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

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الخلاصة

الاهداف: تهدف الدراسة الى معرفة التاثير المضاد للجراثيم لثلاثة اعشاب(السواك Salvadorapersica والحبة السوداء Nigella sativa والهيل Elettariacardamomum) باستعمال ثلاثة طرائق مختلفة للاستخلاص (الاستخلاص المائي والاستخلاص الكحولي والاستخلاص باستعمال جهازالسكسولايت). **المواد وطرائق العمل**: ثمانية وعشرون شخصا من الذين يعانون من بخر الفم (١٤ذكر)و(١٤ انثى) وتتراوح اعمارهم بين(١٨-٦٥) سنة. ثمان وخمسون عينة تم جمعها باستعمال الرؤوس الورقية المعقمة(حجم ٥٠) او مسحات قطنية معقمة ثم نقلها في مرق thioglycolate وزرعت على وسط اكار الدم في ظروف هوائية او لاهوائية لمدة(٤٨-٧٢) ساعة تشخيص الجراثيم تم بالاعتماد على الخصائص الشكلية والصفات الزرعية واختبارات سهولة التاثر بالمضادات الحيوية.تم فحص التاثير الكمي لهذه المستخلصات على اربع عشر نوع من الجراثيم الموجودة في حالة بخر الفم وتم قياس اقطار تثبيط النمو كمؤشرات للفعالية المضادة للجراثيم مقارنة بالكلوروهكسدين كلوكونيت٠,٢٪ (كضابط). **النتائج** : ضمن مستخلصات الاعشاب المختلفة المختبرة على ثلاثة عشر نوعا من الجراثيم Bacteriodes وأنواع Viridans Streptococci) و Peptostreptoco cci و Actinomycescetes و Porphymonos و FusobacteriumVeillonella و (Non-coagulase Staphylococcus و Prevotella و Propinobacterium و Tetragenococci و Eubacterium و عزلة من نوع Staphylococcus aureus. اظهرت النتائج بان المستخلص الكحولي للسواك كان له تاثيرا واضحا مضادا للجراثيم ضد معظم الجراثيم المختبرة (٨٥٪ من الضابط) .اما تاثير المستخلص الكحولي للحبة السوداء فكان (٦,٣٪ من الضابط), وتاثيرالمستخلص الكحولي للهيل كان(٢٢,٩٪ من الضابط) في حين كان تاثير كل من المستخلص المائي للسواك (١٣,٥٪ من الضابط), المستخلص المائي للحبة السوداء(٠٪ من الضابط), المستخلص المائي للهيل (٦,٨٪ من الضابط). اما تاثيرالاستخلاص بطريقة سكسولايت فللسواك كانت ١٤٪ من الضابط,وللحبة السوداء ٨,٥٪ من الضابط, وللهيل ١٢,٦٪ من الضابط.**الاستنتاج**: لقد كان للمستخلص الكحولي للسواك مقارنة بالضابط التاثير المثبط الاقوى للانواع Peptostreptococci, Actinomyces, Staphylococcus aureus. لذلك يمكن اعتبار السواك كعامل فعال مضاد للجراثيم المسببة لبخر الفم في حين لا يمكن اعتبار كل من الحبة السوداء او الهيل كمواد فعالة ضد جراثيم بخر الفم.

ABSTRACT

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 Soxhelt 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., Porphymonos spp., Fusobacterium spp., Veillonella spp., Non-coagulase Staphylococcus, Prevotella spp., Propinobacterium spp., Tetragenococci spp. ,Eubacterium spp., and Staph.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), Soxhelt 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.

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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 report-ed to be as high as 50%. However, only a few patients visit dental clinicians to seek help for halitosis[1,2] .Oral malodor has a complex etiology with extrinsic and intrinsic pathways. Extrinsic causes includetobacco, 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 odor-iferous compounds derived from foods. Intrin-sic causes of bad breath are oral and systemic in origin[3].

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 methylmercaptan. Halito-sis of oral origin is associated with poor oral hygiene, dental plaque, dental car-ies, gingivitis, stomatitis, periodontitis, tongue coating, and oral carcinoma.Dry mouth (xerostomia) may also promote oral malodor, although a correlation is not always observed[4].

