Research Article

Phytochemical, Cytotoxic, and Antimicrobial Evaluation of the Fruits of Miswak Plant, *Salvadora persica* L.

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Salvadora persica L. (Salvadoraceae) is an evergreen shrub growing in the Middle East, Africa, and Southern Asia. It is traditionally known as "miswak" and used as toothbrushes and for the treatment of toothache, gum diseases, boils, chest infection, gonorrhea, headache, spleen troubles, stomachache, and ulcers. To the best of our knowledge, this is the first study aimed at conducting phytochemical, cytotoxic, and antimicrobial investigations of the fruits (berries) of *S. persica* collected from the Jazan region of Saudi Arabia. Analysis of the ethanol extract of *S. persica* fruits using GC-MS showed the presence of six esters (20.71%), seven alkanes (15.47%), tetracosamethyl-cyclododecasiloxane (9.91%), eicosamethyl-cyclodecasiloxane (7.27%), and 1-monolinoleoylglycerol (5.17%). The predominant constituents were acetyl dasycarpidan-1-methanol (10.47%), tetracosamethyl-cyclododecasiloxane (9.91%), eicosamethyl-cyclodecasiloxane (7.27%), and 1-monolinoleoylglycerol (5.17%). The petroleum ether extract of the fruits contained mainly eicosamethyl-cyclodecasiloxane (23.81%), 1-monolinoleoylglycerol (11.78%), (Z,Z,Z)-9,12,15-octadecatrienoic acid ethyl ester derivative (10.56%), and tetracosamethyl-cyclododecasiloxane (9.91%). The cytotoxic properties of the ethanol extract were investigated by MTT assay against the breast MCF7, ovary A2780, and colon HT29 cells. The fruit extract of *S. persica* was selective against the ovarian and colon cancer cells compared to normal fibroblast cells (MRC5) as it showed IC_{50} values 17.50, 8.35, and 5.12, against MCF7, A2780, and HT29 cells, respectively. Interestingly, the fruit extract was also found to possess selective antimicrobial activity for *Streptococcus mutans* isolates with minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values of 3.12 and 6.25 mg/mL, respectively. Interestingly, it was found to be ineffective against other Gram-positive as well as Gram-negative microorganisms. This study provides insight into the bioactive components present in the fruits of the plant that can be utilized for its cytotoxic and antimicrobial properties.

1. Introduction

Salvadora persica L. (family: Salvadoraceae), which has many local names (miswak, peelu, toothbrush tree, and mustard tree), is an ancient native plant found in Africa, Iran, Pakistan, India, Sri Lanka, and the Middle East countries including Saudi Arabia, Oman, Yemen, Jordan, and Syria [1, 2]. It is an evergreen shrub with a wide crown of curved branches, slightly rough bark, greyish-brown stem, greenish-yellow flowers, and fleshy pink fruits [3].

There are many traditional uses reported for the *S. persica* plant. It has mainly been used in dental care to cure toothache and gum diseases and for cleaning teeth. Additionally, it is used to relieve boils, chest diseases, gonorrhea, headache, spleen troubles, stomachache, and ulcers. The root infusion can also be used to increase milk production in lactating women. Leaves of the plant are being utilized as a mouthwash and in treatment of chest pain, cough, tooth and gum problems, body pain, backache, piles, stomachache, stomatitis, and wounds. The bark latex is beneficial to subside skin sores. The seeds are taken as a tonic, and the seed oil is rubbed to treat joint pain, lumbago, and rheumatism. It has also been used to relieve edema, fever, malaria, and worms. The plant juice is used by women as a female contraceptive [1, 4].

The uses of stem and roots of *S. persica* in dentistry could be correlated to the presence of many phytochemical constituents such as tannins that inhibits glucosyltransferase enzyme to reduce plaque and gingivitis [5]. The resins of the chewing stick of the plant protect against dental caries by forming a layer on enamel [6]. Salvadorine isolated from the chewing stick of *S. persica* exerted bactericidal effects and stimulated the gingiva [7]. Recently, five α-amylases were isolated from the roots of the plant which showed good affinity towards the substrates such as starch and glycogen [8]. Moreover, *S. persica* sticks were found to stimulate the saliva flow in humans owing to the mildly bitter taste of essential oils present in it, which act as a buffer. Additionally, high chloride concentrations inhibit the calculus formation and removes stains from the tooth surfaces [7, 9]. Moreover, the use of chewing sticks causes calcium saturation in human saliva leading to promotion in remineralization of tooth enamel [5].

The anticancer activity of *S. persica* was also investigated. In a previous study, *S. persica* aqueous root extract exhibited oral protective activity on the normal periodontal ligament fibroblast (PDL) cell lines, and notably it caused cytotoxicity on oral cancer cells: oral epithelial dysplasia (DOK) and oral squamous cell carcinoma (PE/CA-PJ15), all using the MTT assay [10]. The MTT assay was also used to investigate the cytotoxicity of *S. persica* sticks and bark extracts against HepG2, MCF7, A549, and HCT116 cancer cells. *S. persica* extracted with petroleum ether showed the highest cytotoxicity compared to the same plant extracted with aqueous alcohol, chloroform, and ethyl acetate. The petroleum ether extract yielded two triterpenes (ursolic and oleanolic acids) which showed IC_{50} 10.2–43.6 μg/mL against the four cancer cell lines [11].

