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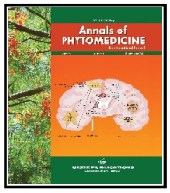

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Antibacterial activity of combined extract of *Salvadora persica* L. and *Zingiber officinale* Rosc. in comparison to their individual extracts on *Streptococcus mutans* as common oral pathogen

Imran Mohammad*,, Arun Kumar*, Prawez Alam***◆ and Mohammed Sarosh Khan****

**Department of Bioscience, Shri Jagdishprasad Jhabarmal Tibrewala University, Jhunjhunu-333010, Rajasthan, India*
*** Department of Basic Medical Sciences, College of Medicine, Prince Sattam Bin Abdulaziz University, Al-Kharj, Saudi Arabia*
**** Department of Pharmacognosy, College of Pharmacy, Prince Sattam Bin Abdulaziz University, Al-Kharj, Saudi Arabia*

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Abstract

Dental caries is a prevalent oral disease caused by *Streptococcus mutans* is one of the most common gram-positive cocci and this is involved in formation of bacterial biofilm on the tooth surface. *S. mutans* is a major etiological agent in dental caries. The use of natural products can be alternative to antibiotics. Natural products has gained more popularity in recent years. In this study, antibacterial activities of combination of extracts of *Salvadora persica* L. (SP) and *Zingiber officinale* Rosc. (ZO) against *S. mutans* (SM) were performed, Along with GC-MS, analysis on individual methanolic extracts and combination of extracts of Miswak and Ginger. The antibacterial activities of combination of extracts were examined using the agar-well diffusion method and the inhibition zones were measured. The results demonstrated that the combination of extracts of *S. persica* and *Z. officinale* have significant antibacterial susceptibility against *S. mutans*, with inhibition zones ranging from 13.0 mm to 14.0 mm, depending on the concentration (solute percentage) of the extract. The minimum concentration (solute percentage) needed for ZO methanolic extract to inhibit the growth of SM 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. GC-MS analysis identified several bioactive compounds that may contribute to the observed antibacterial susceptibility. These results suggest that these natural products may have potential as an alternative to antibiotics for controlling oral-infections. Need further studies on different plants combination on different types solvent for their efficacy, mechanism of action of extracts. So in future, they can be used directly or products as a treatment for oral infections.

1. Introduction

In today's world, the demand for natural and organic health products is increasing unexpectedly very fast because they are safe and natural-based medicinal agents, medicinal plants, or plant-based products (Guimaraes *et al.*, 2021). This type of product has a positive impact on human health and the treatment of many human disorders. Now, because of its positive results with zero (or minimal) side effects and no negative impact on the patient's body as well as the body of a healthy individual, it is widely used. Plant-based or natural goods are in high demand in developing countries, particularly in big cities, for their families' healthcare and home remedies for patients since they are safer. Furthermore, the World Health Organization (WHO) has urged underdeveloped countries to exploit medicinal plants as a natural resource because of their potential health benefits for humans (Alattas *et al.,* 2016). Due to its positive impact on human health after years of research and experimentation on many types of plants, plant-based products such as roots, stems, leaves, flowers and seeds have emerged as a source of natural products and phytochemicals.

Our respiratory system is widely known for being the primary source of infection and exposure in the human body. The mouth and nose are both parts of our respiratory system, but our mouth, in particular, has a dual purpose in our bodies because it is both a member of the respiratory and digestive systems (Voidarou *et al.,* 2021). As the mouth is part of the digestive system, after eating some small and tiny particles of food remain in the mouth and after several hours, the food get start fermenting because of bacterial involvement. Hence, the mouth becomes the source and entry point of many microorganisms (bacteria, fungi and viruses). These are commonly known as normal oral flora and these normal floras are sometimes responsible for minor oral problems and affect the general health status of the patient. They can occasionally cause severe and chronic oral pathological diseases (Lemos *at al.,* 2021). Normal flora can be opportunistic pathogens and can spoil people's healthy lives in the body's functional, psychosocial and economic dimensions; these are all related to oral health. Less healthy oral cavity and craniofacial health affects diet, nutrition, sleep and psychological status, interaction with others, as well as school, college and the workplace. Cleaning the mouth can be accomplished primarily