Although the dorsum of the tongue seems to harbor one of the most com-plex 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 ag-gravates halitosis[5]. 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[6]. SalvadoraPersi-ca, 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 ,antinflamatory and antimycotic ef-fects[7]. Nigella Sativa seeds(Black seeds) have been employed for thou-sands of years as spice and food pre-servatives and found to have medical properties in traditional medicine spe-cially antibacterial action .Its black seeds referred to by the Prophet Mo-hammed (peace be upon him)as having healing power(8). Cardamom (Elettaria cardmommum) whose odor is highly aromatic and pleasant ,the taste is aromatic and pungent .It is reported to have virustatic properties .One of its indications and usages is for inflamma-tion of the mouth and pharynx[9].

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 Chlorhexidineglu-conate mouthwash 0.2% (Al-Mansour ,Iraq) were used .Twenty eight adult pa-tients (14male and 14 female their ages between 18-65 years) attended the Dental educational hospital, oral diagnosis sec-tor , college of dentistry at Mosul Uni-versity 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 care-ful 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 cot-ton 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[4] 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 im-mediately in another sterile screw-capped vials containing 4 ml of thioglycolate 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[10] and in 95%ethanol water[11].

Swabs were streaked on two blood agar plates incubated aerobicaly and an-aerobically for 48-72 hours at 37 ºC. Colonies of different characteristics were isolated and idenified using vari-ous methods.[12]Detection of fluorescence under long wave UV light(360nm) is a useful tool for rapid presumptive identification of some anaerobic bacte-ria.Fresh colonies on blood agar plates were examined under fluorescent mi-croscope (360 ºnm) in a dark room(13). Evaluation of the anti microbial activity of the herb extracts and chlorhexidingluconate 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. Thir-teen 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.(13), viridans Strepto-cocci(10), Peptostreptococci spp. (9), Actinomyces spp.(6), Porphymonos spp.(4), Fusobacterium spp.(4), Veil-lonella spp.(4), Non-coagulase Staphy-lococcus(3) , Prevotella spp.(1), Pro-pinobacterium Spp.(1) , Tetragenococci Spp.(1), Eubacterium spp.(1)and Staphylococcus aureus(1) isolate.

Table (1) : Microscopical Characteristics used for Identification of the Bacteria isolates.

Time	SOV	SS	df	MS
1	Bacteriodes Species	13	Gram negative pleomorphic rods	Gray-white, circular,convex, non-hemolytic
2	Viridans Streptococci	10	Gram positive cocci arranged in chains or pairs&-can grow in MSA	On MSA appear small blue colonies but on blood agar were non-hemolytic
3	Peptostreptococci Species	9	Gram positive large cocci arranged in chains	Gray-white ,opaque colonies with fetid odor
4	Actinomycesspecies	6	Gram-positive coccobacilli & beaded filamentous rods	Small ,convex gray –white colony
5	Porphymonos Species	4	Gram–positive coccobacilli	Dark brown to black &compared to Prevotella were more mucoid
6	Fusobacterium Speices	4	Gram-negative, pale-staining,long spindle with pointed ends	Gray-white colonies fluoresces under UV light(hemolytic or non)
7	Veillonella Species	4	Gram-negative small cocci	Small ,transparent,grayish-white smooth colonies fluoresces under UV light
8	Non-Coagulase Staphylococcus	3	Strongly Gram-positive cocci arranged in irregular clusters	Round ,smooth, raised,&glisting grey to deep golden yellow colonies
9	Prevotella Species	1	Gram-negative short rods or coccobacilli	Small ,dark black, smooth , shiny colonies fluoresces brick red under UV light
10	Propinobacterium Species	1	Pleomorphic ,anaerobic Gram-positive rods	Circular, entire, convex, glisting& opaque colonies
11	Tetragenococci Species	1	Gram–positive spherical tetrads	Smooth, entire ,white ,convex colonies
12	Eubacterium Species	1	Uniform or pleomorphic,-Gram-positive rods	Circular,entire,low-convex,white,smooth colonies
13	Staphylococcus Aureus	1	Strongly Gram-positive cocci arranged in irregular clusters	Grey to white colonies on primary isolation
	Total	58		

Although the microbiology of the human oral cavity has been investigated thoroughly, the oral microbial flora has remained incom-pletely characterized. Most studies fo-cused 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 reac-tions[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, PeptoStreptococ-ci, Actinomyces, Porphymonas, Fuso-bacterium , Veillonella , Non-coagulase Staphylococcus species ,each isolate was tested twice .

