

**Original article:**

**A comparative study of four indigenous medicinal plants of Pakistan against some oral pathogens**

Hina Imran<sup>1</sup>, Tehmina Sohail<sup>2</sup>, Atiq-ur-Rahman<sup>3</sup>, Wasif Iqbal<sup>4</sup>, Nudrat Fatima<sup>5</sup>, Maria Shakir<sup>6</sup>

**Abstract**

**Background:** This study is carried out with an objective to comparative investigation of antibacterial and antifungal potentials of four medicinal plants of Pakistan. The ethanol extract of *Aloe vera* (gel), *Azadirachta indica* (bark), *Salvadora persica* (stem) and *Syzygium aromaticum* (oil) were used against *Streptococcus salivarius*, *Streptococcus pyogenes*, *Streptococcus mutans*, *Streptococcus uberis*, *Streptococcus pneumoniae* and *Candida albican*. **Method:** Antimicrobial activity was evaluated by agar well diffusion assay at 20, 10 and 5mg/ml concentration of test samples. Ampicillin is used as standard drug. The sensitivity of microorganisms to all selected parts of four plants were compared with each other and with standard antibiotics. All plant extracts showed increase in antimicrobial activity with increase in concentration. **Result:** Among all extracts the extract of *A. vera* present maximum antimicrobial activity in dose dependent manner with highest zone of inhibition of 29.1±3.2 at 20mg/ml conc. while *S. persica* exhibited minimum antimicrobial activity with zone of inhibition 17.5±2.5 at 20mg/ml conc. **Conclusion:** The present study suggested that these medicinal plants contain certain compounds which can be used to discover bioactive natural products to cure common dental problems, which are safe and economical.

**Keywords:** *A. vera* (gel); *A. indica* (bark); *S. persica* (stem); *S. aromaticum* (oil); antibacterial activity; antifungal activity

Bangladesh Journal of Medical Science Vol. 19 No. 02 April'20. Page : 284-290

DOI: <https://doi.org/10.3329/bjms.v19i2.45009>

**Introduction**

Good oral health does not just mean you have pretty teeth. Our whole mouth needs care, good health and good oral hygiene. Good oral health is reflection of overall health. Although poor oral hygiene is not a life threatening condition but unfortunately it is directly associated with certain life threatening diseases<sup>1,2</sup>. The main causative factor of poor oral hygiene is dental plaque. Dental plaque is a sticky colorless soft deposit (microbial biofilm) that forms

naturally over tooth surface or other hard surfaces in the oral cavity (removable & fixed restorations) soon after tooth eruption or tooth cleaning<sup>3</sup>. Herbal products have recently undergone more thorough investigation for their potential in preventing oral diseases, predominantly plaque-related diseases<sup>4</sup>. The use of and search for drugs and dietary supplements derived from plants have accelerated in recent years<sup>5,6</sup>. Therefore, there is a need to develop alternative antimicrobial medicines from natural

1. Dr. Hina Imran, Senior Medica Officer, PCSIR Labs Complex Karachi, Pakistan  
Email: [dr\\_hinaimran@yahoo.com](mailto:dr_hinaimran@yahoo.com)
2. Dr. Tehmina Sohail, Senior Scientific Officer, Pharmaceutical Research Centre PCSIR Labs Complex Karachi, Pakistan
3. Dr. Atiq ur Rehman, Principal Scientific Officer, Pharmaceutical Research Centre PCSIR Labs Complex Karachi, Pakistan.
4. Dr. Wasif Iqbal, Ishratul Ebad Khan Institute of Oral Health Sciences, Dow University of Health Sciences, Karachi, Pakistan
5. Dr. Nudrat Fatima, Senior Scientific Officer, Pharmaceutical Research Centre PCSIR Labs Complex Karachi, Pakistan
6. Dr. Maria Shakir, Registrar, United Medical and Dental College Karachi, Pakistan