Corresponding author: Dr. Prawez Alam
Professor, Department of Pharmacognosy, College of Pharmacy, Prince Sattam Bin Abdulaziz University, Al-Kharj, Saudi Arabia
E-mail: p.alam@psau.edu.sa
Tel.: +96-6532771723

through mechanical and chemical action (Sabbagh *et al.,* 2020), with the most common mechanical approach being a teeth cleaning with a toothbrush in combination with toothpaste. Oral and dental hygiene can be maintained and preserved in a variety of ways all around the world, despite the fact that toothbrushes are widely used (Abhary and Al-Hasmi, 2016).

Today's world is more alert and mindful of his health and he is aware of the adverse effects of chemical products used on a regular basis (Nainu *et al.,* 2021), which are often ineffective in restoring a patient's prior healthy state. As a result, the globe is turning to natural products because natural products (chewing sticks) were commonly used to clean and maintain oral hygiene in many traditions and cultures (Sabbagh *et al.,* 2020). Chewing sticks are typically made from plants or trees with significant antimicrobial properties, making them one of the most natural items available. Chewing sticks (Miswak) are a common practice in many parts of the world and the most common and well-known natural medicinal plant is *Salvadora persica* (Miswak) (Balhaddad *et al.,* 2021). *S. persica*, often called "Miswak" or "Siwak" (tooth stick), is a member of the Salvadoracea family. It is most typically seen in coastal areas of the Arabian Peninsula and the Indian Peninsula. Rarely, it is an upright, evergreen little tree or shrub (Mansour *et al.,* 2020). Furthermore, Muslims all over the world use this tree due of its many health benefits their religious instruction. Furthermore, Muslims' last prophet (PBUH) instructed and taught people to brush their teeth five times a day with Miswak. Furthermore, many villagers regard it as their traditional (Sabbagh *et al.,* 2020). Miswak is beneficial because it has therapeutic characteristics and is beneficial for eye, ear and mouth illnesses, cough, asthma, scurvy, piles and other disorders, according to several studies. Miswak has been used by ancient Arabs to keep their mouths clean and whiten their teeth since the Islamic era. *S. persica's* beneficial effects on mouth cleaning and oral hygiene are partially attributable to mechanical action and partially due to chemical activity (Mansour *et al.*, 2020).

Muslims' religious instruction helps us further understand the benefits of oral hygiene, because Muslims' last prophet (PBUH) instructed and taught people to brush their teeth five times a day with Miswak. Furthermore, many villagers regard it as their tradition. Miswak is beneficial because it has therapeutic characteristics and is beneficial for eye, ear and mouth illnesses, cough, asthma, scurvy, piles and other disorders, according to several studies. Miswak has been used by ancient Arabs to keep their mouths clean and whiten their teeth since the Islamic era. *S. persica's* beneficial effects on mouth cleaning and oral hygiene are partially attributable to mechanical action and partially due to chemical activity. A lab analysis of this plant extract reveals several chemical compositions that are linked to its various activities. Saponins, tannins, silica, a little quantity of resin, trimethylamine and alkaloidal components were found in benzyl-isothiocyanate isolated from *S. persica* plant roots. The *S. persica* root has a significant mineral content, according to a previous report (Abhary and Al-Hasni, 2016).

