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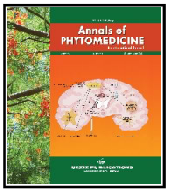

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In vitro investigation of minimum inhibitory concentration and minimum bacterial concentration from combined extract of *Salvadora persica* L. and *Zingiber officinale* Rosc. against *Streptococcus mutans*

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Abstract

Salvadora persica L. (Miswak) and *Zingiber officinale* Rosc. (Ginger) are widely used in traditional medicine for their antimicrobial properties. The present study aimed to investigate the minimum bacterial concentration (MBC) of a combined extract of *S. persica* and *Z. officinale* against *Streptococcus mutans*, a major contributor to dental caries. The extracts were obtained using methanol diluent and the MIC was determined using the microdilution method in 96-well microtiter plates. The results showed that the combined extract had a minimum inhibitory concentration (MIC) of 75 µg + 1.25 mg/ml and a minimum bactericidal concentration (MBC) of 150 µg/ml + 2.5 mg/ml against *Streptococcus mutans*. The findings suggest that the combined extract of *S. persica* and *Z. officinale* may have potential as a natural alternative to synthetic antimicrobial agents for the prevention and treatment of dental caries. Further studies are warranted to investigate the safety and efficacy of the combined extract *in vivo* and to elucidate the mechanism of action.

1. Introduction

Salvadora persica L., also known as Miswak and also known as the toothbrush tree, this is a therapeutic or medicinal plant. It is commonly used most of the people for oral hygiene in many states and nations. Miswak contains several bioactive compounds such as flavonoids, alkaloids and tannins, which is reported by in many research to possess antibacterial and anti-inflammatory properties. *S. persica* has been used for centuries as a natural remedy for oral health problems such as tooth decay, gum disease, and bad breath. It contains compounds that have antimicrobial, anti-inflammatory and antioxidant properties, which can help to promote oral health and prevent oral diseases (AbdEL Rahman *et al.,* 2003).

Zingiber officinale Rosc. commonly known as Ginger, is a widely used spice with several medicinal properties. Ginger contains gingerols and shogaols, which have been reported to possess antibacterial, anti-inflammatory and antioxidant properties. Moreover, this plant has been used as tradition in remedy for its various health benefits (Akullo *et al.,* 2022). *Z. officinale* has been used traditionally for its antioxidant and anti-inflammatory properties. It contains compounds such as gingerols and shogaols, which have been shown to have anti-inflammatory effects and may help to reduce inflammation in the body. It is also known for its ability to help with digestive issues such as nausea and vomiting.

The use of medicinal plants as an alternative to synthetic antibiotics has been gaining popularity due to their natural origin and low toxicity. *S. persica* (Miswak) and *Z. officinale* (Ginger) have been traditionally used for medicinal purposes and their antibacterial properties has reported in several studies (AbdEL Rahman *et al.,* 2003). Several studies have reported the antibacterial activity of *S. persica* and *Z. officinale* against various microorganisms, including *S. mutans*. However, according to our knowledge, there is no previous research, that can show investigations of the *in vitro* antibacterial activity of a combined extract of *S. persica* and *Z. officinale* against *S. mutans*.

When the methanolic extracts from *S. persica* and *Z. officinale* are combined, they have been shown to have even greater health benefits. Studies have shown that the combination has antimicrobial, antioxidant and anti-inflammatory properties, making it useful for a variety of health issues (Saleem *et al.,* 2005). The combination of methanolic extracts from *S. persica* and *Z. officinale* has been the subject of some scientific research. One study published in the Journal of Medicinal Plants Research in 2013 evaluated the antibacterial activity of the combination extract against several strains of bacteria. The results showed that the extract had significant antibacterial activity against all tested strains, including *E. coli* and *Staphylococcus* aureus. Another study published in the Journal of Medicinal Food in 2017 evaluated the anti-inflammatory and antioxidant effects of the

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combination extract in rats. The results showed that the extract had significant antioxidant and anti-inflammatory effects (Saleem *et al.*, 2005). Overall, the combination of methanolic extracts from *S. persica* and *Z. officinale* show promising potential for use in various medicinal applications. However, further studies are required in urgent basis to fully understand its appliance of action and potential effects and side effects. Overall, the combination of methanolic extracts from *S. persica* and *Z. officinale* show promising potential for use in various medicinal applications.

For example, the combination extract has been shown to have antibacterial action against several strains of bacteria with *E. coli* and *Staphylococcus aureus*. It has also been shown to have significant antioxidant and anti-inflammatory effects (Habib *et al.*, 2008), which may help to protect against chronic diseases such as cancer, diabetes and heart disease.

