Characterization of Antimicrobial Metabolites Produced by Salvadora persica and Analysis of Its Chemical Compounds Using GC-MS and FTIR

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ABSTRACT

Meswak (Salvadora persica) is one of the most commonly used medicinal plants for oral hygiene among global Muslim community. The objectives of this study were analysis of the secondary metabolite products using GC-MS and FTIR and evaluation of antimicrobial activity. The FTIR analysis proved the presence of alkyl halides, alkanes, and alkenes which shows major peaks at 960.55, 1029.99, 1097.50, 1141.86, 1321.24, 1373.32, 1723.65, 2852.72, and 2922.16. Our research recorded maximum zone formation was against Staphylococcus aureus (6.635±0.25). Salvadora persica was very highly active against Aspergillus terreus 6.77±0.24. Twenty-one bioactive compounds were identified in the methanolic extract of Salvadora persica. GC-MS analysis of Salvadora persica revealed the existence of the 5α-Androstan-16-one, cyclic ethylene mercaptole, Glycerin, 3,5-Dithiahexanol, 5,5-dioxide, 1H-Pyrazole-1-carbothioamide, 3,5-dimethyl, 17-Octadecynoic acid, Benzenepropanoic acid, α-(hydroxyimino)-Indan-1,2-dione, 4-methyl-, Butanoic acid, 4,4′-dithiobis[2-amino-, [S-(R*,R*)]-, 5-Hydroxymethylfurural, 1H-Pyrrole, 1-(phenylmethyl)-, Cyclopentanone, 2-cyclopentylidene-, Phenol, 2-methoxy-6-(1-propenyl)-, 2,7-Diphenyl-1,6-dioxopyridazino[4,5:2′,3′]pyrrolo[4′,5′-d]pyrida, 2-(4-(But-2-yl)phenyl)propanoic acid, 3H-Cyclodeca[b]furan-2-one, 4,9-dihydroxy-6-methyl-3,10-dim, Phenol, 2,6-dimethoxy-4-(2-propenyl)-, Butylaldehyde, 4-benzyloxy-4-[2,2,4-dioxolanyl]-, Benadryl, 9-Hexadecenoic acid, i-Propyl 11,12-dimethylene-ocadecanoate and 2,6-Bis[2-[2-S-thiosulfuroethylamino]-ethoxy]pyrazine.

Keywords: GC-MS, FT-IR, Salvadora persica, Pseudomonas aerogenosa, Proteus mirabilis, Staph. aureus, Klebsiella pneumonia

INTRODUCTION

Salvadora persica has antiurolithiatic properties. Used for centuries as a natural toothbrush, its fibrous branches have been promoted by the World Health Organization for oral hygiene use1-6. Research suggests that it contains a number of medically beneficial properties including abrasives, antiseptics, astringent, detergents, enzyme inhibitors, and fluoride. Previous studies have reported that S. persica extracts were effective against Streptococcus mutans and Streptococcus faecalis, even using low extract concentrations. Plaque is found preferentially at protected and stagnant surfaces, and these are at the greatest risk of disease7-15. Moreover, the attachment, growth, removal and reattachment of bacteria to the tooth surface are a continuous and dynamic process. Dental plaque, biofilms of microorganisms on tooth surface, plays an important role in the development of caries and periodontal disease16-23. Plaque is found preferentially at protected and stagnant surfaces, and these are at the greatest risk of disease. It was established that mutants group of Streptococci are the key agents