Numerous studies have shown the efficacy of many plant extracts against microorganisms that we know as "antibacterial compounds," which are composed of the components found at some point in greater or lesser numbers in every plant. After extracting plant extracts with the help of different solvents, we obtain chemical substances known as extracts and these chemical substances are known as phytochemicals. Different phytochemicals have different polarities in the presence of different solvents. In this project, we are going to use *S. persica* (SP) and *Z. officinale* (ZO) as research objects. *S. persica* and *Z. officinale* are the scientific names for the Miswak and Ginger plants. The Salvadoraceae family is a subfamily of the Angiosperms (flowering plants) and in this family, the main members are: *Azima, Dobera, Monetia, Platymitium* and *Salvadora*. *Z. officinale* is a member of the Zingiberaceae family and turmeric, cardamom and galangal are additional important members of this Zingiberaceae plant family. Due to their antibacterial properties, phytochemicals are also recognized as an alternative to antibiotics. The benefits of phytochemicals are numerous and they also protect against dangerous pathogens (Jahan *et al.,* 2021). Due to the major problem of increasing multidrug resistance in microorganisms, the world must develop alternative drugs for the treatment of infectious and pathological diseases without worrying about multidrug resistance (Nainu *et al.,* 2021). Particularly helpful as a chemotherapeutic agent in the treatment of infectious disorders is the antibacterial property of phytochemicals with selective toxicity. Numerous scientists have reported the efficacy of several plants against antibacterial. The uses of plant extracts were reported to have antibacterial and anti-inflammatory properties. *Z. officinale* is a most well-known member. *Z. officinale* is used as medicine in numerous cultures throughout the globe. In order to identify the bioactive components responsible for the antimicrobial action of *Z. officinale* (Ginger) extracts against several pathogenic bacteria. This study will examine the extracts' phytochemical composition.

We are all well aware of the inefficacy and no effectiveness of drugs and their significant drawbacks and multi-drug resistance in addition to limited or lower antimicrobial susceptibility associated with the uncontrolled use of systemic antibiotics and numerous medications. This research study on antibacterial, antifungal and antiviral agents is currently receiving attention day-by-day, the number of bacteria that are resistant is increasing. This is an alarming condition for doctors and patients.

2. Materials and Methods

2.1 Plant materials

The plants roots of *Z. officinale* (Ginger) and *S. persica* (Miswak) were purchased from local market, near the residential area in Al Kharj, Saudi Arabia located at 24.16,47.17 and 24° 8' 54" North, 47° 18' 18" East . It has an average elevation: 433 meters/1420.6 feet. considered to have a desert climate. There is virtually no rainfall during the year. The climate here is classified as BWh by the Köppen-Geiger system. The average annual temperature is 26.7°C | 80.0°F in Al-Kharj. The both samples were collected on a sunny day at temperature 32°C (Sophia *et al.,* 2022).

2.2 Extraction methods

100 g A sample of *S. persica* and 100 g B sample of *Z. officinale* plant root were washed and rinsed with distilled water and air dried after that milled to a homogeneous powder and mixed separately with methanol and chloroform solvent than put both of them on a shaker for 72 h. After three time repeat 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 and Oniglinde, 2017). Obtained extract was evaporated to dryness and stored at 4°C in an airtight container for further use.

2.3 Phytochemical screening

The standard methods have been used in this research to screen the phytochemicals (Sofowora, 1993).

2.4 Test for the reduction of sugars (Fehling's test)

In a test tube, an aqueous ethanol extract of 0.5 g in 5 ml water was added to boiling Fehlings solution A and B. For the color reaction, a solution has been observed.

2.5 Examine for flavonoids

Flavonoids were detected using these methods. First, 5 ml of weak ammonia was added to a portion of the extract's aqueous filtrate. 1 mL of concentrated sulphuric acid was added. The presence of flavonoids is indicated by a golden coloration that fades when standing. The filtrate was then treated with a few drops of 1% aluminum solution. The presence of flavonoids is indicated by a yellow coloration. Third, a portion of the extract was cooked for 3 min in a steam bath with 10 ml of ethyl acetate. After filtering the mixture, 4 ml of the filtrate was mixed with 1 ml of weak ammonia solution. The presence of flavonoids is indicated by a yellow coloration.