**Correspondence to:** Dr. Hina Imran, Senior Medica Officer, Pharmaceutical Research Centre PCSIR Labs Complex Karachi, Pakistan. E-mail address is [dr\\_hinaimran@yahoo.com](mailto:dr_hinaimran@yahoo.com)

sources for the treatment of infectious diseases. A study<sup>7</sup> reported that the majority of research studies have been carried out on skin, respiratory and urinary system microorganisms and there has been little research about oral pathogens. Therefore we decided to work on *A. vera*, *A. indica*, *S. persica* and *S. aromaticum* against some oral bacterial and fungal pathogens. The reason behind the selection of these plants was as they were locally available and widely use to cure dental problems in folk medicines.

## Material and Method

### Plant Material

The plant material *A. vera* (gel), *A. indica* (bark), *S. persica* (stem) and *S. aromaticum* (oil) (Avonchem), were purchased from local market of Karachi, Pakistan, and identified by plant taxonomist. Voucher specimens were deposited in the herbarium of Department of Pharmacognosy, University of Karachi (specimen number: AV-03-0708/2008 (*A. vera* gel); AI-04-1109/2008 (*A. indica* bark); SP-05-1010/2008 (*S. persica* stem); SA-06-1110/2008 (*S. aromaticum* oil). Each plant sample (*A. vera*, *A. indica*, *S. persica*) was washed and dried under shaded area cut into small pieces and then finally grinded separately to make coarse powder. The extract of each plant was prepared by mixing 2kg plant powder and 8L ethanol in dry screw capped bottle for 6 weeks then filtered and the solvent was recovered from rotary evaporator. The recovered solvent was again used to soak the residual plant material. The process was repeated three times. The extracts obtained were capped in screw tight bottles for further studies<sup>8</sup>.

### Microorganisms

Standard strain ATCC cultures of *S. salivarius* (ATCC 13419), *S. pyogenes* (ATCC 12384), *S. mutans* (ATCC 25175), *S. uberis* (ATCC 9927) and *S. pneumoniae* (ATCC 27336) were purchased from Musaji Adam & Sons, Pakistan. Fungal strains of *C. albicans* were obtained from PCSIR Labs Karachi, Pakistan.

### Antibacterial activity

Antibacterial activity was carried out by Agar plate diffusion method using 0.5ml of the inoculums containing  $10^8$  bacterial cells respectively. The inoculum was thoroughly mixed with 20ml of molten sterile blood agar media and poured into pre-sterilized Petri dishes. All plates were left to set at 37°C for 30 – 40 minutes. Holes of 6 mm diameter were made in the center of each seeded plates by using a sterilized metallic borer. Different dilutions of the plant extracts prepared in DMSO in the order of 20mg/ml, 10mg/ml and 5mg/ml respectively.

Ampicillin (50mg/ml) was also prepared along side, which served as a positive control. Exactly 0.02ml of each concentration was introduced into each hole with the help of micropipette and was allowed to stand on the bench for about one hour for proper diffusion. It was thereafter incubated at 37°C for 24hrs. The sensitive bacteria grew everywhere except in areas around the holes in the medium. Then, after 24h of incubation the resulting inhibition zones obtained were measured by digital Vernier caliper (in millimeters) and the results were recorded against the corresponding concentrations. All the plates run in triplicate<sup>9</sup>.

### Antifungal Assay

The antifungal activity of test material was determined by the same method<sup>9</sup> and instead of blood agar, Sabouraud dextrose agar was used. The Petri plates of SDA were prepared and 0.1 ml of diluted culture of fungal strain was poured on each plate. The plates were dried for 30 minutes at 37C for 30 – 40 minutes. Holes of 6mm diameter were made in the center of each seeded plates. Different dilutions of the plant extracts prepared in DMSO in the order of 20mg/ml, 10mg/ml and 5mg/ml. Ampicillin (50mg/ml) served as a positive control. Exactly 0.02ml of each concentration was introduced into each hole on the medium and was allowed to stand on the bench for about one hour for proper diffusion. It was thereafter incubated at 37°C for 7 days. The resulting inhibition zones obtained were measured in millimeters and recorded against the corresponding concentrations.