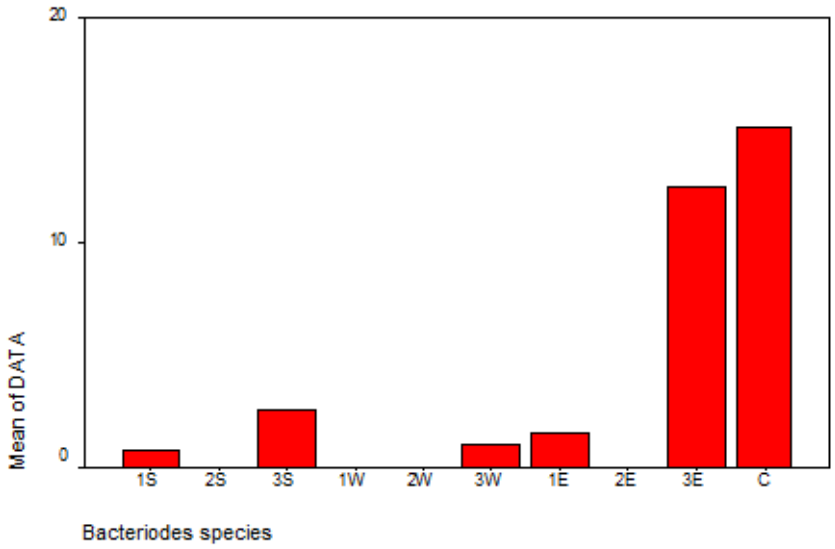

Figure (1) : Comparison Between The Effects of Different Herbs Extracted by Different Methods on Bacteriodes Species(zone of inhibition in millimeters)
C=chlorihexidanegluconate (control), 1S=cardamom extracted by Soxhelt apparatus, 1W= aqueous extract of cardamom ,1E= ethanolic extract of cardamom ,2S=Nigella sativa extracted by Soxhelt apparatus,2W= aqueous extract of Nigella sativa ,2E= etha-nolic extract of Nigella sativa ,3S=Salvadorapersica extracted by Soxhelt apparatus,3W= aqueous extract of Salvadorapersica ,&3E= ethanolic extract of Salvadorapersica .

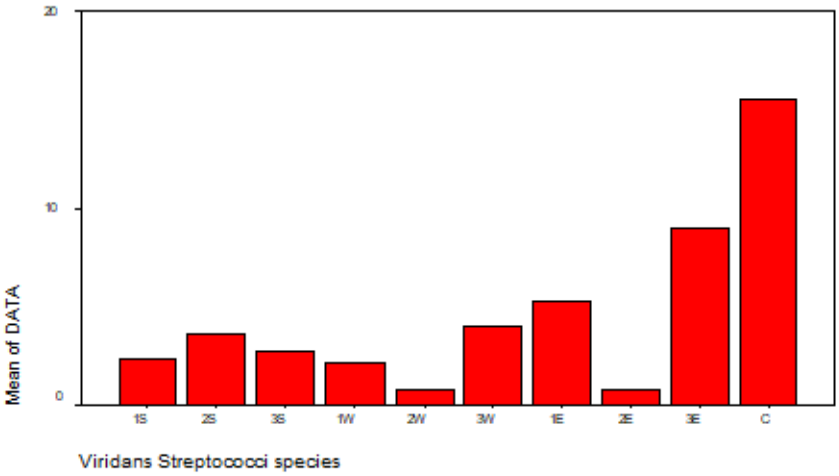

Figure (2) : Comparison Between The Effects of Different Herbs Extracted by Different Methods on Viridans Streptococci Species (zone of inhibition in millimeters)

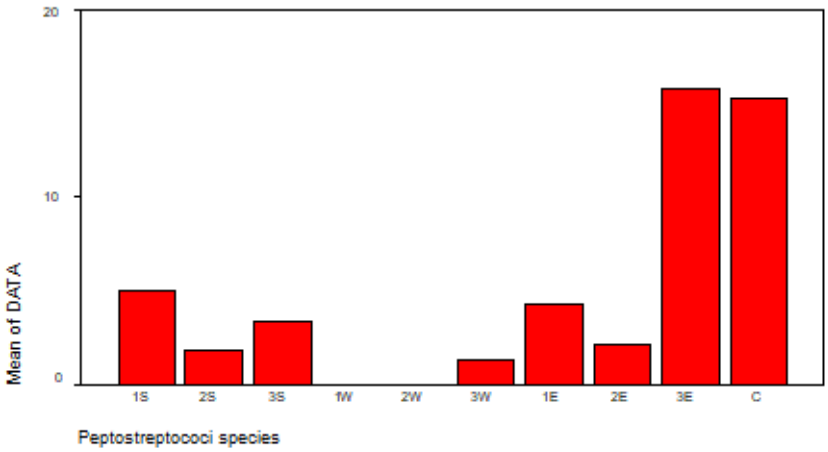

Figure (3) : Comparison Between The Effects of Different Herbs Extracted by Different Methods on PeptoStreptococci Species (zone of inhibition in millimeters)