2.6 Examine for saponins

In a test tube, 0.5 g of extract was mixed with 5 ml of distilled water. The solution was vigorously shaken and looked for a steady stable froth. The foam was combined with three drops of olive oil and vigorously agitated before being examined for the creation of an emulsion.

2.7 Examine for tannins

In a test tube, 0.5 g of the extract was heated in 10 ml of water and then filtered. A few drops of 0.1% ferric chloride were added and the coloration was checked for brownish-green or blue-black.

2.8 Examine for alkaloids

In a test tube, 0.5 g of extract was heated and filtered after being diluted to 10 ml with acid alcohol. 2 ml of dilute ammonia was added to 5 ml of filtrate. To extract the alkaloidal base, 5 ml of chloroform was added and gently shaken. 10 ml of acetic acid was used to extract the chloroform layer. This was split into two parts. Draggendorff's reagent was added to one portion and Mayer's reagent to the other. The presence of alkaloids was determined by the production of a cream (with Mayer's reagent) or a reddish-brown precipitate (with Draggendorff's reagent).

2.9 Examine for cardiac glycosides (Keller-Killiani test)

In a test tube, 2 ml of glacial acetic acid containing one drop of ferric chloride solution was added to 0.5 g of extract, diluted to 5 ml in water. This was followed by 1 cc of pure sulfuric acid. A brown ring at the interface suggested the presence of a cardenolidedeoxy sugar. A violet ring may form beneath the brown ring, whereas a greenish ring may form slightly above the brown ring and eventually expand throughout the acetic acid layer.

2.10 Examine for steroid

Each sample of 0.5 g ethanolic extract was treated with two milliliters of acetic anhydride and two milliliters of H_2SO_4. In certain samples, the color shifted from violet to blue or green, indicating the presence of steroids (Doss, 2009).

2.11 Examine for terpenoid (Salkowski test)

Five milliliters of each extract were combined with two milliliters of chloroform, and three milliliters of pure H_2SO_4 were carefully added to form a layer. Then, we found a reddish-brown coloration of the interface indicating the presence of terpenoid (Doss *et al.*, 2009).

2.12 Antibacterial activities test

The antibacterial susceptibility test of methanolic extract of *Z. officinale* (ZO) and *S. persica* (SP) and combinations extract of *Z. officinale* (ZO) and *S. persica* (SP) were examined against *S. mutans* (SM) using the well-diffusion method. On sterile brain heart infusion (BHI) agar plates supplemented with 2% sucrose and 1% glucose, we prepared three media plates and the plates were swabbed with *S. mutans* (ATCC 25175). Then, all three plates were drilled and five wells were mades in each media plate and loaded with three different concentrations (solute percentage) of extracts from different plants. We used ampicillin (5 µg) as a positive control and methanol as a negative control. After incubation, we read the plates and found zones of inhibition around the sample wells, that indicate the ZO and ZO + SP methanolic extracts have effective levels of antibacterial properties of extracts that means due to presence of phytochemicals inhibits the growth of *S. mutans*.That means these plants can play effective roles in controlling dental carries (Akintobi *et al.,* 2013).

2.13 Gas chromatography-mass spectrum (GC-MS)

The obtained methanolic extracts were analyzed in Shimadzu GC-MS QP2020 Capillary Column DB5 30 system for the presence of bioactive fraction by GC-MS. Before analysis, all the necessary programs were initiated such as temperature, gas and so on. For the analysis, 1 µl of the methanolic extracts were injected in to the GC-MS using microsyringe and the scanning was continued for 28 min. Then, the separated compounds were evaluated from the column and it was detected using detector. Each peaks present in the chromatogram represent individual molecule of the extracts and enter into the mass spectroscopy detector. The compound identifications were done by comparing the retention indices and the mass spectra patterns present in the computer library (Sophia *et al.,* 2022).