Dental caries or tooth decayis well known as oral disease, is a common and chronic disease that affects individuals of all age groups worldwide. *S. mutans* is the most commonly associated bacterium with dental caries (Khalil *et al.*, 2019). This bacterium can produce acid, which damages the enamel and dentin, leading to cavities. Therefore, controlling the growth of *S. mutans* is critical in the prevention and management of dental caries. Dental caries is a multifactorial disease that is influenced by several factors such as diet, oral hygiene practices and genetics (Khalil *et al.*, 2019). Despite the availability of various preventive measures, dental caries remains a significant health problem. The development of new and effective antibacterial agents is necessary for the prevention and treatment of dental caries.

The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) are important parameters in determining the effectiveness of antibacterial agents (Ostrosky *et al.*, 2008). The MIC is the lowest concentration of an antimicrobial agent that inhibits the visible growth of microorganisms, while the MBC is the lowest concentration that results in the death of microorganisms.Therefore, this research aims to diagnose the *in vitro* antibacterial susceptibility of a combined extracts of *S. persica* and *Z. officinale* against *S. mutans* and to determine the MBC of the extract. The MBC is a critical parameter that determines the effectiveness of an antibacterial agent in eradicating bacteria.The results of this research could contribute to the knowledge of the possible use of natural products in the prevention and management of dental caries. Additionally, the study could add to the increasing body of literature on the use of plant-based products in controlling bacterial infections (AL-Saadi, 2016).

Several studies have reported the antibacterial activity of *S. persica* and *Z. officinale* against various microorganisms, including *S. mutans*. However, to the best of our information, there are no previous reports on this that have investigated the *in vitro* antibacterial susceptibility of a combined extracts of *S. persica* and *Z. officinale* against *S. mutans* (Salvat *et al.,* 2001). Therefore, this research aims to diagnose the *in vitro* antibacterial activity of a combined extract of *S. persica* and *Z. officinale* against *S. mutans* and to determine the MBC of the extract. The MBC is a critical parameter that determines the effectiveness of an antibacterial agent in eradicating bacteria. The results of this study will contribute and help to the knowledge of the potential use of medicinal plants in the prevention and management of dental caries.

2. Materials and Methods

2.1 Plant materials

The plant products In Al Kharj, Saudi Arabia, which is located at 24.16, 47.17, and 24° 8' 54" North, 47° 18' 18" East, a local market and a residential neighborhood were the locations where plant samples of *Z. officinale* (Ginger) and *S. persica* (Miswak) were gathered. The elevation there is 433 meters/1420.6 feet on average. Regarded, as has a desert climate. Almost no rain falls throughout the year. The Köppen-Geiger classification for this area's climate is BWh. In Al-Kharj, the yearly average temperature is 26.7°C (80.0°F). Both samples were obtained at 32°C on a sunny day (Sophia *et al.,* 2022).

2.2 Extraction methods

50 g sample A of *S. persica* and 50 g sample B of *Zingiber officinale* plant root were properly cleaned with distilled water two time and keeps in 40.c in for air dry, after properly air drying milled to a powder and mixed with methanol solvent in cleaned beaker than put this beaker on a shaker for 48 h. After three-time repeated extraction process with the same solvent filtered using a Whatman No. 1 filter paper. The final extraction was with the help of Buchi Rotavapor R-215 Rotary Evaporator (Adaramola *et al.*, 2017). The extract obtained was evaporated to dry and stored at 4°C in an airtight container for further use.

2.3 Sample collection and preparation

We have received *S. mutans*, which is coming under ATCC 25175 and it belongs to Microbiology Department of College of Medicine in Prince Sattam University. ATCC 25175 is American Type Culture Collection. Furthermore, this was subcultured in Brain Heart Infusion (BHI) broth and keep it for incubation at 37°C for 18 – 24 h. To achieve a final concentration of 1×10^{-6} colony-forming units per milliliter (CFU/ml), the bacterial solution was diluted with BHI broth after being calibrated to a 0.5 McFarland standard using a spectrophotometer.

2.4 Preliminary Phytochemical screening

Phytochemical preliminary screening is a process of analysing the chemical composition of plant extracts to identify the presence of various bioactive compounds. These compounds are known as phytochemicals and are responsible for the biological activities and therapeutic potential of plants. The screening process involves a series of qualitative chemical tests to detect the presence or absence of various classes of compounds such as flavonoids, alkaloids, reducing sugar, tannin and glycosides. These tests involve the use of specific reagents that react with the different classes of compounds, resulting in characteristic colour changes or precipitate formations.

2.5 Test for reducing sugars (Fehling's test)

Solution A, ofBoiling Fehling's andsolution B of Boiling Fehling's was added to the aqueous methanol extract (1.0 g in 10 ml of water). An examination of the solution's color reaction was done.

