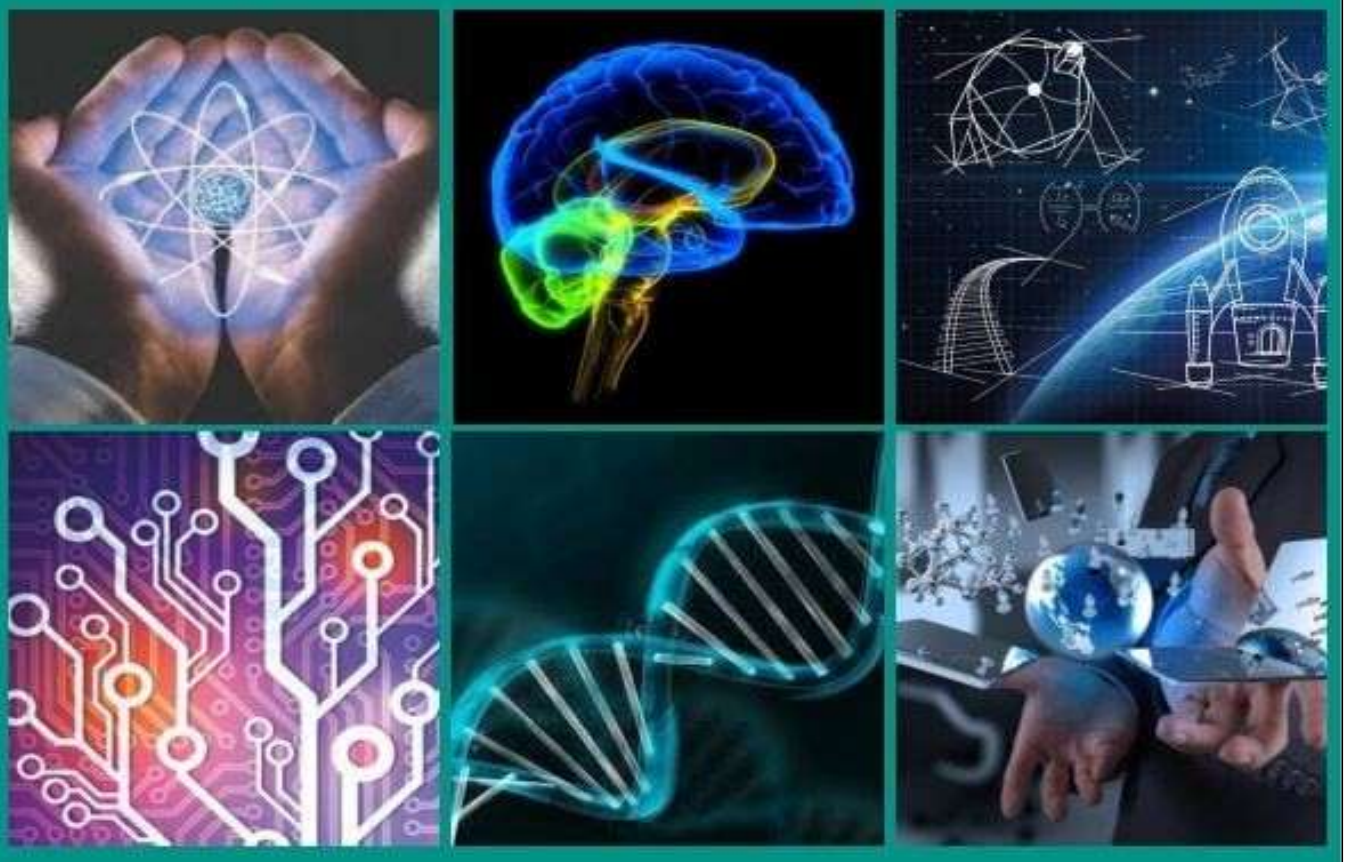

By Universitas Muhammadiyah Sidoarjo

ISSN 2714-7444 (online), https://acopen.umsida.ac.id, published by Universitas Muhammadiyah Sidoarjo