3. Results

3.1 Preliminary phytochemical screening

The mixed extract of *S. persica* and *Z. officinale* and single extracts of *S. persica* and *Z. officinale* have been tested for preliminary phytochemical screening to discover the presence of numerous bioactive compounds (Sofowora, 1993). The extraction changed into completed the use of methanol because the solvent. The screening changed the use of widespread techniques to discover the presence of various styles of phytochemicals bioactive components. We discovered consequences that display the presence of flavonoids, alkaloids, lowering sugar, tannin, and glycosides (Table 1). The consequences of the screening discovered the presence of all 5 compounds with in the extract, indicating the ability of the extract, that is capable of show antimicrobial interest. Alkaloids and flavonoids were said to show antimicrobial interest towards numerous pathogens, consisting of *S. mutans*, that are acknowledged to make contributions to the improvement of dental caries. Tannin and terpenoids have additionally been said to own antimicrobial

properties, at the same time as saponins were said to have anti-inflammatory effects. The presence of those compounds within the extract helps the ability of *S. persica* and *Z. officinale* as herbal options to artificial antimicrobial retailers for the prevention and remedy of dental caries. Further we have to diagnose and discover the extra lively compounds accountable for the discovered interest and elucidate their mechanism of motion (Doss, 2009).

Table 1: Preliminary phytochemical screening of *Z. officinale* (Ginger)

S. No.	Diluent name	Chemical constituents	Results
1.	Hexane	Triterpenoids	-
		Resins	-
2.	Chloroform	Steroids	+
		Triterpenoids	+
		Alkaloids	-
3.	Methanol	Flavonoids	+
		Alkaloids	++
		Reducing sugar	+
		Glycoside	-
		Tannin	+

Table 2: Preliminary phytochemical screening of *S. persica* (Miswak)

S. No.	Diluent name	Chemical constituents	Results
1.	Hexane	Triterpenoids	+
		Resins	++
2.	Chloroform	Steroids	+
		Triterpenoids	++
		Alkaloids	++
3.	Methanol	Flavonoids	+++
		Alkaloids	+++
		Reducing sugar	+
		Glycoside	-
		Tannin	++

3.2 Antibacterial susceptibility

The antibacterial susceptibility was performed on methanolic extracts of *Z. officinale* (ZO), *S. persica* (SP) and combination extract of ZO + SP against *S. mutans* (SM) by agar well diffusion methods. Then evaluated, their antibacterial susceptibility property of individual and combined methanolic extracts of ZO and SP. After inoculation of pure isolate of SM and making 5 wells (sterile glass rod) in 3 sterile BHI agar plate, then we had dispensed extracts 3 different concentrations (solute percentage) with positive control and negative control and then kept in incubation at 37°C for 24 h. Next day, we found zone of inhibitions were formed due to the presence of antibacterial property present in both plants, presented in Table 3 and Figure 1. As seen in figure, the size of the zone of inhibition was increased when increasing the concentration (solute percentage) of *Z. officinale*, *S. persica* and combination of both samples.

Figure 1: Antibacterial susceptibility against *S. mutans* with three methanolic extracts A) *Z. officinale*, B) *S. persica* and C) combination of ZO + SP. Note: V-Vehicle control (methanol) and P- Positive control.

Table 3: Antibacterial susceptibility result table of three methanolic extracts against *S. mutans*

	Z. officinale			*S. persica*			Combination of *Z. officinale* + *S. persica*		
Compounds/concentration (solute percentage)	300 µg	350 µg	400 µg	5 mg	7.5 mg	8 mg	L (300 µg + 5 mg)	M(300 µg + 7.5 mg)	H(300 µg + 8 mg)
Zone of inhibitions in mm	11	12	13	10	11	12	14	14	13