Studies related to the importance of *S. persica* fruits as nutraceutical are very scarce and less investigated [12]. The nutritional value of the *S. persica* fruits has been demonstrated by the presence of sugar metabolites, minerals, amino acids, vitamins (ascorbic acid and carotenoids), polyphenols, and flavonoids. Previously, these metabolites were proved to have considerable antimicrobial, antihyperglycemic, antitumor, and antioxidant properties. The fermented fruit juice is a general body tonic and acts as strong aphrodisiac [12–14].

In spite of the ethnopharmacological importance of *S. persica*, the research on this plant has been focusing only on the stem and root of the plant. To the best of our knowledge, this is the first study to emphasize the cytotoxicity and antimicrobial properties of the fruits of *S. persica*. This study was conducted to identify the constituents of the plant using GC-MS. The cytotoxic and antimicrobial properties of the fruit extract of *S. persica* were evaluated keeping in mind the high reputation and traditional ethnopharmacological uses of the stem and roots of the plant.

2. Materials and Methods

2.1. Plant Collection and Extraction. Reddish purple fruits of *S. persica* were collected from Jazan region of Saudi Arabia in March 2018 and were identified by Dr. Yahiya Masrahi, Botany Department, Faculty of Science, Jazan University, Saudi Arabia. A voucher with specimen no. JU/COP/18–2 was deposited in the herbarium of the Department.

The fruits of *S. persica* were extracted using previously described method by Harborne [15] with some modifications. Fresh fruits of *S. persica* (500 g) were grinded, macerated, and dipped in 2.5 liters of 80% ethanol and petroleum ether separately for 48 h at room temperature with constant shaking. The supernatant obtained was filtered using Whatman filter paper (0.45 μm). This process was repeated two times in the same conditions. Both extracts were mixed and dried at room temperature. The dry weight yield was calculated to be 10%. The extracts were then refrigerated at 4°C in dark bottles for consequent experiments.

2.2. GC-MS Analysis of the Extract. The extract was diluted in methanol (1 : 10 v/v) and was analyzed using Thermo Scientific GC-MS equipped with AS 3000 autosampler, Trace GC ultra, and ISQ detector. Thermo Scientific TR 5MS with dimensions of 30 m × 0.25 mm (internal diameter) × 0.25 μm (film thickness) was used for separation of the components. Helium, at a flow rate of 1.2 mL/min (constant flow mode), was used as carrier gas. A volume of 2 μL of sample extracts was injected in splitless mode. The injection port was set at 320°C and temperature of oven was initially set at 70°C for 5 minutes, which was subsequently ramped to 205°C at rate of 5°C/min and held for 5 minutes, then increased to 280°C at rate of 5°C/min and held for 5 minutes, then to 290°C at rate of 5°C/min and again held for 5 minutes, and finally to 300°C at rate of 5°C/min and held for 5 minutes. The maximum oven temperature was set at 320°C. The mass spectrometer was operated in an electron ionization (EI) mode within the mass range of 60–900 amu with 0.6 scan times (min). The MS ion source temperature and transfer line temperature were set at 320°C and 350°C, respectively, with electron multiplier voltage of 1 Kv.

2.3. Identification of Phytochemical Constituents. The mass spectra were interpreted using Xcalibur software, and the fragmentation patterns in the mass spectra obtained for all constituents were compared with the data stored in the instrument database using the NIST, MAINLIB, and REPLIB built-in libraries. The constituent percentages were measured based on the peak area. The components were identified upon comparison with the structures available in the computer library. The reported biological activities of the constituents listed (Tables 1 and 2) are taken from Dr. Duke's Phytochemical and Ethnobotanical Databases [16].

2.4. Cytotoxicity Assay. Three cancer cell lines, MCF7 (human breast carcinoma cells), A2780 (human ovary carcinoma cells), and HT29 (human colon carcinoma cells), were used in this study to evaluate the cytotoxic properties of the fruit ethanol extract in addition to the effects on MRC5 (normal human fibroblast) cells. The three tumor cell lines were subcultured in RPMI-1640 media (prepared in 10% Fetal Bovine Serum (FBS)), whereas MRC5 was subcultured in Eagle's Minimum Essential Medium (EMEM) (in 10% FBS at 37°C temperature, 5% CO_2, and 100% relative humidity). The cytotoxic properties of the ethanol extract were evaluated using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay according to previous report [17, 18]. The three cancer cell lines as well as the normal human fibroblast were cultured separately in 96-well (3×10^3/well) plates and placed for incubation at 37°C overnight. Thereafter, the final extract concentrations 0–100 μg/mL were prepared and added in triplicate to the wells. Plates were then incubated again for 72 h, followed by addition of MTT to each well. Finally, the plates were incubated for 3 h and DMSO was added to each well. Absorbance was measured at 550 nm using BIORAD, PR 4100 multiplate reader. The optical density was directly related to the number of viable cells. The cytotoxicity was indicated by the decrease in the optical density as compared to control. Extract concentration which causes 50% inhibition in comparison to control (cell growth = 100%) was determined as IC_{50}.