2.6 Test for flavonoids

These methods were used to identify the presence of flavonoids. An aqueous filtrate of the extract was in first level treated with diluted ammonia (5 ml). 1 ml of concentrated sulfuric acid was applied. Flavonoids are indicated by a golden coloration that fades when standing. In second level, a fraction of the filtrate was mixed with a

few drops of a 1% aluminum solution. The presence of flavonoids, that showed by the presence of a yellow coloration. In third level, a portion of the extract was heated for 3 min in a steam bath with 10 ml of ethyl acetate.Than the mixturebeing filtered, 1 ml of diluted ammonia solution was added to 4 ml of the filtrate. The presence of flavonoids is indicated by the presence of a yellow coloration.

2.7 Test for tannins

In a test tube, 20 ml of water and about 1.0 g of the extract were heated, and after filtering the mixture then A few drops of 0.1% ferric chloride were added, and the color of the mixture was checked for brownish green or blue-black hues (Sofowora, 1993).

2.8 Test for alkaloids

In this test, needs to dilute acid alcohol with 1.0 g of extract to 20 ml, than this was heated and filtered. 4 ml of diluted ammonia were added to 10 ml to the received filtrate from filtration. To extract the alkaloidal base, 5 ml of chloroform was mixed slowly and gently shaken. 10 cc of acetic acid were used to extract the layer of chloroform. This was divided into two parts. Draggendorff's reagent was used on one portion, and Mayer's reagent on the other. According to the precipitation of a cream (when using Mayer's reagent) or a reddish brown (when using Draggendorff's reagent) was regarded as positive for the presence of alkaloids (Sofowora, 1993).

2.9 Test for cardiac glycosides (Keller-Killiani test)

The 1.0 g of extract was diluted to 10 ml in water, and then 2 ml of glacial acetic acid containing one drop of ferric chloride solution was added. Underneath this was 1 cc of pure sulfuric acid. The presence of a brown ring at the interface demonstrated the existence of a deoxysugar characteristic of cardenolides. A violet ring may occur beneath the brown ring in the acetic acid layer, whereas a greenish ring may form slightly above the brown ring and eventually extend across this layer.

2.10 Determination of minimum inhibitory Concentration (MIC)

The Minimum Inhibitory Concentration (MIC) is the lowest concentration of an antimicrobial drug capable of obstructing a microorganism's ability to grow visibly *in vitro* under controlled circumstances. The MIC is calculated by serially diluting the antimicrobial agent in a liquid growth medium, adding a standardized inoculum of the test microorganism to the medium, and then culturing the culture for a predetermined amount of time (AL-Saadi, 2016). The next step is to ascertain the MIC by inspecting the culture for growth or lack thereof. Typically, turbidity or other parameters are measured, as well as additional tools like a spectrophotometer or automated microbial growth monitoring systems. MIC values are used to help choose the best antibiotic treatment for diseases brought on by a particular bacteria. The MIC value can also reveal whether a microbe is susceptible to or resistant to a specific antimicrobial agent. In general, the antimicrobial agent is more effective against bacteria the lower the MIC. The strain of the microbe, the type and concentration of the antimicrobial agent used, and the growth circumstances can all affect the MIC. In order to obtain accurate and repeatable results, MIC testing must. Therefore, it is essential to perform MIC testing under standardized conditions to ensure accurate and reproducible results (AL-Saadi, 2016).

2.11 Determination of minimum bactericidal Concentration (MBC)

The MBC of the *S. persica* and *Z. officinale* and the combined extract of these two against *S. mutans* was determined by sub-culturing the bacterial suspension from the wells with no visible growth onto sterile BHI agar plates (AL-Saadi, 2016). The plates were incubated for 24 h at 37°C and the MBC was determined as the lowest concentration of the extract that completely inhibited the growth of the bacteria on the agar plates.The MBC is an important parameter in determining the effectiveness of an antimicrobial agent against a particular microorganism. It provides information about the concentration of the antimicrobial agent required to completely eliminate the microorganism rather than just inhibit its growth. A high MBC value may indicate the development of antimicrobial resistance in the microorganism, which could limit the effectiveness of the antimicrobial agent in treating infections caused by that microorganism.It is important to note that the MBC assay requires more time, resources and expertise compared to the MIC assay and is usually only performed when additional information about the antimicrobial agent's killing ability is required.

3. Results

3.1 Preliminary phytochemical screening

The combined extract of *S. persica* and *Z. officinale* and also individual extracts of these two were subjected to examination for preliminary phytochemical screening to identify the presence of various bioactive compounds (Sofowora, 1993). The extraction was performed using methanol as the solvent. The screening was conducted using standard methods to identify the presence of different types of phytochemicals bioactive components. We found results that show the presence of flavonoids, alkaloids, reducing sugar, tannin, and glycosides (Table 1). The results of the screening revealed the presence of all five classes of compounds in the extract, indicating the potential of the extract, which is able to exhibit antimicrobial activity. Alkaloids and flavonoids have been reported to exhibit strong antimicrobial activity against various pathogens, including *S. mutans*, which are known to contribute to the development of dental caries. Tannin and Terpenoids have also been reported to possess antimicrobial properties, while saponins have been reported to have anti-inflammatory effects. The presence of these compounds in the extract supports the potential of *S. persica* and *Z. officinale* as natural alternatives to synthetic antimicrobial agents for the prevention and treatment of dental caries. More detailed study are needed to diagnose and identify the more active compounds, which is responsible for the observed activity and to elucidate their mechanism of action (Doss, 2009).