3.3 Gas chromatography-mass spectrum (GC-MS)

Analysis for GC-MS was performed on methanolic extracts of *Z. officinale* (ZO) and *S. persica* (SP) and combination of ZO + SP plants. After the completion GC-MS analysis on three types of extracts, the results have shown plenty of peaks have been formed. Each peak refers to chromatogram and this will help us to mass spectra of molecules. The same has been mentioned in Figure 3A, 3B, 3C and Table 4. As seen in Figure 3A, the chromatogram of 25 peaks of the different compounds was observed in extract of *Z. officinale* in methanol. Same way, 35 peaks of individual compounds were observed in methanolic extract of *S. persica* in Figure 3B. As seen in Figure 3C, chromatographic GC-MS analysis of combination of both ZO + SP revealed the presence of 25 peaks that show availability of various types of phytochemicals. In this study, we have focuses on the synergic mixture of phytochemicals where the plants methanolic extracts as source is found to have an significant antibacterial susceptibility. Analysis of GC-MS showed the availability of a several types of potent molecules like Tris (2,4-di-tert-butylphenyl) phosphate uses 33% area and octadecanoic acid, 2,3-dihydroxypropyl ester uses 12% area (Sophia *et al,.* 2022).

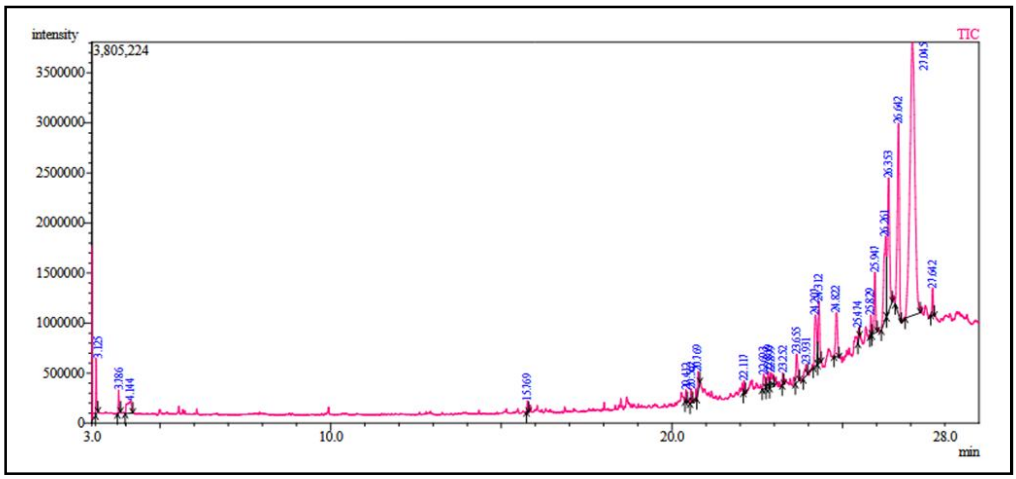

Figure 3A: Analysis of GC-MS of methanolic extracts of *Z. officinale* with 25 peaks.