2.5. Antimicrobial Assays

2.5.1. Test Microorganisms. The ethanol extract was further tested against three standard Gram-positive bacterial strains including *Staphylococcus aureus* (ATCC 254996), *Enterococcus faecalis* (ATCC 254602), and *Streptococcus pneumonia* (ATCC 254657), four standard Gram-negative bacterial strains, *Escherichia coli* (ATCC 254607), *Proteus mirabilis* (ATCC 257440), *Pseudomonas aeruginosa* (ATCC 254992), and *Klebsiella pneumoniae* (ATCC 254656), one standard fungal strain, *Candida albicans* (ATCC 254625), and two clinical strains *Streptococcus mutans* and *Lactobacillus* spp. The tested organisms were procured from the Department of Microbiology, King Fahad Hospital, Jazan, Saudi Arabia.

2.5.2. Preparation of the Test Organisms. A loopful of isolated bacterial colonies were inoculated into 4 mL peptone water and incubated at 37°C for 4 h. The turbidity of actively growing bacterial suspension was adjusted to match the turbidity standard of 0.5 McFarland units [19]. The fungal cultures were maintained on Sabouraud dextrose agar, incubated at 25°C for 4 days. The fungal growth was harvested and washed off with 100 mL sterile normal saline, and the suspension was stored in the refrigerator at 4°C until used.

2.5.3. In Vitro Antimicrobial Activity Testing of Extracts. To test the antibacterial efficacy of the extract, a measured quantity (1 mL) of the standardized stock suspension of bacteria having 10^5–10^6 CFU per milliliter was mixed with 100 mL of Mueller–Hinton agar medium maintained at 45°C. Aliquots (20 mL) of the resulting inoculated Mueller–Hinton agar medium were divided into previously sterilized plates. The agar medium was allowed to set at room temperature and 4 cups of 10 mm diameter were made in each of these plates using a sterilized cork borer (No. 4). Agar discs were removed and the hole was filled with different concentrations (100 μg/mL and 500 μg/mL) of ethanol extract and left undisturbed at room temperature to diffuse for 2 h. The resulting plates were allowed to incubate at 37°C overnight. The plates were then observed for any inhibition in the bacterial growth and the diameter of zone of inhibition was measured. The method used for antibacterial study was also adopted for testing the extract for antifungal activity. The only difference in the method is that instead of Mueller–Hinton agar medium, another medium (Sabouraud dextrose agar medium) was used and the inoculated medium was maintained at 25°C [20].

2.5.4. Determination of MIC. Determination of MIC for the ethanol extract was accomplished against *Streptococcus mutans*. Tested bacterial cultures (100 μL of bacterial culture containing 10^5 CFU/mL) were inoculated into tubes containing different concentrations (3.12, 6.25, 12.5, and 25 mg/mL) of ethanol extract and incubated at 37°C for 24 h. The values were determined by detecting the inhibition of visible growth in the culture tubes. Minimum Bacterial Concentration (MBC) was assayed by subculturing the broth onto freshly prepared solid media and further incubated at 37°C for 24 h [21].

3. Results and Discussion

The total ion chromatogram of ethanol extract of the *S. persica* fruits is shown in Figure 1, which represents retention times of all the components detected in the extract. The chemical composition and documented biological activities of various components of ethanol extract of the fruits of *S. persica* are tabulated in Table 1. The ethanol extract consisted mainly of six esters (20.71%), seven alkanes (15.47%), tetracosamethyl-cyclododecasiloxane (9.91%), eicosamethyl-cyclodecasiloxane (7.27), and 1-monolinoleoylglycerol (5.17). The predominant constituents were acetyl dasycarpidan-1-methanol (10.47%), tetracosamethyl-

TABLE 1: GC-MS analysis of the ethanol extract of *S. persica* fruits.