**Ethical clearance:** This research study obtained ethical approval from the ethics Committee of the Pharmaceutical Research Centre PCSIR Labs Complex Karachi, Pakistan.

### Results

All test drugs showed varying degrees of antimicrobial activities. The results of present study revealed that most potent extract against all test microorganisms was *A. vera* gel extract. It showed maximum zone of inhibition  $29.1 \pm 3.2$  mm at 20mg/ml conc. followed by  $27.3 \pm 3.41$ mm and  $25.3 \pm 3.4$ mm zone of inhibition at 10 and 5mg/ml conc. respectively. *A. indica* exhibited maximum antimicrobial activity at 20mg/ml conc. by showing inhibition zone of  $29.1 \pm 1.4$ mm but at lower conc. (10, 5mg/ml) it exhibited less activity  $25.8 \pm 2.1$  and  $22.7 \pm 1.96$ mm inhibition zones. *S. aromaticum* showed  $26.2 \pm 3.3$ ,  $23.0 \pm 2.8$  and  $20.58 \pm 3.26$ mm zone at 20, 10 and 5mg/ml conc. respectively. *S. persica* displayed least antimicrobial activity as compared to other test drugs by showing  $17.5 \pm 2.5$ ,  $14.5 \pm 1.66$  and  $13.1 \pm 0.80$ mm zone at 20,

10 and 5mg/ml conc. respectively (Table: I, II, III; Graph: I, II, III).

### **Discussion**

Worldwide major cause of morbidity and mortality are infectious disease. Oral infectious diseases fall a great impact on general health. Dental plaque is major causative factor involve in dental problems. Plaque is a natural sticky biofilm contain numerous microorganisms. Daily two times teeth cleaning not only inhibit plaque formation to improve oral hygiene but also prevent initiation of infectious diseases like gingivitis, periodontitis, halitosis, dental caries etc. Dental plaque cannot be removed by rinsing alone, daily brushing and flossing is needed to remove dental plaque<sup>10</sup>. Generally 0.2% chlorhexidine is considered as best plaque removal solution but now many side effects are reported with the use of chlorhexidine<sup>11</sup>. Hence a natural alternative is safe, economical and feasible. Herbal medicine is both promotive and preventive in its approach<sup>12</sup>. Numerous medicinal plants extracts or phytochemicals have been shown to inhibit the formation of dental biofilms by reducing the adhesion of microbial pathogens to the tooth surface, which is a primary event in the initiation and the progression to dental decay<sup>13,5</sup>. Different dilutions of plant extracts were made by dissolving in DMSO and antibacterial and antifungal activities were conducted. DMSO is a highly polar solvent, which is capable of dissolving both polar and nonpolar components. It has a proven safety record in humans, is an effective cell penetration enhancer and helps to bring out the properties of all the components of the herb being dissolved. DMSO showed no antibacterial activity in the agar diffusion test<sup>14</sup>. It was observed by this study that all plants possess antimicrobial activities against test microorganisms. The antimicrobial activity was increased with increasing concentrations. However, the antibacterial activity observed by the extract was low when compared with that of the standard. The results of this current study indicated that extract of *A. vera* gel produced comparatively largest zones of inhibition for all selected bacterial and fungal pathogens as compared to other plants. It was observed that *A. vera* and *A. indica* exhibited almost equal antimicrobial activity at 20mg/ml conc. (29.1±3.2, 29.1±1.4mm zone inhibition) but overall results revealed that *A. vera* showed most significant antimicrobial activity by showing maximum inhibition zone 29.1±3.2 mm, 27.3±3.41mm and 25.3±3.4mm zone of inhibition at 20, 10 and 5mg/ml conc. respectively. The extract of *S. persica* displayed least antimicrobial activity as compared to