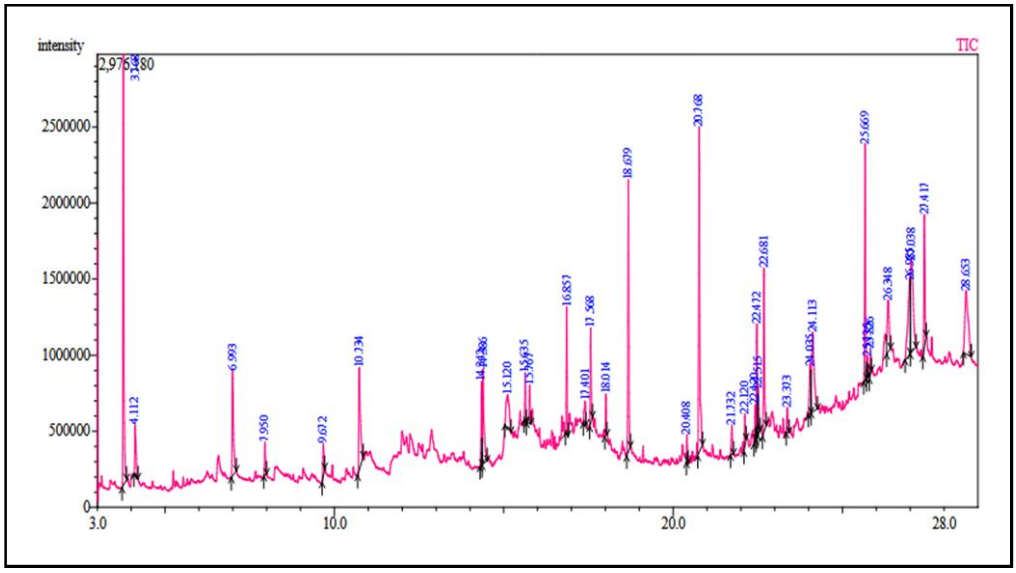

Figure 3B: GC-MS analysis of methanolic extracts of *S. persica* with 35 peaks.

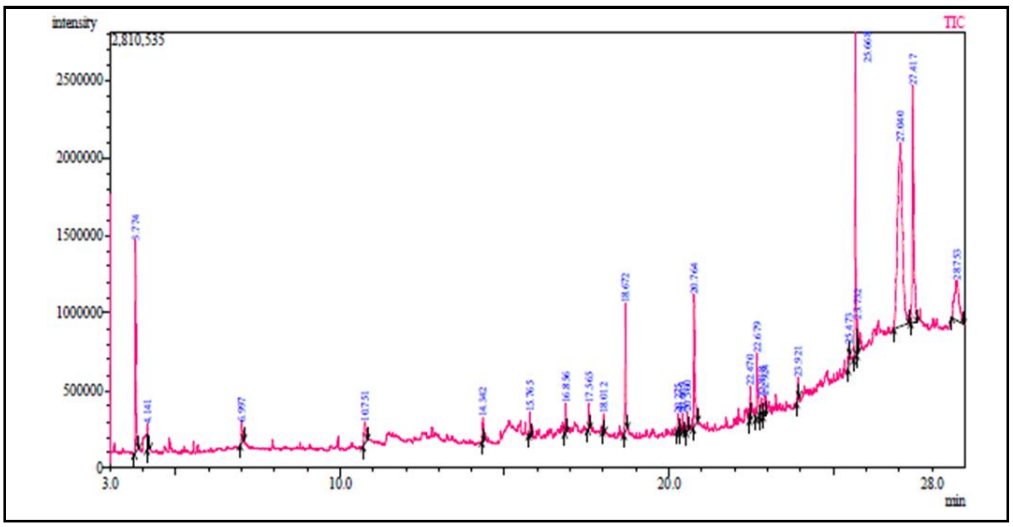

Figure 3C: GC-MS analysis of methanolic extracts of *Z. officinale* combined with *S. persica*.

Table 4: The phytochemicals presented in the combined methanolic extract of (SP + ZO) by GC-MS technique