S. no.	Retention time	Chemical constituents	% area	Molecular formula	MW	Reported activity*
1	8.40	3,3-Diisopropoxy-1,1,1,5,5,5-hexamethyltrisiloxane	0.82	$C_{12}H_{32}O_4Si_3$	324	—
2	10.87	Benzoic acid, methyl ester	0.84	$C_8H_8O_2$	136	Antibacterial
3	18.22	2,2,4-Trimethyl-1,3-pentanediol	1.1	$C_{16}H_{30}O_4$	286	Antimicrobial
4	18.90	Propanoic acid, 2-methyl-, 3-hydroxy-2,4,4-trimethylpentyl ester	1.42	$C_{12}H_{24}O_3$	216	
5	24.36	Cyclooctasiloxane, hexadecamethyl-	0.76	$C_{16}H_{48}O_8Si_8$	592	Antimicrobial
6	20.51	Cycloheptasiloxane, tetradecamethyl-	1.09	$C_{14}H_{42}O_7Si_7$	518	Preservative
7	27.66	Cyclononasiloxane, octadecamethyl-	1.09	$C_{18}H_{54}O_9Si_9$	666	Antifungal
8	28.24	3,5-Di-tert-butyl-4-hydroxybenzaldehyde	1.00	$C_{15}H_{22}O_2$	234	Antioxidant
9	31.27	7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione	3.76	$C_{17}H_{24}O_3$	276	Antioxidant
10	31.72	Nonadecane, 2-methyl-	4.76	$C_{20}H_{42}$	282	Antioxidant
11	32.08	Heptacosane	3.33	$C_{27}H_{56}$	380	Antibacterial
12	32.26	Eicosane, 7-hexyl-	1.30	$C_{26}H_{54}$	366	Antiandrogenic, aldose reductase inhibitor
13	37.05	Eicosane, 2-methyl-	1.26	$C_{21}H_{44}$	296	Antitumor, hemolytic activity, cytotoxic, antioxidant
14	43.13	Octadecane, 3-ethyl-5-(2-ethylbutyl)-	2.33	$C_{26}H_{54}$	366	Antimicrobial, antifungal
15	43.37	Tetratetracontane	2.51	$C_{44}H_{90}$	618	Antioxidant, hypoglycemic
16	48.68	1-Monolinoleoylglycerol trimethylsilyl ether	5.17	$C_{27}H_{54}O_4Si_2$	498	Antimicrobial
17	50.50	psi,psi-Carotene, 7,7′,8,8′,11,11′,12,12′,15,15′-decahydro-	0.78	$C_{40}H_{66}$	546	Antioxidant
18	52.05	Cyclodecasiloxane, eicosamethyl-	7.27	$C_{20}H_{60}O_{10}Si_{10}$	742	Antimicrobial
19	52.37	Dasycarpidan-1-methanol, acetate (ester)	10.49	$C_{20}H_{26}N_2O_2$	326	Anti-inflammatory
20	55.93	Heptasiloxane, hexadecamethyl-	0.72	$C_{16}H_{48}O_6Si_7$	532	Antimicrobial
21	56.57	á,psi-Carotene, 3′,4′-didehydro-1′,2′-dihydro-1′,2′-dihydroxy-, (2′R)-	1.24	$C_{40}H_{56}O_2$	568	Anticancer, antioxidant
22	60.44	Milbemycin B, 5-demethoxy-5-one-6,28-anhydro-25-ethyl-4-methyl-13-chloro-oxime	1.2 1	$C_{32}H_{44}ClNO_7$	589	Antibacterial, antifungal
23	66.02	9-Octadecenoic acid (Z)-, 3-[(1-oxohexadecyl)oxy]-2-[(1-oxooctadecyl)oxy]propyl ester	1.61	$C_{55}H_{10}4O_6$	860	Emulsifying agent
24	66.45	9,12,15-Octadecatrienoic acid, 2,3-bis [(trimethylsilyl)oxy]propyl ester, (Z,Z,Z)-	3.49	$C_{27}H_{52}O_4Si_2$	496	Anticancer, hepatoprotective
25	66.63	9-Octadecenoic acid, 1,2,3-propanetriyl ester, (E,E,E)-	2.86	$C_{57}H_{10}4O_6$	884	Antibacterial, anticancer
26	67.74	Formyl colchicine	2.22	$C_{23}H_{25}NO_7$	427	Anti-inflammatory, antioxidant, cytotoxic activity
27	69.86	psi,psi-Carotene, 1,1′,2,2′-tetrahydro-1,1′-dimethoxy-	3.33	$C_{42}H_{64}O_2$	600	Antioxidant

*Activity reported from Dr. Duke's Phytochemical and Ethnobotanical Databases [42].

TABLE 2: GC-MS analysis of petroleum ether extract of *S. persica* fruits.

S. no.	Retention time	Chemical constituents	% area	Molecular formula	MW	Reported activity*
1	8.42	Cyclotrisiloxane, hexamethyl-	0.74	$C_6H_{18}O_3Si_3$	222	Anticancer
2	10.90	Benzoic acid, methyl ester	0.66	$C_8H_8O_2$	136	Antibacterial
3	18.95	Propanoic acid, 2-methyl-, 2,2-dimethyl-1-(2-hydroxy-1-methylethyl)propyl ester	1.51	$C_{12}H_{24}O_3$	216	
4	27.49	Heptadecane, 2,6,10,15-tetramethyl-	1.12	$C_{21}H_{44}$	296	
5	31.32	7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione	2.52	$C_{17}H_{24}O_3$	276	Antioxidant
6	31.77	Eicosane, 2-methyl-	1.7	$C_{21}H_{44}$	296	Antitumor, hemolytic activity, cytotoxic, antioxidant
7	32.13	Nonadecane, 2-methyl-	1.61	$C_{20}H_{42}$	282	Antioxidant
8	32.31	1-Iodo-2-methylundecane	0.76	$C_{12}H_{25}I$	296	
9	37.23	Cyclononasiloxane, octadecamethyl-	2.33	$C_{18}H_{54}O_9Si_9$	666	Antifungal

TABLE 2: Continued.