Academia Open
Vol. 11 No. 1 (2026): June
DOI: 10.21070/acopen.11.2026.13849

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ISSN 2714-7444 (online), https://acopen.umsida.ac.id, published by Universitas Muhammadiyah Sidoarjo

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DOI: 10.21070/acopen.11.2026.13849

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Academia Open
Vol. 11 No. 1 (2026): June
DOI: 10.21070/acopen.11.2026.13849

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Academia Open
Vol. 11 No. 1 (2026): June
DOI: 10.21070/acopen.11.2026.13849

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Academia Open
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Antibacterial Activity of Natural Mouthwash Against Bacteria Isolated from the Oral Cavity: Aktivitas Antibakteri Obat Kumur Alami Terhadap Bakteri yang Diisolasi dari Rongga Mulut

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Abstract

General Background: Oral hygiene is essential for preventing oral diseases, and interest in herbal formulations has increased due to concerns regarding side effects of chemical mouthwashes. **Specific Background:** Plant-derived ingredients such as pomegranate peel, clove, peppermint oil, rose water, and rock salt contain antimicrobial compounds that may support oral health. **Knowledge Gap:** However, limited studies have evaluated combined natural formulations against bacteria isolated from the oral cavity. **Aims:** This study aimed to examine the antibacterial activity of a natural mouthwash formulation against oral bacteria and compare it with a synthetic mouthwash. **Results:** Six concentrations of the formulation were tested against Klebsiella pneumoniae, Proteus mirabilis, and Enterococcus faecalis. The results showed that K. pneumoniae and P. mirabilis were sensitive to all concentrations, with inhibition zones reaching 26 mm and 28 mm respectively, while E. faecalis showed resistance to several concentrations. **Novelty:** The study presents a combined formulation of pomegranate peel and clove extracts as a natural mouthwash with antibacterial activity against multiple oral bacteria. **Implications:** These findings indicate that herbal mouthwash formulations may provide a natural alternative for oral hygiene applications.

Highlights:

- Certain natural formulations produced bacterial inhibition zones up to 28 mm
- Gram-negative isolates showed broader susceptibility than Enterococcus faecalis
- Combined plant extracts and mineral components demonstrated measurable antimicrobial activity

Keywords: Natural Mouthwash; Antibacterial Activity; Oral Cavity Bacteria; Pomegranate Peel Extract; Clove Extract

Published date: 2026-03-11

Introduction

Dinasti politik adalah kekuasaan yang secara turun temurun dilakukan dalam kelompok keluarga dengan tujuan untuk mempertahankan atau mendapatkan kekuasaan. Keadaan politik oligarki selalu mempengaruhi dinamika politik di Indonesia. Pada kenyataannya kekuatan politik seringkali mendominasikan pada mereka yang mempunyai kekuasaan atau yang mempunyai finansial yang cukup, walaupun seperti yang kita ketahui prinsip demokrasi membuka kesempatan yang sama bagi semua individu untuk berpartisipasi dalam proses politik [1] . Peristiwa ini tidak hanya memperkuat pola pikir nepotisme didalamnya, tetapi juga mencerminkan masalah struktural dalam sistem politik indonesia.

Chemotherapy in the modern period can have a variety of side effects, including immunosuppression and allergies. Natural herbal products and their many components have been shown to lessen the negative effects of chemical medications; as a result, they are favored as an alternative treatment [1].

Because they target oral germs, provide immediate pain relief, and have fewer adverse effects, herbal mouthwashes are in high demand. Chemical mouthwashes contain hydrogen peroxide, chlorine ioxide, and cetylpyridinium chloride as an instant whitener, sterilizer, and tooth pain reliever; however, they are inexpensive and often cause tooth discolouration and adverse effects. Cavities, enamel eruption, gum swelling, gum bleeding, and the development of hollow black eruptions on the tooth surface are all signs of oral infections [2].