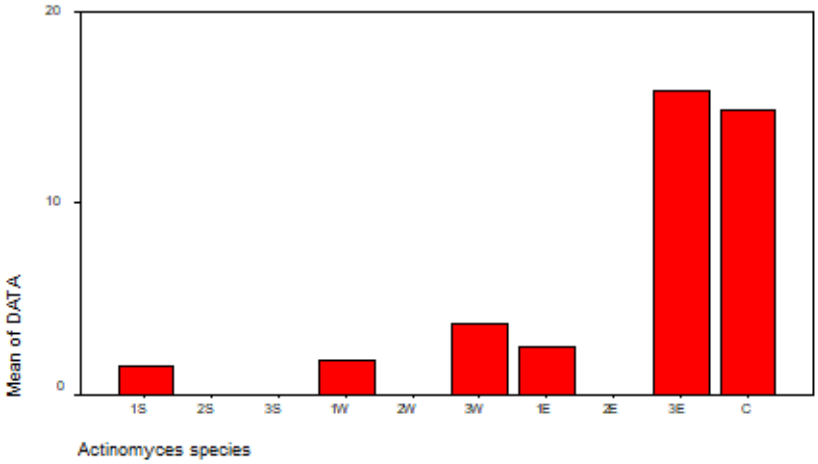

Figure (4) : Comparison Between The Effects of Different Herbs Extracted by Different Methods on Actinomyces Species

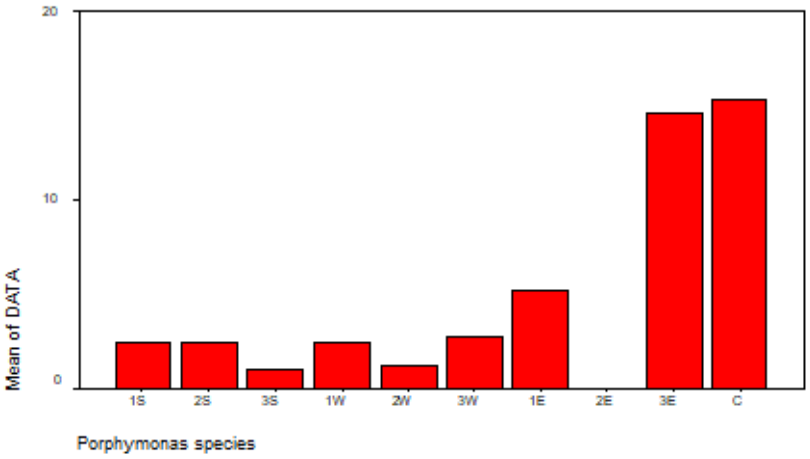

Figure (5) : Comparison Between The Effects of Different Herbs Extracted by Different Methods on Porphymonas Species(zone of inhibition in millimeters).

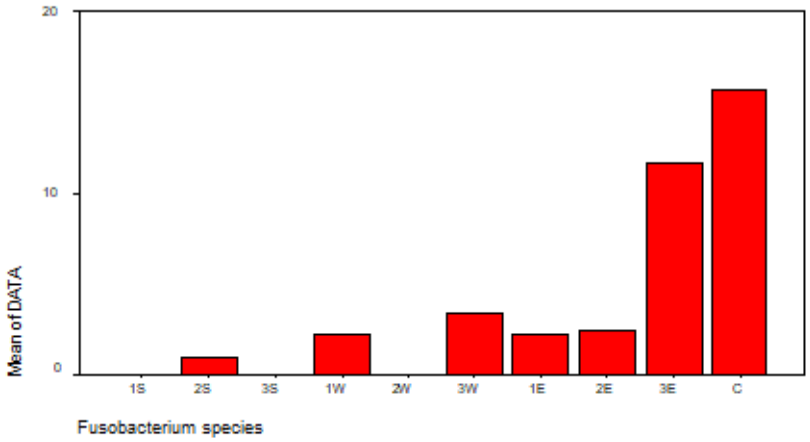

Figure (6) : Comparison Between The Effects of Different Herbs Extracted by Different Methods on Fusobacterium Species