Table 1: Preliminary phytochemical screening of *Z. officinale*

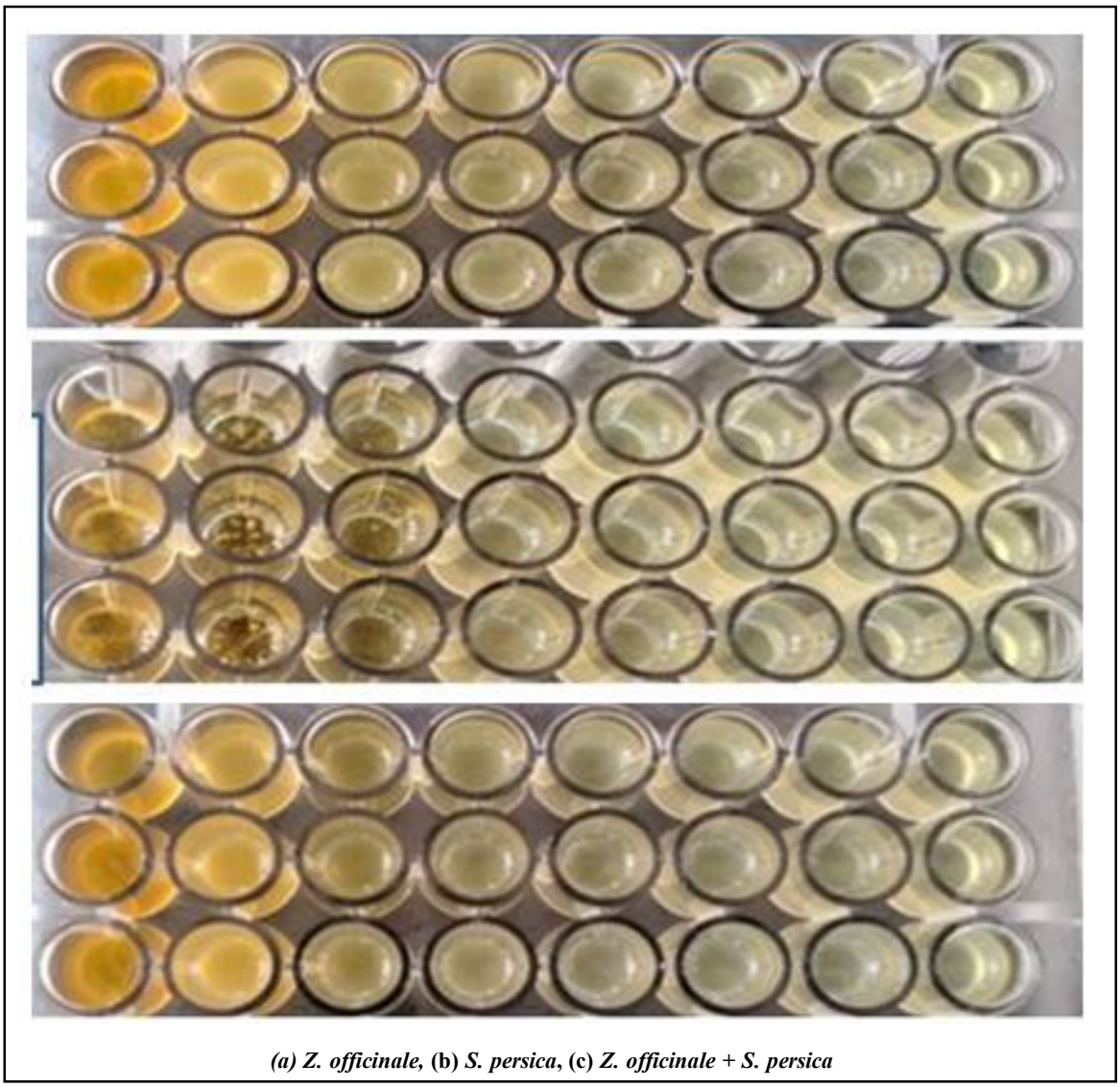
S. No.	Solvent name	Chemicalconstituents	Results
1.	Methanol	Flavonoids	+
		Alkaloids	++
		Reducing sugar	+
		Glycoside	-
		Tannin	+