Liquids with anti-inflammatory properties are called mouth washes. antimicrobial and analgesic properties. Mouthwashes come in two varieties: chemical and herbal. Phytochemicals, a natural component of herbal mouthwash, have the desired anti-inflammatory and antimicrobial properties. Herbal mouthwash is becoming more and more popular because it doesn't include alcohol, artificial flavors, colors, or preservatives. Because it contains natural substances that naturally clean and cure teeth and gums. Many herbal mouthwashes contain antimicrobial herbs, such as peppermint, which has a cooling impact on the mouth, and clove, which has long been used for oral health due to its antiseptic, antibacterial, and antiviral properties. Alcohol and fluoride, which are hazardous to our bodies in excess, are found in almost all chemical mouthwashes. Therefore, the majority of herbal mouthwashes are safe substitutes for children, diabetics, those with dry mouth, and pregnant women [3].

Because they target oral germs, provide immediate pain relief, and have fewer adverse effects, herbal mouthwashes are in high demand. The Herbal Mouthwash's objective It can stop tooth decay, lessen the amount of plaque in your mouth, and lower your risk of gum infection [4]. The two primary uses of mouthwash are medicinal and preventative. While short-term use is typically adequate in therapy, long-term use is necessary in prevention. In preoperative or postoperative care, as well as in aesthetic dentistry (anti-stain and whitening properties), it also serves to relieve certain disorders [5]. For thousands of years, natural substances have been successfully utilized in traditional medicine all across the world. Numerous of them possess pharmacological characteristics, including cytostatic, antibacterial, and anti-inflammatory actions.

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Their utility in human medicine has been proven [6]. Herbal Mouthwash: Herbal mouthwashes are made from natural plant extracts. A range of plant leaves, fruits, seeds, and tree oils are used to make the natural extract used in herbal mouthwashes. The use of herbal mouthwashes has surpassed that of chemical mouthwashes due to its non-irritating, non-staining, and alcohol-free properties [7].

Halitosis is an unpleasant mouth odor that comes from the oral cavity. If left untreated, it can lead to significant sickness and a decline in self-confidence. The bacterial breakdown of organic substrates left in the mouth, which mostly results in volatile sulfuric compounds, is responsible for around 80% of all instances of halitosis. It has been proposed that using mouthwash with antibacterial properties can help treat bad breath by killing bacteria, rinsing away food particles, and removing plaque from the oral cavity [8].

Pomegranate extracts inhibit the enzyme that breaks down sucrose, and by both competitive and noncompetitive inhibition, the organisms cause plaque to form. Pomegranate's polyphenolic flavonoids are useful for preserving dental health and preventing gingivitis [9].

Eugenol, an analgesic, antibacterial, antiseptic, phenolic, and flavonoid, is found in clove leaves. Among the many advantages of cloves are their antibacterial, antiviral, antifungal, antiplatelet, anticancer, antihistamine, and antioxidant properties. Because it contains beta caryophilene. The antioxidant activity and flavonoid chemicals that counteract free radicals are also attributed to the phenolic compounds found in clove leaves [10].

Material and methods

A. Sample Collection

Samples were collected from 20 patients by using a swab from oral cavity under the supervision of a specialist dentist then samples were taken by Brain Heart Infusion (BHI) broth which utilized as transport medium.

B. Preparation of Media

In our study, MacConkey (Himedia) MH081 and brain heart infusion (Himedia) M211 agar were utilized to isolate bacterial species from oral cavity, and then experiments were performed on them later.

C. Gram Stain

Gram staining was performed on isolates which obtained from the oral cavity according to the manufacturer's instructions (microxpress).

D. Biochemical Tests

Two Biochemical tests were performed:

Catalase Test: A small amount of pure colony collected with a wooden stick from suspicious isolates into clean slide, then a drop of catalase reagent was applied. The emergence of gas bubbles suggests a positive result [11].