S. no.	Retention time	Chemical constituents	% area	Molecular formula	MW	Reported activity*
10	42.64	Octadecane, 3-ethyl-5-(2-ethylbutyl)-	2.53	$C_{26}H_{54}$	366	
11	52.08	Cyclodecasiloxane, eicosamethyl-	23.81	$C_{20}H_{60}O_{10}Si_{10}$	742	Antimicrobial, antihelmintic, antioxidant
12	56.22	Tetracosamethyl-cyclododecasiloxane	9.91	$C_{24}H_{72}O_{12}Si_{12}$	890	Hepatoprotective, antispasmodic, antirheumatic
13	48.68	1-Monolinoleoylglycerol trimethylsilyl ether	11.78	$C_{27}H_{54}O_4Si_2$	498	Antimicrobial
14	65.99	9,12,15-Octadecatrienoic acid, 2-[(trimethylsilyl)oxy]-1-[[(trimethylsilyl)oxy]methyl]ethyl ester, (Z,Z,Z)-	10.56	$C_{27}H_{52}O_4Si_2$	496	Anticancer, anti-inflammatory

*Activity reported from Dr. Duke's Phytochemical and Ethnobotanical Databases.
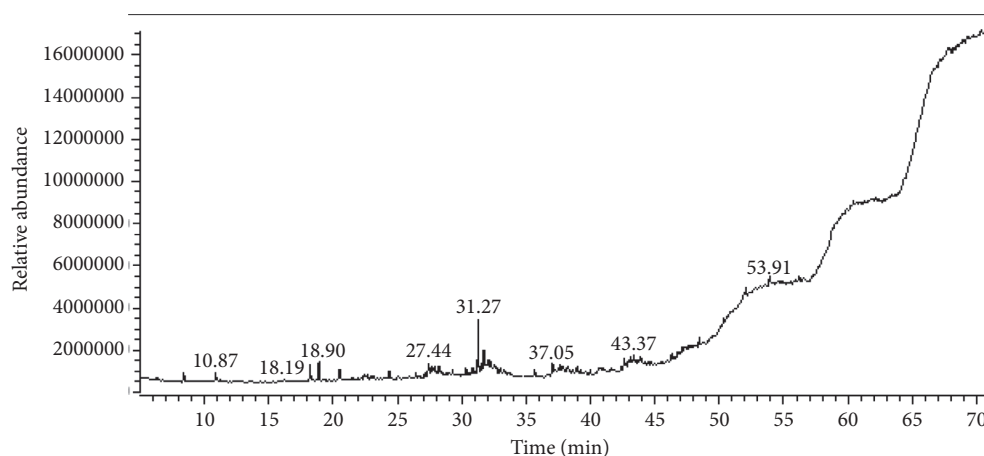

FIGURE 1: Total ion GC-MS chromatogram of the ethanol extract of *S. persica* fruits.

cyclododecasiloxane (9.91%), eicosamethyl-cyclodecasiloxane (7.27%), and 1-monolinoleoylglycerol (5.17). The major alkanes detected were 2-methyl nonadecane (4.76%) and heptacosane (3.31%). Five compounds were detected in trace amounts. Two carotene derivatives, (2′R)-3′,4′-didehydro-1′,2′-dihydro-1′,2′-dihydroxy-α-*psi*-carotene (1.24%) and 1,1′,2,2′-tetrahydro-1,1′-dimethoxy-*psi,psi*-carotene (3.33%), were also detected for the first time in the *S. persica* fruits. Formyl colchicine (2.22%) was an alkaloidal constituent present in the fruits. The fruits were found to be devoid of phytosterol, fatty acids, saponins, and mono-, di-, and triterpenoids. The chemical components methyl benzoate (0.66%), 2-methyl nonadecane, 2-methyl eicosane, 3-ethyl-5-(2-ethylbutyl)-octadecane, 7,9-di-tert-butyl-1-oxaspiro (4,5)deca-6,9-diene-2,8-dione (2.52%), and 1-monolinoleoylglycerol (11.78%) were also detected in the ethanol extract of the fruits.

Similarly, the total ion chromatogram of petroleum ether extract was obtained using GC-MS and is shown as Figure 2, whereas the chemical constituents and major biological activities of the petroleum ether extract of the *S. persica* fruits are summarized in Table 2. The prominent constituents characterized in the petroleum ether were eicosamethyl-cyclodecasiloxane (23.81%), 1-monolinoleoylglycerol (11.78%), (Z,Z,Z)-9,12,15-octadecatrienoic acid ethyl ester

derivative (10.56%), and tetracosamethyl-cyclododecasiloxane (9.91%). Four alkanes identified in the extract included 2,6,10,15-tetramethyl heptadecane (1.12%), 2-methyl eicosane (1.7%), 2-methyl nonadecane (1.61%), and 3-ethyl-5-(2-ethylbutyl)-octadecane (2.53%). Figure 3 represents various important chemical constituents detected in ethanol and petroleum ether extracts of fruits of *S. persica* along with their structure.

