Antimicrobial Effects of some Plants on Bacteria Isolated from Oral Halitosis Patients

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Aims: The purpose of this study was to evaluate the antimicrobial activities of three herbs (Salvadora persica, Nigella sativa, and Elettaria cardamomum) by using three different methods of extractions (aqueous, ethanolic, and Soxhlet apparatus technique). Materials and methods: Twenty eight subjects suffered from oral halitosis their ages range(18-65) years. Fifty eight samples were collected by sterile paper points(size 50) or sterile cotton swab and transported in thioglycolate broth and cultured on blood agar in aerobic or anaerobic conditions for 48-72 hours. The herbal extracts were qualitatively examined against thirteen microbial strains, zones of growth of inhibition were measured as indicators of anti-microbial activity compared to chlorohexidin gluconate 0.2%(as control). Results: Thirteen microbial species were isolated in this study: (Bacteriodes species, Viridans Streptococci, Peptostreptococci spp., Actinomyces spp., Porphyromonas spp., Fusobacterium spp., Veillonella spp., Non-coagulase Staphylococcus, Prevotella spp., Propionibacterium spp., Tetragnococcii spp., Eubacterium spp., and Staphylococcus aureus). Ethanolic extraction of S. persica exhibited notable antimicrobial activities against most of the tested strains(85% to the control), N. sativa was (6.3% of the control) and E cardamomum was (22.9% of the control). Aqueous extraction of S. persica was (13.5% of the control), N. sativa was (about 0% of the control) and E cardamomum was (6.8% of the control). Soxhlet apparatus extraction method of S. persica was (14% of the control), N. sativa was (8.5% of the control), and E. cardamomum was (12.6% of the control). Conclusion: Ethanolic extraction of S. persica has the first inhibitory effect compared to the control in the species of Peptostreptococci, Actinomyces, and Staphylococcus aureus. So, S persica can be considered as an effective antimicrobial agent in inhibiting the growth of oral halitosis including pathogens, while neither E. cardamomum nor N. sativa can be considered as effective antimicrobial agents in inhibiting the growth of oral bacteria causing halitosis.

Key words: halitosis, medicinal plants, soxhlet apparatus, ethanolic, aqueous.

ABSTRACT

INTRODUCTION

Halitosis, fetor oris, oral malodor or bad breath are the general terms used to describe unpleasant breath emitted from a person's mouth regardless of whether the odorous substances in the breath originate from oral or non-oral sources. The prevalence of halitosis has been reported to be as high as 50%. However, only a few patients visit dental clinicians to seek help for halitosis. Oral malodor has a complex etiology with extrinsic and intrinsic pathways. Extrinsic causes include tobacco, alcohol and certain foods such as onions, garlic and certain spices. Substances absorbed into the circulatory system may be released in pulmonary air or saliva as volatile odoriferous compounds derived from foods. Intrinscic causes of bad breath are oral and systemic in origin.

Reviews in research reports now agree, in the vast majority of cases, halitosis (80 to 90%) originates within the oral cavity, where anaerobic bacteria degrade sulphur-containing amino acids to the foul smelling volatile sulphur compounds (VSC), namely hydrogen sulphide and methyl mercaptan. Halitosis of oral origin is associated with poor oral hygiene, dental plaque, dental caries, gingivitis, stomatitis, periodontitis, tongue coating, and oral carcinoma. Dry mouth (xerostomia) may also promote oral malodor, although a correlation is not always observed.

Although the dorsum of the tongue seems to harbor one of the most complex microbiological niches in human ecology, knowledge of the role of tongue flora in health and disease is also very limited. The papillary structure of the dorsum represents a unique ecological niche in the oral cavity, offering a large surface area that favors the accumulation of oral debris and microorganisms that aggravates halitosis. Despite the availability of a wide range of antimicrobial agents for clinical use, development of new anti-microbial agents remains important and many studies have been aiming at the discovery and development of new antimicrobial agents. Salvadorapersica, is a medical plant whose roots, twigs or stems have been used for centuries as oral hygiene tools in many parts of the world particularly Saudia Arabia. Many studies have demonstrated that extracts of S. persica possess various anti plaque, anti periopathic, anticaries, antiinflammatory and antymycotic effects. Nigella Sativa seeds (Black seeds) have been employed for thousands of years as spice and food preservatives and found to have medical properties in traditional medicine especially antibacterial action. Its black seeds referred to by the Prophet Mo-hammed (peace be upon him) as having healing power.