Oxidase Test: A filter paper was soaked with several drops of oxidase reagent 1% then a small part of the colony was extracted with a sterile wooden stick rubbed on moistened filter paper. A good reaction is evidenced by the emergence of dark or deep purple color within 10-20 seconds [11].

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E. The VITEK 2 System

The VITEK 2 system device was used to diagnose the bacteria growing on plates.

Bacteria used in mouthwash testing

Three types of bacteria, Proteus, Klebsiella and Enterococcus were used which were diagnosed by the VITEK device, to study the extent of the effect of mouthwash.

F. Preparing a Natural Mouthwash:

1. Prepare the pomegranate peel and clove extracts separately, adding 4 ml of distilled water for every 1 gram of pomegranate peel or clove.

2. Boil them for 15 minutes.

3. Filter them well and leave them to cool.

4. Place rose water in an empty, sterile container, add the pomegranate peel and clove extracts, and mix well.

5. Then add rock salt and a few drops of peppermint oil and mix well.

6. Once finished, the natural mouthwash is ready and can be refrigerated.

Table 1. *Different concentrations were prepared from first natural mouthwash.*

Materials	Concentration (1)	Concentration (2)	Concentration (3)	Concentration (4)	Concentration (5)	Concentration (6)
Pomegranate peel (ml)	2	4	5	5	10	2
Cloves ml	1	2	10	5	5	4
Rock salt (ml)	1	1	1	1	1	1
Rose water (ml)	21	18	9	14	9	18
Mint oil (drop)	2	2	2	2	2	2
Total volume (ml)	25	25	25	25	25	25

Molar Hinton agar plates were prepared, a bacterial solution was formulated to achieve an absorbance equivalent to the McFarland 0.5 standard, corresponding to a bacterial concentration of $1.5x10^8$ cells/cm^3, then three types of bacteria (K. pneumoniae, P. mirabilis and E. faecalis) cultured on the plates, and made

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holes in the plates to pour the natural mouthwash and compare it with the synthetic mouthwash (BiOFRESH), taking into account sterile conditions. The plates were incubated for 24 hours at 37 C.

G. Result:

1. Primary Isolation:

Twenty samples were collected from the College of Dentistry / Dental Teaching Hospital. Nineteen out of twenty root canal samples (95 %) (19/20) showed the growth during primary isolation. After that, the isolates were purified on the media used in the study (Figure 1), three types of bacteria were identified using the VI TEK system, obtaining one isolate each of Klebsiella pneumoniae, Enterococcus faecalis, and Proteus mirabilis, with a percentage of (5%) for each.

Figure 1. *A. Growth K. pneumoniae on MacConkey agar*
B. Growth P. mirabilis on MacConkey agar
C. Growth Enterococcus faecalis on Brain Heart Infusion agar

A B C

2. Gram stain:

The result of the Gram staining test showed that two of the bacteria targeted in our study were Gram-negative (K. pneumoniae and P. mirabilis), while the third was Gram-positive (E. faecalis) (Figure 2).

Figure 2. *A. Gram negative cells (100x) of P. mirabilis*
B. Gram positive cells (100x) of E. faecalis
C. Gram negative cells (100x) of K. pneumoniae