The previous phytochemical screening studies showed that the stem of *S. persica* yielded octacosanol, 1-triacantanol, β-sitosterol, 3-O-β-D-glucopyranoside salvadourea, *m*-anisic acid [22, 23], pyrrolidine, pyrrole, and piperidine derivatives [24], salvadoside and salvadoraside glycosides [25], and flavonoids, which included quercetin, rutin, kaempferol, and quercetin glucoside [26], while the roots of *S. persica* yielded β-sitosteryl fatty esters and β-sitosteryl-3-vanilloyl-4′-fatty esters [27]. The roots, leaves, and stem contained ascorbic acid, butanediamide, salvadorine, salvadourea, lignans, fatty acids, trimethyl amine, cyanogenic glycosides, steroidal esters, tannins, aniline derivative, spiculesporic acid, methyl hexadecanoate, and homo-γ-linolenic acid [28]. Additionally, the stem essential oil is reported to consist mainly of 1,8-cineole (eucalyptol) (46%), β-pinene (6.3%), and 9-*epi*-(E)-caryophyllene (13.4%) [29]. The roots and stems also possessed oleic,
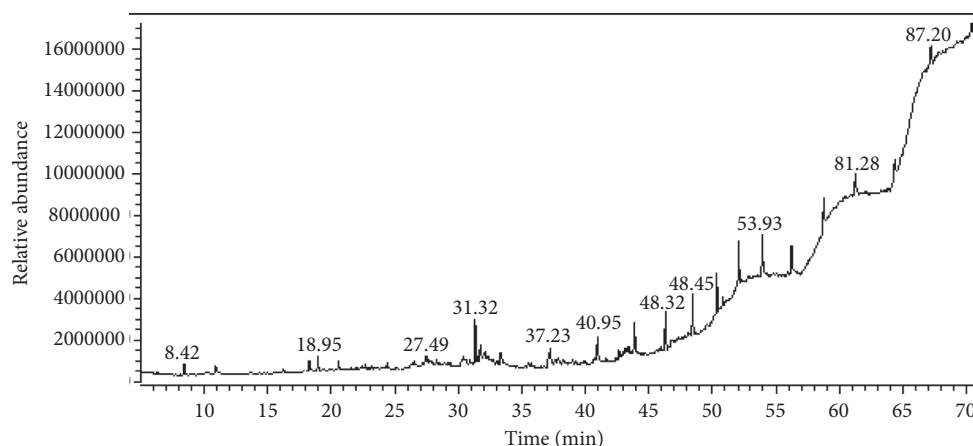
Figure 2: Total ion GC-MS chromatogram of the petroleum ether extract of *S. persica* fruits.

linoleic, and stearic acids as well as methyl and ethyl esters of fatty and other organic acids [30].

The ethanol fruit extract of *S. persica* was tested for cytotoxic effects using MTT assay (72 h), which showed activity against the breast, ovary, and colon cancer cells (IC50: 5.12–17.50 μg/mL). However, it was observed to be more selective against the ovarian and the colon cancer cell lines compared to the normal cells MRC5 (Table 3, Figure 4). This result was in agreement with the previous reports showing the antitumor activity of *S. persica* root, sticks, and bark extracts against oral carcinoma, HepG2, MCF7, A549, and HCT116 cancer cells [31–36]. Both *S. persica* fruit extract used in this study and *S. persica* root extract previously tested have exhibited protection to the normal lung and oral cells, thus indicating the safety profile of this commonly used plant. Eicosane, psi-carotene, 3′,4′-didehydro-1′,2′-dihydro-1′,2′-dihydroxy-(2′R)-9,12,15-octadecatrienoic acid, 2,3-bis[(trimethylsilyl)oxy]propyl ester, (Z,Z,Z)-, and formyl colchicine were all identified in the GC-MS analysis of the ethanol extract of *S. persica* fruits in this study. These constituents were previously reported to exert anticancer activities according to Dr. Duke's Phytochemical and Ethnobotanical Databases (Table 1) [16].

The antibacterial activity of the ethanol extract was determined using the disc diffusion method. The plant extract was found to possess selective antimicrobial activity, as it was ineffective at both the tested concentrations (100 μg/mL and 500 μg/mL) against the isolates of *Staphylococcus aureus* and *Enterococcus faecalis* as well as against the Gram-negative microorganisms, *Escherichia coli*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, and *Klebsiella pneumonia*. Interestingly, it was found to be selectively effective against the isolates of *Streptococcus mutans*, which was resistant to the standard drug Ampicillin. The zone of inhibition at the higher concentration 500 μg/mL was found to be 25 ± 2.1 mm which was comparable to the zone of inhibition shown by ampicillin for other bacterial strains

at the same concentration (Table 4). The MIC and MBC values of the extract were 3.12 and 6.25 mg/mL, respectively, whereas the standard drug ampicillin was found to have nonsignificant activity against the tested strain. This could mainly be due to the phytochemical differences between the constituents in the fruits of *S. persica* collected from this region or to the characteristic differences between *S. mutans* and other bacterial strains used in this study. This could also be attributed to any potential to new characters that could have been gained by the clinical strains that showed resistance against *S. persica* extracts. On the other hand, the sensitivity of *S. mutans* to the extract of *S. persica* supports the traditional use of this plant in oral health as it is considered one of the most common oral microbes that cause caries.