Peak#	R.Time	Area	Area%	Height	Height %	Name
1	3.774	2386352	6.59	1365320	12.40	Glycerin
2	4.141	394912	1.09	159912	1.45	2,2-dimethoxy butane
3	6.997	392020	1.08	133388	1.21	1,2-cyclooctanedione
4	10.751	525028	1.45	138663	1.26	1-Methyl-1-(3-methylbutyl)oxy-1-silacyclobutane
5	14.342	239649	0.66	151559	1.38	Tetradecane
6	15.765	226168	0.62	150527	1.37	2,4-Di-tert-butylphenol
7	16.856	305593	0.84	188396	1.71	Heptadecane
8	17.565	273015	0.75	162773	1.48	1,4-Methanobenzocyclodecene, 1,2,3,4,4a,5,8,9,12,12a
9	18.012	169771	0.47	114206	1.04	Eicosane
10	18.672	1544455	4.26	825339	7.50	Tetradecanoic acid
11	20.275	304159	0.84	109394	0.99	Octadecane
12	20.409	282486	0.78	125843	1.14	Hexadecanoic acid, Methyl ester
13	20.560	246917	0.68	126226	1.15	1-Methyl-3-Hydroxy carbonyl-4-azaphe
14	20.764	2100196	5.80	851329	7.73	n-Hexadecanoic acid
15	22.470	441004	1.22	214935	1.95	9-Hexadecenoic acid, phenylmethyl ester, (Z)-
16	22.679	1181212	3.26	416514	3.78	Octadecanoic acid
17	22.818	377372	1.04	116806	1.06	Triacontylheptafluorobutyrate
18	22.924	536071	1.48	127412	1.16	Benzene, (2-decyldodecyl)-
19	23.921	247424	0.68	142849	1.30	1-(4-Hydroxy-3-methoxyphenyl)dec-4-en-3-one
20	25.473	166686	0.46	105790	0.96	Glycerol, 2-TMS-
21	25.668	4041843	11.15	2092557	19.01	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl
22	25.732	490188	1.35	219914	2.00	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl
23	27.040	12195547	33.66	1180912	10.73	Tris(2,4-di-tert-butylphenyl) phosphate
24	27.417	4611057	12.73	1526118	13.87	Octadecanoic acid, 2,3-dihydroxypropyl ester
25	28.753	2554407	7.05	260055	2.36	Tetracosamethyl-cyclododecasiloxane
		36233532	100.00	11006737	100.00	

4. Discussion

After performing different types of laboratory experiments on methanolic extracts of *Z. officinale* (ZO), *S. persica* (SP) and combination of ZO + SP plants, it hsas been found that methanolic extracts of ZO + SP has shown significant antibacterial susceptibility against *S. mutans* as compared to *S. persica* methanolic extracts show less antibacterial susceptibility against *S. mutans.* It can be clearly noticed by looking the unit of concentration (solute percentage) of ZO used in µg/ml and on other hands, SP in mg/ml against *S. mutans* as a common oral pathogen in this research. The synergistic effect of the two combination of extracts have a potential therapeutic property on *in vitro* application as natural antimicrobial agents. The current research focuses on the synergic mixtures of phytochemicals where the methanolic extracts of the plants as source is found to have an significant antibacterial susceptibility. Analysis of GC-MS showed the presence of a several types of potent molecules like Tris(2,4-di-tert-butylphenyl) phosphate uses 33% area and octadecanoic acid, 2,3-dihydroxypropyl ester uses 12% area. Phytochemicals that might have occurred due to synergism of the extracts. The MIC assay was performed on BHI broth against *S. mutans* by using individual and combined methanolic extracts of *Z. officinale* (ZO) and *S. persica* (SP) to identify the range of minimum inhibitory concentrations (solute percentage). MIC value of tested sample, that can show efficacy of particular extracts that may help in this research and motives other researchers to go deeper in this type of research concept. The antibacterial action of extracts which is showing *in vitro* effectiveness against *S. mutans.* It has possibility of treatment in human illness without any other side effects on human health.

5. Conclusion

We are now in a opinion to suggest further study on individual and combined extracts. Some of the data, of this research study could be used in future research study. However, more studies are needed to investigate the safety and efficacy of the combined extract *in vivo* and in clinical settings. Nonetheless, the results of this provides preliminary evidence for the use of Miswak and Ginger as a natural alternative to conventional antibiotics for the treatment of oral disease which is caused by *S. mutans. S. mutans* is one of the most common gram-positive cocci and this is involved in formation of bacterial biofilm on the tooth surface. *S. mutans* is a major etiological agent in dental caries.

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Conflict of interest

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

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