other plant extracts by showing maximum inhibition zone 17.5±2.5mm at 20mg/ml while 13.1±0.80mm inhibition zone and at 5mg/ml conc. (Table: I, II, III; Graph: I, II, III). The present study revealed the presence of medicinally active constituents such as alkaloids, sulfur compounds, tannins, saponins, cyanoglycosides, nitrate, terpenoids, oleic and stearic acids, salicylic acid enhance antimicrobial activity of medicinal plants<sup>15,16,17</sup>. The presence of these natural chemicals in selected plants gives conferment of their antimicrobial activities<sup>18,19</sup>. A scientific document<sup>20</sup> reported that Neem could reduce the ability of Streptococcal bacteria to colonize on the surface of teeth. Xavier and Vijayalakshmi<sup>21</sup> reported that ethanol extracts of *Allium sativum* bulb and *A. indica* leaf extracts exhibited high level of inhibition effect on *S. mutans*. Some more studies<sup>22,23</sup> on *A. indica* reveals its antimicrobial activity even at minimum conc. (5% and 1 w/v %). All these reports support our results that *A. indica* bark at low conc. exhibited antimicrobial activity. Wynn<sup>24</sup> reported that antimicrobial activity of *A. vera* is due to the presence of anthraquinones, aloe emodin, aloetic acid, aloin, anthracene, anthranol, barbalion, chrysophanic acid, cinnamonic acid, isobarbaloin and resistannol. They inhibit protein synthesis by bacterial cells and has shown the inhibitory effects on some periodontopathic, cariogenic and opportunistic pathogenic bacteria<sup>25,26</sup>. *S. persica* showed least and dose dependent antimicrobial activity. Almas and Al-Bagieh<sup>27</sup> reported that *S. persica* pulp, bark and whole plant showed antimicrobial activity at higher conc. (10 and 50%) not at lower conc. (1%). In another research study it was reported that sulfated compounds and isothiocyanate present in *S. persica* are responsible for antibacterial effects<sup>28</sup>. Jajarm<sup>29</sup> reported that *S. persica* mouthwash cannot alter oral microbes especially *Streptococcus mutans*. Our results also did not show activity of *S. persica* against *S. mutans* and *S.pneumoniae* (Table: I, II, III; Graph: I, II, III). Eugenol and caryo-phyllene present in *S. aromaticum* are known to possess antibacterial and antifungal properties. Aneja and Joshi<sup>30</sup> reported that *S. aromaticum* oil exhibited significant activity at 50% conc. against *S. mutans* and our studies also exhibited same results on this organism in dose dependent ratio.

### **Conclusion**

The results obtained from this study confirm the traditional approach of the antimicrobial effectiveness and therapeutic applications of the examined plants. To the best of knowledge, as few studies have been

conducted on antimicrobial effects of medicinal plants against oral pathogens it is better that the effect of herbal extracts on other oral bacteria that have cariogenic activity be studied. It is suggested that more research should be carried out to find plants with antimicrobial activity for producing herbal oral care formulations, which can have comparable preventive potential to the synthetic agents and can be alternative for people who wish to avoid alcohol, artificial preservatives, artificial flavors and colors.

**Conflict of interest:** None

**Acknowledgement**

We would like to pay our gratitude and respects to Late Prof. Dr Mansoor Ahmed (my supervisor). He was a dedicated professor in the Department of

Pharmacognosy at University of Karachi Pakistan. He was honored with numerous awards and recognitions for both his teaching and his research. May Allah rest his soul in peace and give strength to his family.