Interestingly, previous reports of *in vitro* antibacterial and antifungal studies on roots and stem extracts of *S. persica* on cariogenic bacteria and periodontal pathogens showed that the plant was effective against *S. faecalis*, *S. aureus*, *S. pyogenes*, *S. mutans*, *P. aeruginosa*, *L. acidophilus*, *P. gingivalis*, *H. influenzae*, *A. actinomycetemcomitans*, and *C. albicans* [37–39]. However, this study utilized the fruits of *S. persica* and included additional bacterial strains such as *E. faecalis*, *E. coli*, *P. mirabilis*, and *K. pneumonia* for antibacterial activity, but the fruits extract of the collected plant was ineffective against all these strains.

Previously, the roots and stem of *S. persica* used as toothbrushes showed antimicrobial, antiplaque, aphrodisiac, analgesic, alexiteric, antipyretic, anti-inflammatory, and astringent effects, which help in cleaning the teeth and decreasing tooth decay [40, 41]. In another study, the bioactive compounds extracted from *S. persica* roots and stems showed wide scope of pharmacological activities including antimicrobial, antioxidant, antiulcer, anticonvulsant, sedative, antiplasmodia, antimalarial, analgesic, antitumor, scurvy, gonorrhea, boils, antiosteoporosis, enzyme inhibitory activity, anti-inflammatory, hypoglycemic,

Table 3: Cytotoxic activity of the ethanol extract of the *S. persica* fruits against three cancer cell lines and one normal fibroblast (MTT 72 h IC$_{50}$ ± SD µg/mL).

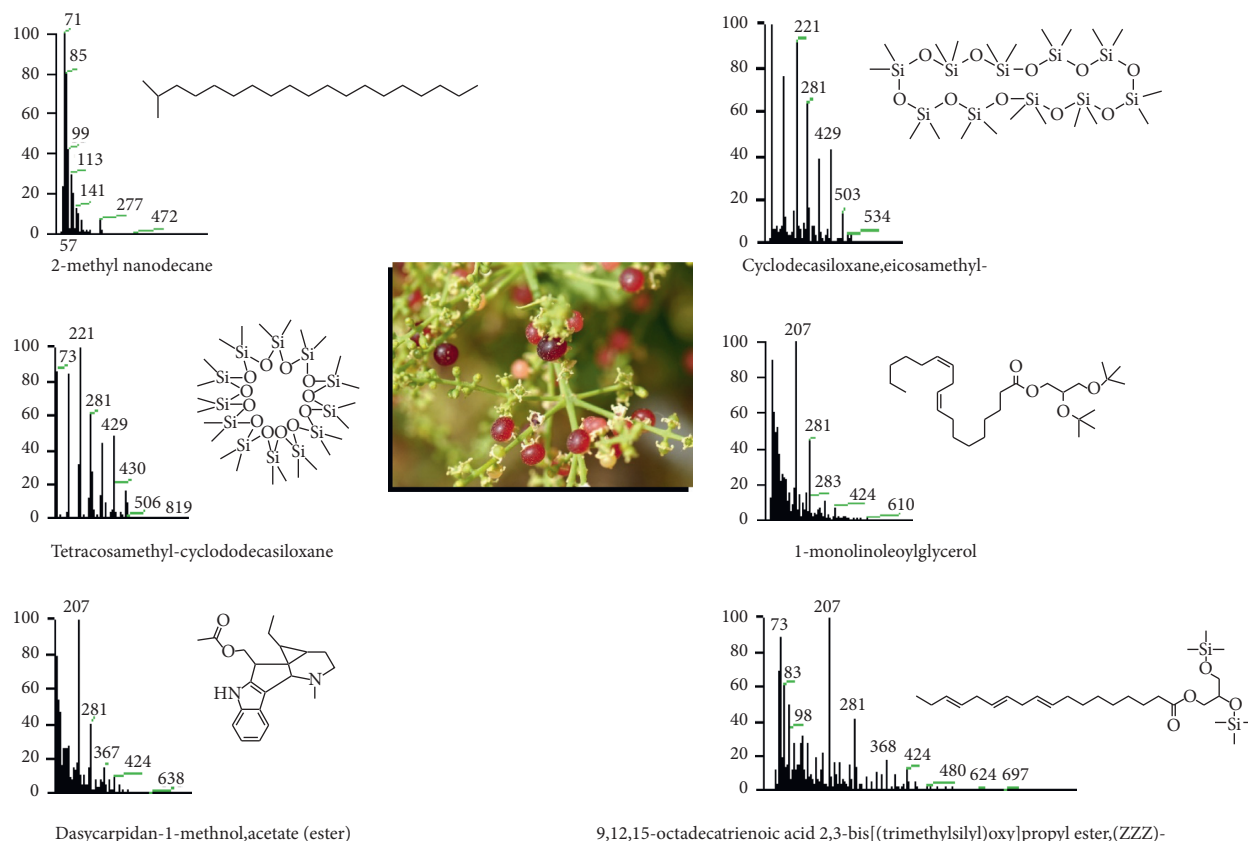| Extract	IC$_{50}$ (µg/mL)			
	MCF7	A2780	HT29	MRC5
Fruits of *S. persica* L.	17.50 ± 0.46	8.35 ± 0.77	5.12 ± 1.37	13.74 ± 2.27