Table 2: Preliminary phytochemical screening of *S. persica*

S. No.	Solvent name	Chemicalconstituents	Results
1.	Methanol	Flavonoids	+++
		Alkaloids	+++
		Reducing sugar	+
		Glycoside	-
		Tannin	++

3.2 Minimum inhibitory concentrations (MIC)

The MIC were performed on of *Z. officinale* (ZO), *S. persica* (SP) and combination of ZO + SP plants methanolic extracts by microdilution method by 2 folds with BHI broth against *S. mutans* (SM) and the obtained results are presented in Table 3, Figure 1 Graph 1. The minimum concentrations needed for ZO methanolic extract to inhibit the growth of *S. mutans*were found to be 300 µg/ml and for SP and Combination ZO + SP were found to be 5 mg/ml and 75 µg combined with 1.25 mg/ml, respectively (AL-Saadi, 2016).

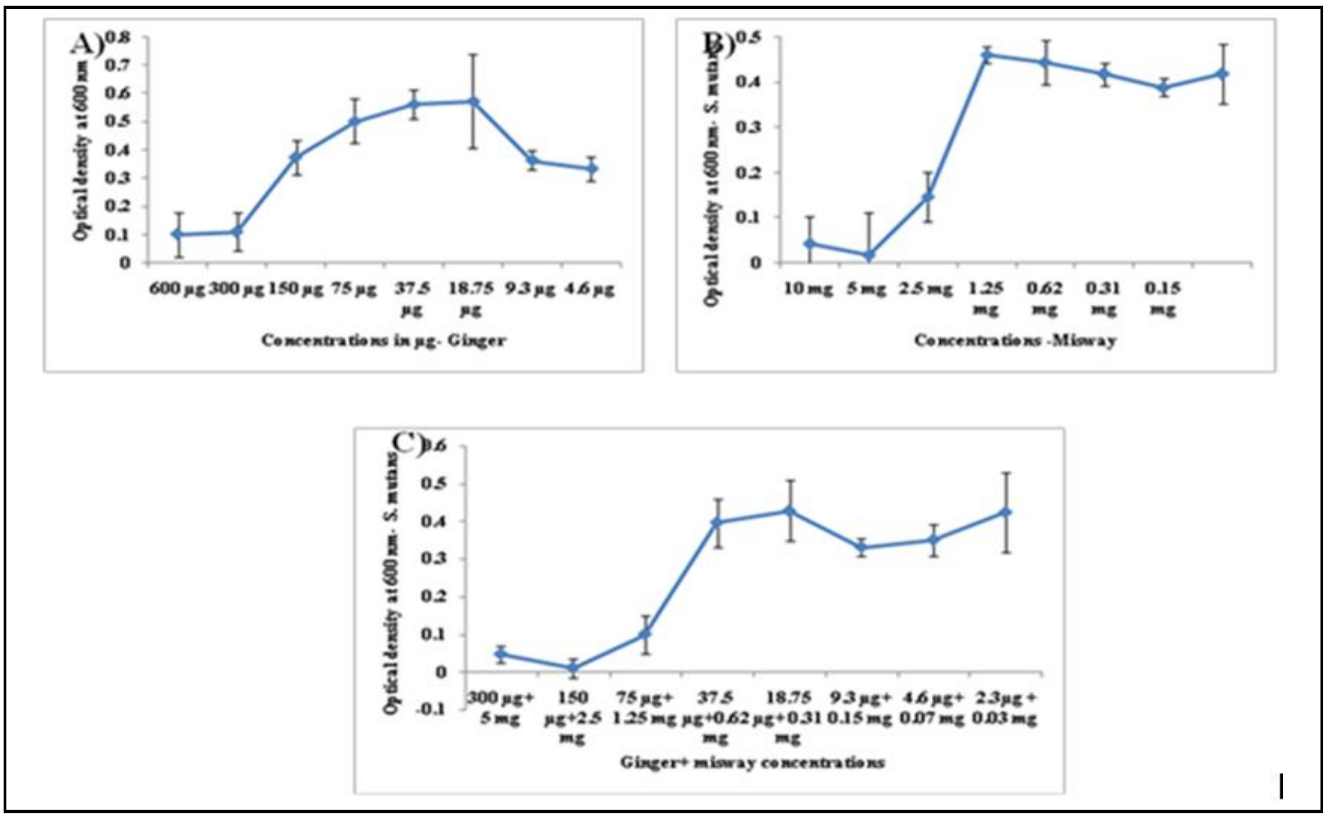


(a) Z. officinale,* (b) *S. persica*, (c) *Z. officinale* + *S. persica

Figure 1: MIC by microdilution of three methanolic extracts against *S. mutans.*

Table 3: MIC of three methanolic extracts against *S. mutans*

S.No	Sample name	MIC result
1.	*Z. officinale* (ZO)	300 µg/ml
2.	*S. persica* (SP)	5 mg/ml
3.	Combination of (ZO + SP)	75 µg + 1.25 mg/ml



Graph 1: MIC of three methanolic extracts against *S. mutans*.