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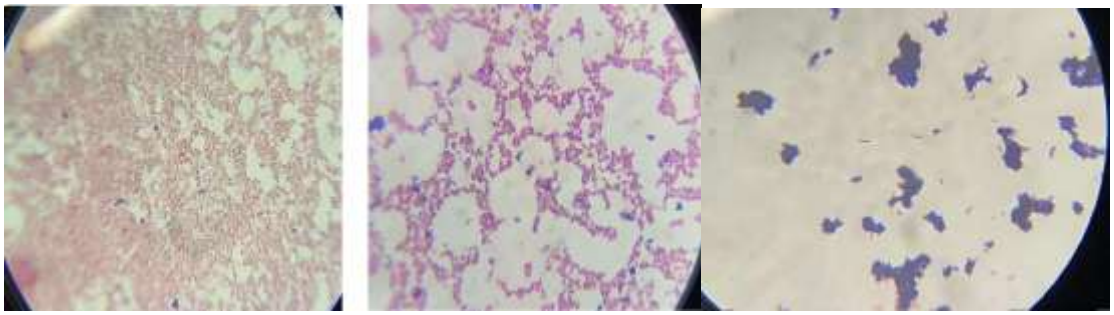

A B C

3. Biochemical Tests:

The results of the biochemical tests for the three isolated targeted in our study were negative for the oxidase test, while the results varied for the catalase test (Table 2).

Table 2. *Biochemical Tests results*

Bacteria	Catalase	Oxidase
P. mirabilis	Positive	Negative
K. pneumoniae	Positive	Negative
E. faecalis	Negative	Negative

The data in (Table 3) clarified the following observations: The effect on increasing progressively the efficacy of the natural mouthwash was noted with the change in concentration of 1 to 5 with the inhibition zone acquiring a maximum diameter of 28 mm when concentration was at concentration 5. This increase was directly proportional to the addition volume of pomegranate peel which attained 10ml at the same concentration (Figure 3).

Figure 3. *Illustrates the different concentrations of the natural mouthwash before use while they are in the container.*

Table 3. *The different concentrations of natural mouthwash*

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First: Analysis of Bacterial Reaction of K. pneumoniae and P. mirabilis: the Gram-negative K.

Bacteria	Con.1	on.2	on.3	on.4	on.5	on.6
K. pneumoniae	+(17mm)	+(16mm)	(23mm)	(26mm)	(24mm)	(20mm)
P. mirabilis	+(16mm)	+ (18mm)	(25mm)	(26mm)	(28mm)	(20mm)
E. faecalis	-	-	(17mm)	(15mm)	-	-

pneumoniae and P. mirabilis were extremely sensitive to all six concentrations. K. pneumoniae attained peak zone of inhibition at the fourth concentration (26mm) and P. mirabilis at fifth concentration (28mm). Such results indicate that the active components may have a strong ability to diffuse inside the walls of such kind of this bacterium. E. faecalis was the most resistant, as it responded only to concentrations (3) and (4) (Figure 4).

Figure 4. *The antimicrobial effect of natural mouthwash*

Second: Connecting the findings to the components (Why do concentrations 3 and 4 work?) On reviewing the concentration table, could see the following: Concentration 3: The largest amount of cloves (10ml) is added to this concentration. The tissue of clove contains a lot of eugenol that is a powerful antimicrobial agent and that explains the better inhibition of the resistant E. faecalis (17mm). Concentration 4: In this formulation, a high concentration of pomegranate peel (5ml) was mixed with cloves (5ml). Tannins in pomegranate peel was synergistic with eugenol of clove, and the combination of the two provides the most widespread antibacterial effect of all the tested pathogens.

Role of the fixed components (rock salt and peppermint oil): The amount of the rock salt (1 mL) and peppermint oil (2 drops) were fixed in all the concentrations in the experiment. The identified change in the

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inhibition thickness can, therefore, only be explained by the changes in the ratios of pomegranate and clove, and the rock salt and peppermint oil remain constant auxiliary agents, which increase osmotic pressure and improve the organoleptic characteristics.

Concentration 4 was the only formulation which showed an inhibition to all taxa of bacteria in the study at a similar and higher rate (26mm, 26mm and15mm) of K. pneumoniae, P. mirabilis and E. faecalis respectively. The statistical perspective of this phenomenon was referred to as the comprehensive effective concentration, which is an equilibrated solution of pomegranate peel and clove constituents (5ml of each). Perception of resistance. E. faecalis presents itself as a stubborn strain, which requires a specific chemical combination between eugenol and tannin to disrupt its defense system.