**Authors' contribution:**

Data gathering and idea owner of this study: Imran H, Sohail T, Rahman A, Iqbal W

Study design: Imran H, Sohail T, Rahman A, Iqbal W, Fatima N, Shakir M

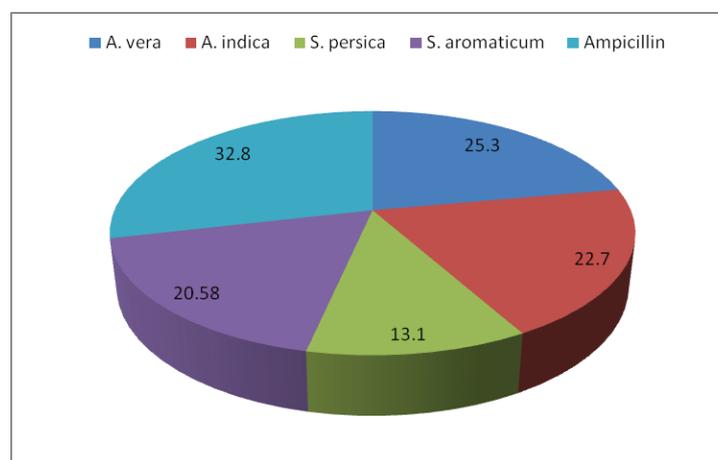
Data gathering: Imran H, Sohail T, Rahman A, Iqbal W, Fatima N, Shakir M

Writing and submitting manuscript: Imran H, Sohail T, Rahman A

Editing and approval of final draft: Imran H, Sohail T, Rahman A, Iqbal W, Fatima N, Shakir M

**Table I: Antimicrobial activity of *A. vera*, *A. indica*, *S. persica* and *S. aromaticum* at 5mg/ml conc.**

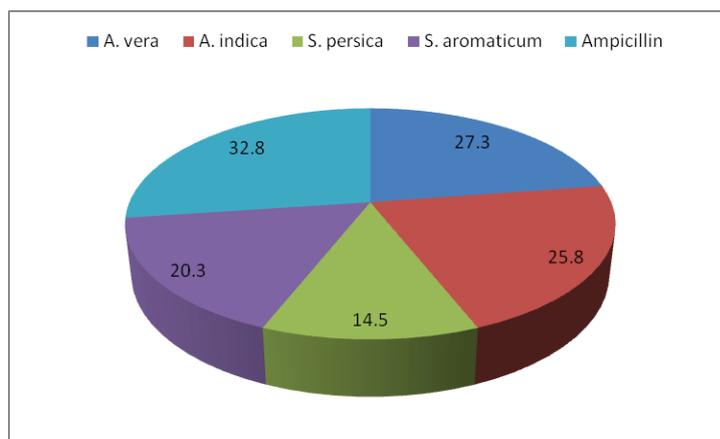
Test organisms	Mean zone of inhibition in mm and standard deviation (SD)					
	<i>A. vera</i> Mean±SD	<i>A. indica</i> Mean±SD	<i>S. persica</i> Mean±SD	<i>S. aromaticum</i> Mean±SD	Ampicillin Mean±SD	Negative control (DMSO)
<i>S.salivarius</i>	26.8±1.1	22.2±0.34	13±0.2	25.4±0.35	34±0.17	-ve
<i>S.pyogenes</i>	19±0.3	20±0.30	--	17.4±0.50	33±0.2	-ve
<i>S.mutans</i>	24.5±0.5	21.1±0.36	--	17.2±0.25	35±0.2	-ve
<i>S.uberis</i>	28±0.5	25.1±0.56	12.5±0.7	23.4±0.40	32.8±0.26	-ve
<i>S.pneumoniae</i>	25.2±0.4	24±0.75	--	20.1±1.25	32.5±0.45	-ve
<i>C.albicans</i>	28.5±0.79	24±0.70	14±0.35	20±1	29.5±0.36	-ve
Average	25.3±3.4	22.7±1.96	13.1±0.80	20.58±3.26	32.8±1.86	-ve



Graph I: Shows average zone of inhibition (mm) against various microorganisms at 5mg/ml conc.