3.3 Minimum bactericidal concentrations (MBC) of three methanolic extracts

The MBC of the *S. persica* and *Z. officinale* and the combined extract of both against *S. mutans* was examined for MBC. Briefly, for the MBC assay, compounds 1 concentrations below MIC and 3 concentrations above MIC were added in BHI broth supplemented with sucrose and glucose and *S. mutans* culture was added to each tube and incubated (AL-Saadi, 2016). After incubation, 100 µl aliquot from each sample was added to sterile BHI plates and spreading on plate was done and incubated for 24 h at 37°C and the MBC was determined as the lowest concentration of the extract that completely inhibited the growth of the bacteria on the agar plates.

3.3.1 Concentrations used

Z. officinale **:** below **MIC**-200 µg, MIC-300 µg, above MIC-600 µg and 1 mg.

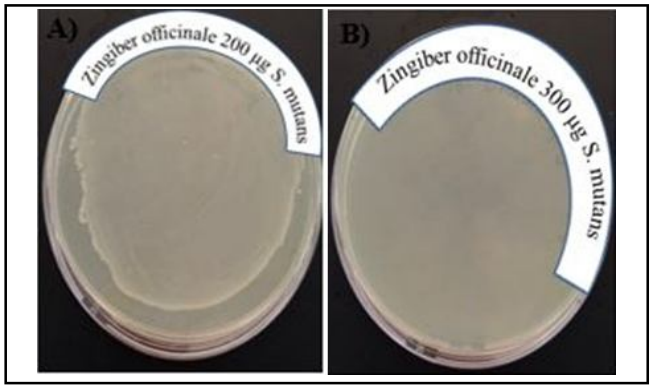
S. persica : below MIC-2.5 mg, MIC-5 mg, above MIC-7.5 and 9 mg.

Z. officinale + *S. persica* : below MIC-75 µg + 1.25 mg, MIC-150 µg + 2.5 mg, above MIC-300 µg + 5 mg and 300 µg +7.5 mg

The MBC of three methanolic extracts of *Z. officinale* and *S. persica*, and combination of both determined against *S. mutans* are presented in Figures 1-4 and Table 1. The MBC might be same as MIC value or above MIC. As seen in Figure 1A-D, the methanolic extract of ginger showed no colony formation till their MIC level and growth was observed below MIC level indicating the killing concentration of ginger was found to be 300 µg/ml. Similarly, no colony formation was observed till their MIC level after treatment with methanolic extract of misway and colony was seen in below MIC indicating 5 mg/ml of misway extract was needed to kill the *S. mutans* (Figure 2A-D). Same way, as shown in Figure 3A-D, when both the extracts were combined against *S. mutans,* 150 µg/ml of ginger extract along with 2.5 mg/ml misway extract was needed to kill the *S. mutans.*

Table 4: Minimum bactericidal concentration of three methanolic extracts of *Z. officinale*, *S. persica* and combinations of both (*Z. officinale* + *S. persica*) against *S.mutans*

S.No	Samples name	MBC result
1	*Z. officinale*	300 µg/ml
2	*S. persica*	5 mg/ml
3	*Z. officinale* + *S. persica*	150 µg/ml + 2.5 mg/ml



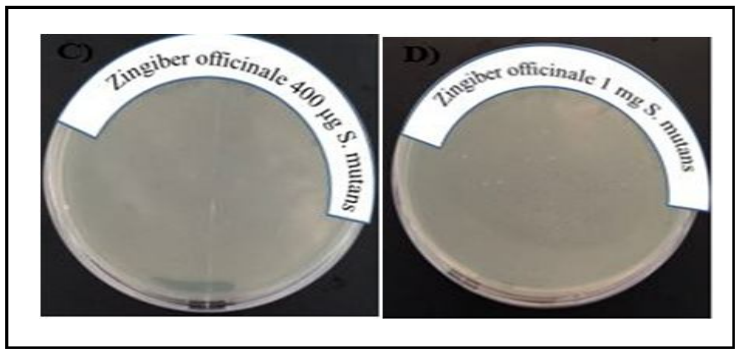


Figure 2: MBC of methanolic extract of *Z. officinale* against *S. mutans*.