Our study found that Biofresh mouthwash, which contains the active ingredient (sodium fluoride), did not achieve any results in inhibiting the growth of bacterial species.

Discussion

In this study, the fifth concentration, which contains a large amount of pomegranate peel extract (10ml), had the strongest inhibitory effect on P. mirabilis with a diameter of (28mm), this result aligns with several others, including one conducted by Nihal Hamdi on a range of natural mouthwashes, including pomegranate peel, to test their antimicrobial activity. our study found that pomegranate exhibited higher antimicrobial activity against Streptococcus mutans than other mouthwashes [12]. It also agrees with a study by Anis Irmawati, who found that pomegranate mouthwash can inhibit periodontal bacteria as an alternative treatment for bad breath by inhibiting plaque formation and the growth of periodontopathogens [13].

The third concentration, containing the highest volume of clove extract that had the highest inhibitory effect on P. mirabilis and also had the highest effect on E. faecalis, despite the high resistance of this isolate, this was consistent with the study conducted by Anshul Shah and his colleagues, which aimed to evaluate the effectiveness of alcoholic clove extracts against E. faecalis. At a concentration of 5%, the clove extract showed no antimicrobial activity, while concentrations of 10% and 50% showed antimicrobial activity. It was concluded that clove has antimicrobial activity against E. faecalis [14]. Also, in a study conducted by Shafi Ahmed and others, which included natural substances such as cloves, the antibacterial activity was evaluated, the diameter of the inhibition zone was (15 mm, 12 mm, 10 mm, 9 mm for S. mutans) and (20 mm, 20 mm, 16 mm, 12 mm for S. salivarius). This variation in diameters was due to the volume and concentration of the mouthwash was used [15].

However, at the fourth concentration, when used large and equal amounts of clove extract and pomegranate peel (5 ml each), it had a significant and equal antimicrobial effect against both K. pneumoniae and P. mirabilis,this can be attributed to a synergistic interaction between the two components, and it is considered the strongest concentration against K. pneumoniae.

Rose water was used in varying quantities depending on the amount of pomegranate peel and cloves extract to dilute the natural mouthwash, balance the flavor and take advantage of its antimicrobial properties and according to a study conducted by Ramezanalizadeh and his colleagues, the results showed that rose

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water had a significant effect on bacterial adhesion but did not affect their growth, and it was unable to eliminate the biofilm after it had formed [16].

Adding two drops of peppermint oil to all concentrations of natural mouthwash provides a refreshing flavor and aroma, and it is known to inhibit bacterial growth. According to a study conducted by Kataria and Malayapan using a mouthwash with peppermint oils, the mint oils of peppermint species were found to possess antimicrobial activity against several clinical isolates studied, and thus could be a good natural antimicrobial source [6].

A study by Sarker and others showed that rock salt can be used as a remedy for bad breath, as a teeth whitener, or as a mouth freshener. Gargling with rock salt provides an easy remedy for bad breath and relief from a sore throat [17].

However, according to a study conducted by group of researchers to compare the effectiveness of a natural coconut mouthwash with Biofresh mouthwash, it showed a good result in inhibiting bacteria which was Streptococcus mutans and this does not match with current study [18].

Conclusion

The natural mouthwash was manufactured in six different concentrations and tested on three bacterial isolates from the oral cavity: K. pneumoniae, P. merabilis, and E. faecalis. All six concentrations of the natural mouthwash demonstrated good antibacterial effects, unlike the synthetic mouthwash tested. The pomegranate peel extract and clove extract showed the greatest effect, suggesting their potential use in formulating a natural mouthwash with very few side effects compared to synthetic chemical mouthwashes. While synthetic chemical mouthwashes can be effective initially, over time and with frequent use, they can lead to adverse oral health effect.

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