This study was to evaluate the antimicrobial activity of the different herb extracts on the bacteria isolated from patients suffering from halitosis.

MATERIALS AND METHODS

Two thousands grams of Siwak sticks available in Iraqi markets as (Muslim siwak, Suadi Arabia), 750 g of Cardamom, 750 g of Black seeds available in Iraqi markets in herbal stores and 50 ml of Chlorhexidine gluconate mouthwash 0.2% (Al-Mansour, Iraq) were used. Twenty eight adult patients (14 male and 14 females, their ages between 18-65 years) attended the Dental educational hospital, oral diagnosis sector, college of dentistry at Mosul University were enrolled in the study. All patients suffered from halitosis besides chief complains. Patients, who received antibiotic during the last two weeks, eat any meal that generates strong odors on the previous day or in the morning of the test, smoked within an hour before test, chewed tasteful gum, wore scented personal-care products, brushed or rinsed with strong odorous compounds, were excluded.

Dental examination was performed on the dental chair at the oral diagnosis sector under artificial light. When careful clinical examination was ended, the examiner had to prospect the origin of halitosis; deep pockets, heavy calculus, large destructive carious tooth, and retained root. After isolation with cotton rolls, single sterile point size 50 was inserted for 30 seconds in the prospect-ed site by using sterile twizzer and placed immediately in sterile screw-capped vials containing ml of thio-glycolate broth as reducing transport media for anaerobic bacteria. The tongue was scraped several times with a sterile tongue scraper. This scraping produced thick brown fluid. Another sterile point was inserted for 30 seconds in this brown fluids and by using sterile twizzer, it was
placed immediately in another sterile screw-capped vials containing 4 ml of thio-
glycolate broth. This meant that we had two vials for each patient but in case of
good oral hygiene (healthy gingivae and sound teeth), only one vial containing
sample from tongue scrapings. The herbs (S. persica, N. sativa, and E cardamomum)
were extracted by a Soxhlet apparatus and in 95% ethanol water.

Swabs were streaked on two blood agar plates incubated aerobically and
anaerobically for 48-72 hours at 37 ºC. Colonies of different characteristics were
isolated and identified using various methods. Detection of fluorescence under
long wave UV light (360 nm) is a useful tool for rapid presumptive identification
of some anaerobic bacteria. Fresh colonies on blood agar plates were examined
under fluorescent microscope (360 nm) in a dark room. Evaluation of the anti-
microbial activity of the herb extracts and chlorhexidine gluconate mouth wash 0.2%
as control, using disc diffusion method, the diameters of zone of inhibitions were
measured using a ruler. ANOVA, Duncan multiple & Dunnetts tests were used as
statistical methods.

RESULTS AND DISCUSSION

In this study, the sample consisted of twenty eight subjects (14 males and 14
females their ages ranged (18-65) years, who suffered from oral halitosis as side
chief complain. They attended College of Dentistry at Mosul University asking for
diagnosis and treatment. Thirteen bacterial species were isolated and identified,
including Gram-positive and Gram-negative, aerobic and anaerobic. Fifty eight
bacterial samples were isolated as showed in Table 1, Bacteriodes spp., viridans
Streptococci (10), Peptostreptococci spp. (9), Actinomyces spp. (6), Porphymonas
spp. (4), Fusobacterium spp. (4), Veillonella spp. (4), Non-coagulase Staphylococcus (3)
, Prevotella spp. (1), Pro-pinobacterium Spp. (1), Tetragenococci Spp. (1),
Eubacterium spp. (1) and Staphylococcus aureus (1) isolate.
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Table (1) : Microscopical Characteristics used for Identification of the Bacteria isolates.