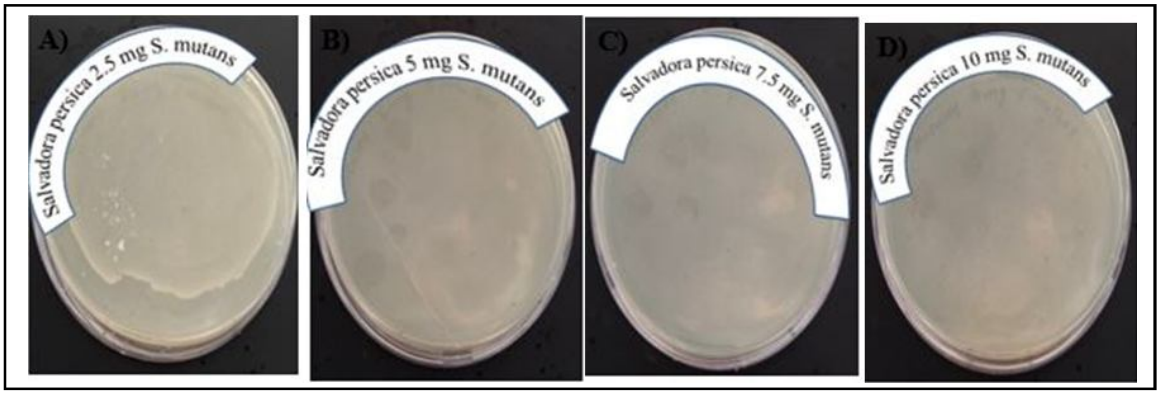


Figure 3: MBC of methanolic extract of *S. persica* against *S. mutans*

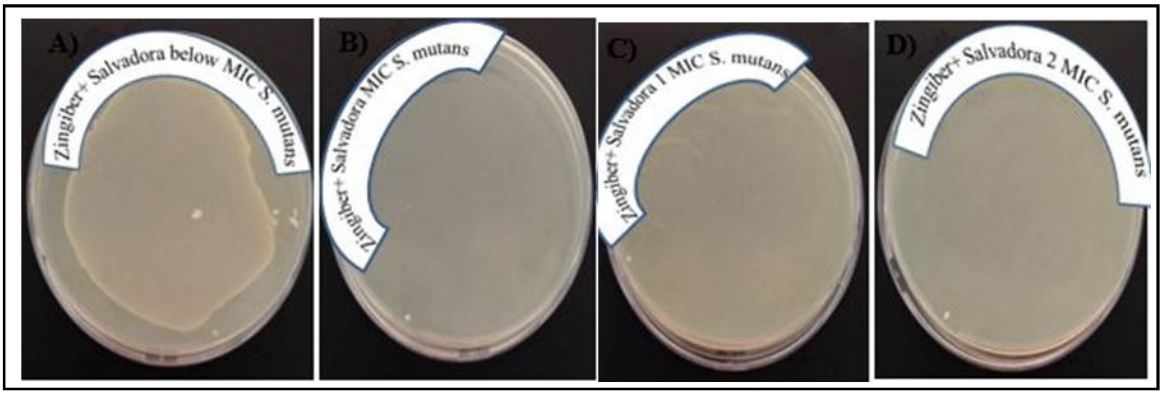


Figure 4: MBC of combination of *Z. officinale* + *S. persica* methanolic extract against *S.mutans*.

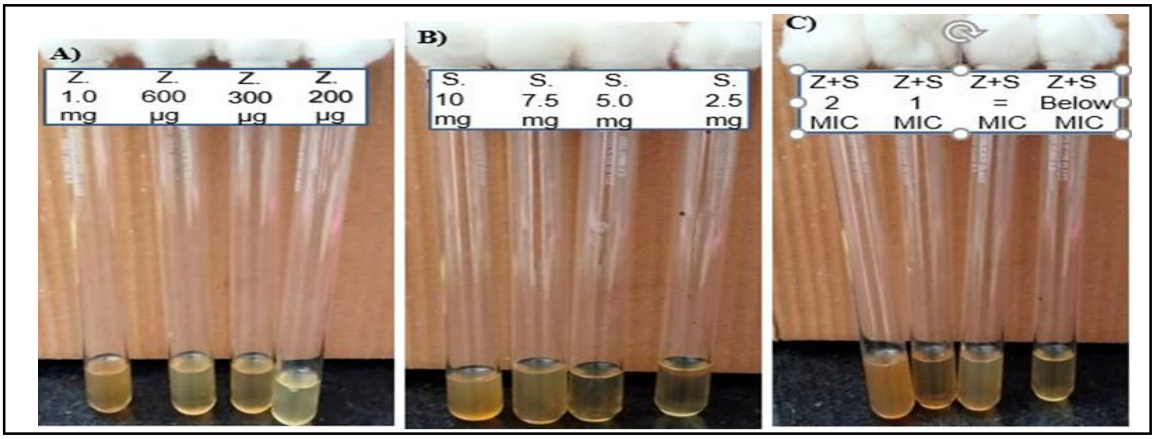


Figure 5: Visual effect of MBC of three methanolic extracts. A. *Z. officinale*, B. *S. persica*, C. *Z. officinale* + *S. persica*.