**Table II: Antimicrobial activity of *A. vera*, *A. indica*, *S. persica* and *S. aromaticum* at 10 mg/ml conc.**

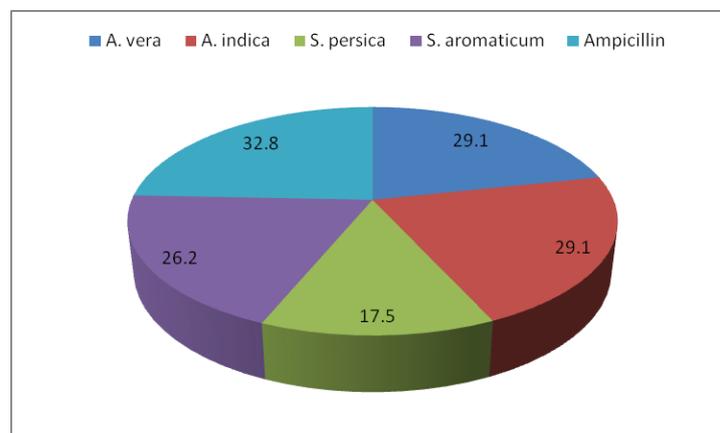
Test organisms	Mean zone of inhibition in mm and standard deviation (SD)					
	<i>A. vera</i> Mean±SD	<i>A. indica</i> Mean±SD	<i>S. persica</i> Mean±SD	<i>S. aromaticum</i> Mean±SD	Ampicillin Mean±SD	Negative control (DMSO)
<i>S.salivarius</i>	28.5±0.43	25±1.10	13.3±0.40	27.4±0.89	34±0.17	-ve
<i>S.pyogenes</i>	21±0.3	25.1±0.26	13±0.5	20±0.2	33±0.2	-ve
<i>S.mutans</i>	28.2±0.26	22.2±0.49	--	20.8±0.32	35±0.2	-ve
<i>S.uberis</i>	30.5±0.75	27±0.70	16.5±0.50	25.6±0.28	32.8±0.26	-ve
<i>S.pneumoniae</i>	26.2±0.88	28±0.40	--	22.3±0.28	32.5±0.45	-ve
<i>C.albicans</i>	29.5±0.65	27±0.75	15.3±0.43	22.1±0.96	29.5±0.36	-ve
Average	27.3±3.41	25.8±2.1	14.5±1.66	23.0±2.8	32.8±1.86	-ve



Graph II: Shows average zone of inhibition (mm) against various microorganisms at 10mg/ml conc.

**Table III: Antimicrobial activity of *A. vera*, *A. indica*, *S. persica* and *S. aromaticum* at 20 mg/ml conc.**

Test organisms	Mean zone of inhibition in mm and standard deviation (SD)					
	<i>A. vera</i> Mean±SD	<i>A. indica</i> Mean±SD	<i>S. persica</i> Mean±SD	<i>S. aromaticum</i> Mean±SD	Ampicillin Mean±SD	Negative control (DMSO)
<i>S.salivarius</i>	29.83±0.25	30.1±0.32	15±1	31.4±0.41	34±0.17	-ve
<i>S.pyogenes</i>	24±0.2	27±0.6	16.5±0.7	23.1±0.28	33±0.2	-ve
<i>S.mutans</i>	29.5±0.45	27.5±0.20	--	22.2±0.7	35±0.2	-ve
<i>S.uberis</i>	33.5±0.36	30±0.30	21.1±0.23	27.5±0.45	32.8±0.26	-ve
<i>S.pneumoniae</i>	27.2±1.7	30.2±0.32	--	26.5±0.51	32.5±0.45	-ve
<i>C.albicans</i>	31±0.2	29.8±0.50	17.5±0.45	27±0.5	29.5±0.36	-ve
Average	29.1±3.2	29.1±1.4	17.5±2.5	26.2±3.3	32.8±1.86	-ve



Graph III: Shows average zone of inhibition (mm) against various microorganisms at 20mg/ml conc.

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