<table>
<thead>
<tr>
<th>Time</th>
<th>SOV</th>
<th>SS</th>
<th>df</th>
<th>MS</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Bacteriodes Species</td>
<td>13</td>
<td></td>
<td>Gram negative pleomorphic rods</td>
</tr>
<tr>
<td>2</td>
<td>Viridans Streptococci</td>
<td>10</td>
<td></td>
<td>Gram positive cocci arranged in chains or pairs &amp; can grow in MSA</td>
</tr>
<tr>
<td>3</td>
<td>Peptostreptococci Species</td>
<td>9</td>
<td></td>
<td>Gram positive large cocci arranged in chains</td>
</tr>
<tr>
<td>4</td>
<td>Actinomyces species</td>
<td>6</td>
<td></td>
<td>Gram-positive cocccbacilli &amp; beaded filamentous rods</td>
</tr>
<tr>
<td>5</td>
<td>Porphymonas Species</td>
<td>4</td>
<td></td>
<td>Gram-positive coccobacilli</td>
</tr>
<tr>
<td>6</td>
<td>Fusobacterium Species</td>
<td>4</td>
<td></td>
<td>Gram-negative, pale-staining, long spindle with pointed ends</td>
</tr>
<tr>
<td>7</td>
<td>Veillonella Species</td>
<td>4</td>
<td></td>
<td>Gram-negative small cocci</td>
</tr>
<tr>
<td>8</td>
<td>Non-Coagulase Staphylococcus</td>
<td>3</td>
<td></td>
<td>Strongly Gram-positive cocci arranged in irregular clusters</td>
</tr>
<tr>
<td>9</td>
<td>Prevotella Species</td>
<td>1</td>
<td></td>
<td>Gram-negative short rods or cocccbacilli</td>
</tr>
<tr>
<td>10</td>
<td>Propinobacterium Species</td>
<td>1</td>
<td></td>
<td>Pleomorphic, anaerobic Gram-positive rods</td>
</tr>
<tr>
<td>11</td>
<td>Tetragenococci Species</td>
<td>1</td>
<td></td>
<td>Gram-positive spherical tetrads</td>
</tr>
<tr>
<td>12</td>
<td>Eubacterium Species</td>
<td>1</td>
<td></td>
<td>Uniform or pleomorphic, Gram-positive rods</td>
</tr>
<tr>
<td>13</td>
<td>Staphylococcus Aureus</td>
<td>1</td>
<td></td>
<td>Strongly Gram-positive cocci arranged in irregular clusters</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>58</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Although the microbiology of the human oral cavity has been investigated thoroughly, the oral microbial flora has remained incompletely characterized. Most studies focused on cultivable microorganisms, which constituted only 1 to 10 percent of all microbial species. Consequently, these studies have been biased toward “what grows” and have ignored “what does not grow”\(^{(14,15)}\). An advantage of herbal medicinal plants is that they provide a complex of natural compounds to the patients which have smoother action and are better tolerated than synthetic drugs, and produce few allergic reactions\(^{(16)}\).

Figures (1, 2, 3, 4, 5, 6, 7 and 8) showed the effects of different herbs extracts on the isolates of Bacteriodes, viridans Streptococci, PeptoStreptococci, Actinomymes, Porphymonas, Fusobacterium, Veillonella, Non-coagulase Staphylococcus species, each isolate was tested twice.

**Figure (1):** Comparison Between The Effects of Different Herbs Extracted by Different Methods on Bacteriodes Species (zone of inhibition in millimeters)

C=chlorihexidane gluconate (control), 1S=cardamom extracted by Soxhelt apparatus, 1W=aqueous extract of cardamom, 1E=ethanolic extract of cardamom, 2S=Nigella sativa extracted by Soxhelt apparatus, 2W=aqueous extract of Nigella sativa, 2E=ethanolic extract of Nigella sativa, 3S=Salvadorapersica extracted by Soxhelt apparatus, 3W=aqueous extract of Salvadorapersica, &3E=ethanolic extract of Salvadorapersica.