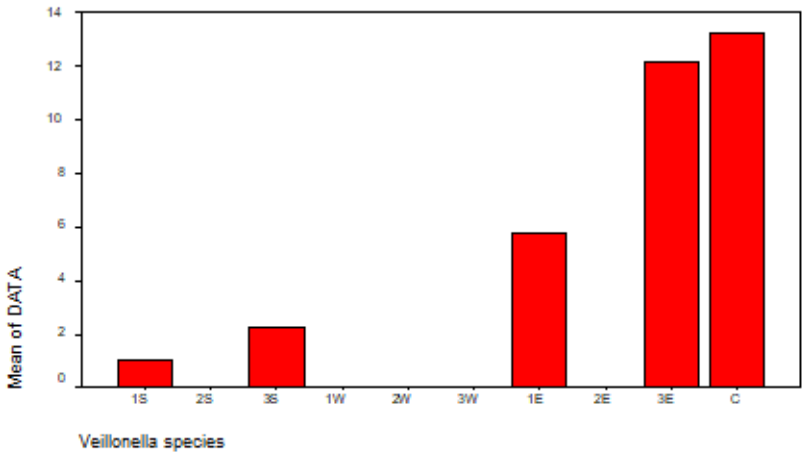

Figure (7) : Comparison Between The Effects of Different Herbs Extracted by Different Methods on Veillonella Species(zone of inhibition in millimeters)

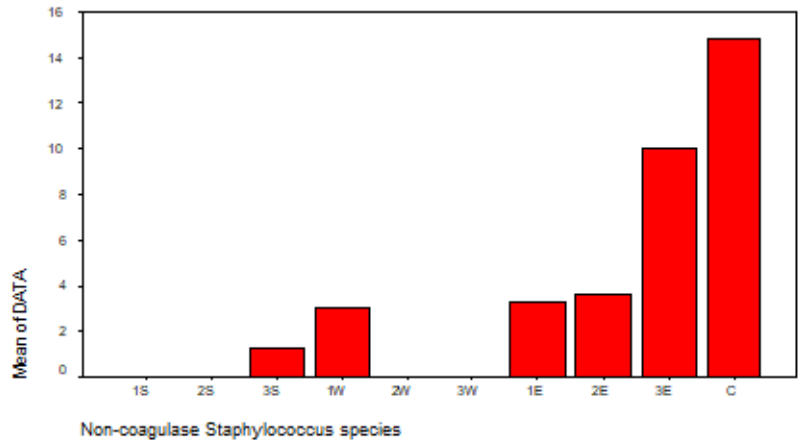

Figure (8) : Comparison Between The Effects of Different Herbs Extracted by Different Methods on Non-coagulase Staphylococcus Species (zone of inhibition in millimeters)

The control (chlorhex-idinegluconate 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, restora-tions, 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 medic-inal plants for the maintenance oral hy-giene[18].This study indicated that the etha-nolic extraction of S. persica demon-strated a reasonable range of inhibitory effects on the test bacteria. Although the chlorhexidinegluconate rinse had the greatest effect, the ethanolic extraction of S. persica compared favorably as it inhibit the growth of the microraginsms safely and with less side effects .Ethanolic extraction of S. persica inhib-ited 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 re-markable antibacterial activity but less than chlorhexidine.[19] The profound antibacterial effects of S. persica is be-lieved 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[20].

In summary, the rational explanation of the attractiveness of S. persica chewing stick as a tool for teeth clean-ing 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[21], their different extractions showed low effects against bacteria causing halitosis isolated in this study .Different crude extracts of Nigel-la 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.[22]

In relation to Elettariacardamomum, 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 charac-teristic 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 compo-nents 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 con-tacts with air(23,9). For these above rea-sons, Elettariacardamomum 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 (Chlorohexidanegluconate) and Salvadorapersica extractions but had little great effect than Nigella sativa extraction. An oral anti-halitosis preparations usually design to desorbe (inhibit) microorganisms and/or to absorb materials causing halitosis produced by thesemicroorganisms. 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 chlorohexidanegluconate 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 com-pounds to non- volatile sulfur com-pounds. 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 Elettariacardamomum which has considerable antioxidant property.

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