Figure 3: Major bioactive components detected in *S. persica* fruits using GC-MS.

hypolipidemic, anticonvulsion, diuretic, and bitter stomachic activities, in addition to antiurolithiatic properties [31–36, 42, 43]. Benzyl isothiocyanate and benzyl nitrile were major active compounds purified from *S. persica* with powerful antibacterial and antifungal activity [27, 44, 45]. Benzyl isothiocyanate is extracted from the mouth when chewing sticks were used more than once for 10 minutes. The essential oils present in the sticks showed positive effect on gingival fibroblasts and resistance towards oral keratinocytes [46]. *N*-Benzylbenzamide isolated from the roots of *S. persica* exhibited a considerable inhibitory activity against the collagen-induced platelet aggregation in human and a considerable antiseptic against *E. coli* [47]. *S. persica* stem was also reported to act as a protectant against the pentylenetetrazol-induced convulsions in humans by extending the sleeping period and decreasing the induction time induced by sodium pentobarbital [36]. Moreover, Persicaline, a sulphur-containing imidazoline alkaloid, and three furan derivatives with hydroxyl groups were identified from the root of *S. persica*, which showed promising antioxidant activity as compared to ascorbic acid [48, 49].

The prevalence of dental caries was reported to be remarkably low in the users of *S. persica* stem due to the presence of strong antimicrobial thiocyanate component along with other substances such as potassium chloride, sodium chloride, tannins, and saponins [50]. The ethanol extract of *S. persica* stem exhibited substantial decrease in the cariogenic bacterial growth and dental decay [50]. Additionally, data obtained from controlled clinical trials

TABLE 4: Antimicrobial activity of the ethanol extract of S. *persica* fruits against Gram-positive and Gram-negative microorganisms.

| Extract/compound	Concentration (μg mL^{-1})	Zone of inhibition (mm) (mean ± SEM)									
		Gram +ve bacteria			Gram –ve bacteria				Clinical bacteria		Fungal strain
		S. aureus	E. faecalis	S. pneumonia	E. coli	P. mirabilis	P. aeruginosa	K. pneumoniae	S. mutans	Lactobacillus sp.	C. albicans
Fruits of S. *persica* L.	100	NS	NS	NS	NS	NS	NS	NS	10 ± 1.72	NS	NS
	500	NS	NS	NS	NS	NS	NS	NS	25 ± 2.12	NS	NS
Ampicillin	500	23.0 ± 0.21	17.1 ± 1.19	14.4 ± 0.23	20.0 ± 0.27	27.1 ± 1.22	19.3 ± 1.26	13.1 ± 1.10	NS	17.6 ± 1.19	NT
Griseofulvin	500	NT	NT	NT	NT	NT	NT	NT	NT	NT	16 ± 0.27

NS: not significant (<5 mm); NT: not tested.
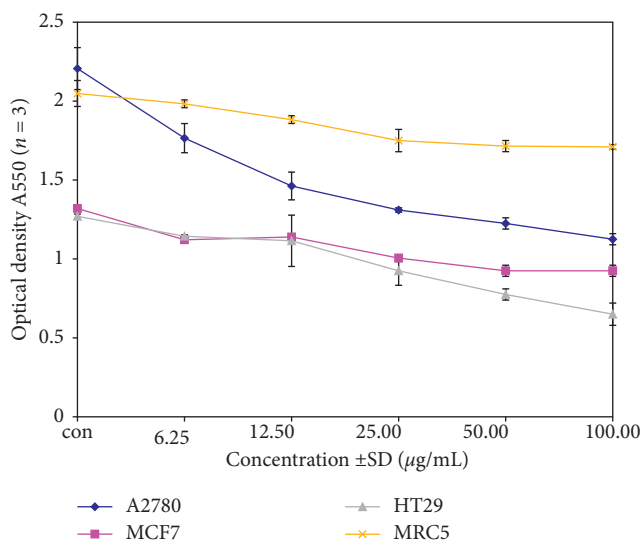
FIGURE 4: Cytotoxic effects of ethanol extract of S. persica fruits on A2780, MCF7, HT29, and MRC5 cells. Experiments were repeated three times.

revealed that the extract of *S. persica* roots and stem is also effective antimicrobial if used as an irritant for treating necrotic pulp teeth [51–53].

4. Conclusion

Forty-one phytochemicals were identified from the ethanol and petroleum ether extracts of the fruits of *S. persica* grown in the southern region of Saudi Arabia using GC-MS. The ethanol extract showed good cytotoxic properties against ovary and colon cancer cells and lesser cytotoxicity against the MRC5 normal cells. Further studies are required to establish the cytotoxic effects of the extract and identification of components acting as cytotoxic. The fruit extract of *S. persica* has also demonstrated potential antimicrobial activity against *S. mutans* which was resistant to the standard drug ampicillin. This study suggested a new potential ethnopharmacological use of *S. persica* fruits along with the roots and stem of the plant in dental care and for other related ailments.

Data Availability

All the data related to the study are available through the corresponding author and can be provided upon request.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

Acknowledgments

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