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causing dental caries\textsuperscript{24-29}. According to studies, mutans Streptococci can colonize the tooth surface and initiate plaque formation by their ability to synthesize extracellular polysaccharides from sucrose, using glucosyl transferase. This sucrose dependent adherence and accumulation of cariogenic streptococci is critical to the development of pathogenic plaque\textsuperscript{30-33}. Some types of interactions are thought to be of primary importance in the colonization of the periodontal environment. The further accumulation of plaque around the gingival and subgingival region may lead to a shift in its microbial composition from Streptococcus-dominated to a larger number of Actinomyces spp., and an increased number of capnophilic and obligatory anaerobic bacteria, such as Porphyromonas gingivalis. Several in vitro studies have indicated that Salvadora persica contains substances that possess dental plaque inhibiting properties against oral microbes\textsuperscript{34-38}. The fresh leaves can be eaten as part of a salad and are used in traditional medicine for cough, asthma, scurvy, rheumatism, piles and other diseases. The flowers are small and fragrant and are used as a stimulant and are mildly purgative\textsuperscript{39-42}. The berries are small and barely noticeable; they are eaten both fresh and dried. The aims of our research were analysis of the secondary metabolite products and determination of antibacterial and antifungal activity.

### MATERIAL AND METHOD

Gas chromatography–Mass Spectrum and Fourier transform infrared spectrophotometer analysis: Salvadora persica GC–MS analysis were carried out in a GC system (Agilent 7890A series, USA). The flow rate of the carrier gas, helium (He) was set to 1 mL min\(^{-1}\), split ratio was 1:50. Interpretation of mass spectrum was conducted using the database of National Institute of Standards and Technology (NIST, USA). The powdered sample of Salvadora persica was treated for FTIR spectroscopy (Shimadzu, IR Affinity 1, Japan)\textsuperscript{43,44}. The sample was run at infrared region between 400 nm and 4000 nm. Results of the study were based on analysis of variance (ANOVA) using Statistica Software. A significance level of 0.05 was used for all statistical tests.

Antibacterial and antifungal activity of natural compounds of Salvadora persica: The test pathogenic bacteria were swabbed in Müller-Hinton. Seventy \(\mu\)L of plant extract was loaded on the bored wells. Antifungal activity was evaluated by measuring the zone of inhibition against the test fungi. Methanol was used as solvent control. Amphotericin B and fluconazole were used as reference antifungal agent. The tests were carried out in triplicate. The antifungal activity was evaluated by measuring the inhibition-zone diameter observed after 48 h of incubation\textsuperscript{45-47}.

### Table 1: Major phytochemical compounds identified in methanolic extract of Salvadora persica

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Phytochemical compound</th>
<th>RT (min)</th>
<th>Molecular Weight</th>
<th>Exact Mass</th>
<th>Pharmacological actions</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>5α-Androstan-16-one , cyclic ethylene mercaptole</td>
<td>3.264</td>
<td>350</td>
<td>350.210194</td>
<td>antimicrobial activity</td>
</tr>
<tr>
<td>2.</td>
<td>Glycerin</td>
<td>3.310</td>
<td>92</td>
<td>92.047344</td>
<td>anti-inflammatory</td>
</tr>
<tr>
<td>3.</td>
<td>3,5-Dithiahexanol 5,5-dioxide</td>
<td>3.379</td>
<td>170</td>
<td>170.007136</td>
<td>anti-tumour activity</td>
</tr>
<tr>
<td>4.</td>
<td>1H-Pyrazole-1-carbothioamide ,3,5-dimethyl-</td>
<td>3.625</td>
<td>155</td>
<td>155.051719</td>
<td>anti-inflammatory</td>
</tr>
<tr>
<td>5.</td>
<td>17-Octadecenoic acid</td>
<td>3.768</td>
<td>280</td>
<td>280.24023</td>
<td>anti-diarrheal</td>
</tr>
<tr>
<td>6.</td>
<td>Benzenepropanoic acid , α-(hydroxyimino)-</td>
<td>4.975</td>
<td>179</td>
<td>179.058243</td>
<td>antimicrobial activity</td>
</tr>
<tr>
<td>7.</td>
<td>Indan-1,2-dione , 4-methyl-</td>
<td>5.353</td>
<td>160</td>
<td>160.052429</td>
<td>antimicrobial, antitumor, anti-inflammatory, antiviral</td>
</tr>
<tr>
<td>9.</td>
<td>5-Hydroxymethylfurfural</td>
<td>6.366</td>
<td>126</td>
<td>126.031694</td>
<td>Anti-inflammatory properties</td>
</tr>
<tr>
<td>10.</td>
<td>1H-Pyrrole , 1-(phenylmethyl)-</td>
<td>7.424</td>
<td>157</td>
<td>157.089149</td>
<td>anti-Candida activities</td>
</tr>
</tbody>
</table>
**RESULTS AND DISCUSSION**