**Figure (2):** Comparison Between The Effects of Different Herbs Extracted by Different Methods on Viridans Streptococci Species (zone of inhibition in millimeters)
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Figure (3) : Comparison Between The Effects of Different Herbs Extracted by Different Methods on PeptoStreptococci Species (zone of inhibition in millimeters)

Figure (4) : Comparison Between The Effects of Different Herbs Extracted by Different Methods on Actinomyces Species

Figure (5) : Comparison Between The Effects of Different Herbs Extracted by Different Methods on Porphymonas Species (zone of inhibition in millimeters).
Figure (6) : Comparison Between The Effects of Different Herbs Extracted by Different Methods on Fusobacterium Species

Figure (7) : Comparison Between The Effects of Different Herbs Extracted by Different Methods on Veillonella Species (zone of inhibition in millimeters)

Figure (8) : Comparison Between The Effects of Different Herbs Extracted by Different Methods on Non-coagulase Staphylococcus Species (zone of inhibition in millimeters)
The control (chlorhexidine gluconate mouthwash 2%) was the most effective agent followed by ethanolic extraction of S. persica. Chlorhexidine is a mouthwash with a broad spectrum of activity, being active against both gram +ve and gram -ve bacteria. Long-term maintenance use of chlorhexidine can cause local side effects such as calculus formation and staining of teeth, restorations, or tongue. Giannelli et al. 1999(17) suggested that chlorhexidine is highly cytotoxic in vitro and advise a more cautious use of the antiseptic in oral surgical procedures because of these undesirable side effects. There is now widespread interest in the use of medicinal plants for the maintenance oral hygiene(18). This study indicated that the ethanolic extraction of S. persica demonstrated a reasonable range of inhibitory effects on the test bacteria. Although the chlorhexidine gluconate rinse had the greatest effect, the ethanolic extraction of S. persica compared favorably as it inhibit the growth of the microorganisms safely and with less side effects. Ethanolic extraction of S. persica inhibited the growth of the species of Pepto-streptococci, Actinomyces, and Staph. aureus in a rate more effectively even by chlorhexidinegluconate 0.2%. S. persica alcoholic extract produced remarkable antibacterial activity but less than chlorhexidine. The profound antibacterial effects of S. persica is believed to be due to its high chemical contents of benzylthiocynate, nitrate, trimethylamine, chloride, tannins and sulphur. The different reactions of each strain to the various extracts indicated that each solvent extracted different chemical components of S. persica(19).

In summary, the rational explanation of the attractiveness of S. persica chewing stick as a tool for teeth cleaning is cheapness, safeness, its shape is like a brush, contains chemical constituents with variable actions. It seems to be two in one, which means it, gathers the tooth paste and tooth brush in one implement. As well as Nigella sativa seeds have many medicinal properties such as anti-bacterial, antifungal, analgesic, anti-inflammatory and immune-potentiating(20), their different extractions showed low effects against bacteria causing halitosis isolated in this study. Different crude extracts of Nigella sativa were tested for antimicrobial effectiveness against different multiple resistance bacterial isolates (16 gram negative and 6 gram positive). The crude extracts of N. sativa showed a promising effect against the tested organisms.(21)

In relation to Elettaria cardamomum, the most functionally important constituent of it is the volatile oil. The volatile oil content of seeds varies from 6.6–10.6%. The volatile oil gives its characteristic aroma and described generally as comphory, sweet, and aromatic spicy. When the spice is chewed, it does have a slight astringent and pungent taste. The astringent sensation can arise from intense release of many components of the volatile oil when seeds are chewed and or from phenolics that are usually present in seeds. However, the oil is reported to develop some off flavor when it contacts with air(23,9). For these above reasons, Elettaria cardamomum had been used from a long times to cover the bad mal-odor. In this study, cardamom had low effect against oral bacterial responsible for halitosis comparing to both control (Chlorohexidinegluconate) and Salvadorapersica extractions but had little great effect than Nigella sativa extraction. An oral anti-halitosis preparations usually design to desorb (inhibit) microorganisms and/or to absorb materials causing halitosis produced by the microorganisms. The preparations which act directly on inhibition of the microorganisms can effectively cure oral halitosis as they prevent the source of volatile sulfur compounds so no odor can be smelled. This is greatly occur with chlorohexidinegluconate 0.2% and Salvadorapersica and to a lesser extent with Nigella sativa. The preparation which acts on materials produced by the microorganisms can mask the oral halitosis temporarily for short times. Their indirect action is accomplished by converting the volatile sulfur compounds to non-volatile sulfur compounds. The absence of bad odor is not due to removal of the cause but due to the change of volatility of the sulfur compounds specially if the preparations had a strong smell. This greatly occurs with Elettaria cardamomum which has considerable antioxidant property.

REFERENCES