4. Discussion

Phytochemical screening found out the presence of alkaloids, flavonoids, tannins, terpenoids and saponins within the extract, that have been suggested to own antimicrobial properties. The located pastime of the blended extract in contrast to *S. mutans* may be attributed to the presence of those bioactive compounds. We have investigated the minimal bacterial concentration (MBC) of a combined extract of *S. persica* and *Z. officinale* against the *S. mutans*, a pathogen implicated withinside the improvement of dental caries. The microdilution approach turned into used to decide the MBC and the effects confirmed that the combined extract had a 75 µg + 1.25 mg/ml minimal inhibitory concentration (MIC) and150 µg/ml + 2.5 mg/ml of minimal bacterial concentration (MBC) in against to *S. mutans.*

The MBC of 150 µg/ml + 2.5 mg/ml for the combined extract is one of the better choices in comparison to different herbal antimicrobials, which have been suggested before against S. mutans. However, the capacity of the extract to function as an herbal opportunity to artificial antimicrobial sellers for the prevention and remedy of dental caries must now no longer be dismissed. *S. persica* and *Z. officinale* are extensively used historically in different cultures for medicinal properties.

5. Conclusion

In conclusion, the existing observation shows proof of the capacity of *S. persica* and *Z. officinale* extract combination betternatural and herbal options in comparison to artificial antimicrobials. These artificial antimicrobial agents know for causing adverse effects after long terms uses. Therefore, *S. persica* and *Z. officinale* extract combination may help in the prevention and cure of dental cariese specially against *S. mutans* and as well as in other diseases.

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Conflict of interest

The authors declare no conflicts of interest relevant to this article.

References

AbdELRahman, H. F.; Skaug, N.; Whyatt, A. M. and Francis, G. W. (2003). Volatile compounds in crude *Salvadora persica* extracts. Pharmaceutical biology, **41**(6):399-404.

Adaramola, B. and Onigbinde, A. (2017). Influence of extraction technique on the mineral content and antioxidant capacity of edible oil extracted from ginger rhizome. Chem. Int., **3**(1):1-7.

Akullo, J. O.; Kiage, B.; Nakimbugwe, D. and Kinyuru, J. (2022). Effect of aqueous and organic solvent extraction on *in vitro* antimicrobial activity of two varieties of fresh ginger (*Zingiber officinale*) and garlic (Allium sativum). Heliyon. **8**(9):e10457.

AL-Saadi, Z. N. (2016). Estimation of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of cell-free extracts of bifidobacterium species against methicillin-resistant Staphylococcus aureus *in vitro*. American Journal of Biomedical and Life Sciences, **4**(5):75-80.

Doss, A. (2009). Preliminary phytochemical screening of some Indian medicinal plants. Ancient Science of Life, **29**(2): 12.

Habib, S. H. M.; Makpol, S.; Hamid, N. A. A.; Das, S.; Ngah, W. Z. W. and Yusof, Y. A. M. (2008). Ginger extract (*Zingiber officinale*) has anticancer and anti-inflammatory effects on ethionine-induced hepatoma rats. Clinics, **63**(6):807-813.

Khalil, M. A.; El-Sabbagh, M. S.; El Naggar, E. B. and El-Erian, R. H. (2019). Antibacterial activity of *Salvadora persica* against oral pathogenic bacterial isolates. Nigerian Journal of Clinical Practice, **22**(10):1378-1387.

Ostrosky, E. A.; Mizumoto, M. K.; Lima, M. E.; Kaneko, T. M.; Nishikawa, S. O. and Freitas, B. R. (2008). Methods for evaluation of the antimicrobial activity and determination of minimum inhibitory concentration (MIC) of plant extracts. Revista Brasileira de Farmacognosia, **18**: 301-307.

Saleem, M.; Kim, H. J.; Ali, M. S. and Lee, Y. S. (2005). An update on bioactive plant lignans. Natural Product Reports, **22**(6):696-716.

Salvat, S.; Antonnacci, A.; Fortunato, F.; Suarez, S. and Godoy, G. (2001). Screening of some plants from Northern Argentina for their antimicrobial activity. Letters in Applied Microbiology, **32**(5):293-297.

Sofowora, A. (1993). Screening plants for bioactive agents. Medicinal Plants and Traditional Medicinal in Africa. 2nd Ed. Spectrum Books Ltd, Sunshine House, Ibadan, Nigeria, pp:134-156.

Sophia, A.; Faiyazuddin, M.; Alam, P.; Hussain, M. T. and Shakeel, F. (2022). GC-MS characterization and evaluation of antimicrobial, anticancer and wound healing efficiency of combined ethanolic extract of Tridaxprocumbens and Acalyphaindica. Journal of Molecular Structure, **1250**:131678.

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