**Identification of biochemical compounds:** Analysis of compounds was carried out in methanolic extract of *Salvadora persica*, shown in Table 1. Chromatogram GC-MS analysis of the methanol extract of *Salvadora persica* showed the presence of thirty one major peaks and the components corresponding to the peaks were determined as follows. All peaks were determined to be 5α-Androstan-16-one, cyclic ethylene mercaptole, Glycerin, 3,5-Dithiahexanol 5,5-dioxide, 1H-Pyrazole-1-carbothioamide, 3,5-dimethyl-1H-Pyrrole, 1-(phenylmethyl)-, 1H-Pyrrrole, 1-(phenylmethyl)-, Cyclopentanone, 2-cyclopentylidene-2-methoxy-6-(1-propenyl)-, 2,7-Diphenyl-1,6-dioxopyridazino[4,5:2’,3’]pyrrolo[4’,5’,d]pyrida, 2-(4-(But-2-yl)phenyl)propanoic acid, 3H-Cyclodeca[b]furan-2-one, 4,9-dihydroxy-6-methyl-3,10-dim-2,6-dimethoxy-4-(2-propenyl)-, Butylaldehyde, 4-benzyloxy-4-[2,2,4-dimethyl-4-dioxolanyl]-, Benadryl, 9-Hexadecenoic acid, i-Propyl 11,12-methylene-octadecanoate and 2,6-Bis[2-[2-S-thiosulfuroethylamino ] ethoxy]pyrazine. The FTIR analysis of *Salvadora persica* leaves proved the presence of alkyl halides, alkanes, and alkenes which shows major peaks at 960.55, 1029.99, 1097.50, 1141.86, 1321.24, 1373.32, 1616.35, 1723.65, 2852.72 and 2922.16. In the current study, the anti-microbial activity of *Salvadora persica* methanolic extract was determined by zone of inhibition against many bacteria, fungi and yeast. Clinical pathogens *Klebsiella pneumonia*, *Escherichia coli*, *Staphylococcus aureus*, *Salmonella typhi*, *Bacillus subtilis*, *Streptococcus faecalis*, *Proteus mirabilis* and *Streptococcus pyogenes* were selected for antibacterial activity. Our research found maximum zone formation was against *Staphylococcus aureus* (6.635 ± 0.25). Antifungal activities against *Aspergillus niger*, *Penicillium expansum*, *Trichoderma viride*, *Saccharomyces cerevisiae*, and *Microsporum canis*. *Salvadora persica* recorded high antifungal activity against *Aspergillus terreus* (6.77±0.24). Medicinal properties of *Salvadora persica* extract is due to presence of bioactive metabolites. Twenty one phytoconstituents were identified by Gas chromatography – Mass Spectrum and Fourier transform infrared spectrophotometer. This plant derived bioactive compounds used as source of antibiotic properties and pharmaceutical industries used for drug formulation.
CONCLUSION

Twenty one bioactive chemical compounds were identified by GC-MS and FT-IR analysis. This Salvadora persica derived bioactive compounds used as source of antibiotic properties and pharmaceutical industries used for drug formulation.

Financial Disclosure: There is no financial disclosure.

Conflict of Interest: None to declare.

Ethical Clearance: In this study, all experimental protocols were approved under the Department of Biology, College of Science for women, University of Babylon, Hillah city, Iraq and all experiments were carried out in accordance with approved guidelines.

REFERENCES


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