Antibacterial Potency of Aqueous Plant Extracts against Streptococcus mutans

Hêro F. S. Akrayi[1]

[1]Department of Biology, College of Education, University of Salahaddin, Erbil-Iraq.

ABSTRACT

In this study the inhibition effect of aqueous extracts of nine plants (Coriander, Black Tea, Bitter Fennel, Cubeb, Dry Black Lime, Ginger, Nutmeg, Turmeric and Senna) was tested against Streptococcus mutans, which obtained from Dental caries patient; the results showed that the antibacterial effect of these extracts was better in case of adding the extract to the medium than the case of evaporating the extract and then adding it to the medium except in case of Black tea and Nutmeg (was better in case two than in case one).

The results of the well diffusion test indicated that crude aqueous extracts of plants under study showed different degrees in inhibiting bacterial growth. The aqueous extract of Black dry lime showed the broadest antibacterial activity by inhibiting growth of tested isolate and at both cases, followed it Black tea and Cubeb. While the aqueous extract of Nutmeg, showed moderate antibacterial activity against the tested bacteria, followed it Ginger, Turmeric and Senna. The aqueous extract of Coriander did not show inhibition activity against S. mutans, while Bitter fennel showed inhibiting growth of S. mutans in case two at concentration 5000µg/ml. The MIC was determined for all extracts against tested bacteria, where the lowest value of MIC was for Black dry Lime and the highest value was for Cubeb, Ginger and Turmeric.

Keywords: Streptococcus mutans, Antibacterial activity, Plant extracts, Well diffusion technique, MIC.

INTRODUCTION

Oral diseases continue to be a major health problem worldwide. Dental caries and periodontal diseases are among the most important global oral health problems. Bacterial plaque plays the primary role in the pathogenesis of the disease. Dental plaque is a general term for the diverse microbial community (predominantly bacteria) found on the tooth surface, embedded in a matrix of polymers of bacterial and salivary origin. Plaque develops naturally on teeth, and forms part of the defense systems of the host by helping to prevent colonization of enamel by exogenous (and often pathogenic) microorganisms (colonization resistance) (1-3). As the principal etiological agent of human dental caries, Streptococcus mutans has developed multiple mechanisms to colonize the tooth surface and, under certain conditions, to become a numerically significant species in cariogenic biofilms (1, 4).

In drug discovery, most studies have examined on the antimicrobial potential of medicinal plants and other natural products measured as either killing or inhibiting the microbial growth. Natural products including medicinal plants are still major sources of innovative therapeutic agents for the various conditions of human diseases. The populations in rural developing countries rely heavily on traditional healers and medicinal plants as a basis to treat various maladies in spite of the availability of modern medicine. The world health organization reported that 80% of the world populations rely mainly on traditional medicine. Herbal medicine of natives in every country forms a major part of the world heritage of the plant material medical (5).

Correspondence:
Hêro F. S. Akrayi
Department of Biology, College of Education, University of Salahaddin, Erbil, Kurdistan Region of Iraq.
e-mail: herobio2013@gmail.com

There is an increasing interest in phytochemicals as new sources of natural antimicrobial agents (6). Some plant-derived compounds inhibit peptidoglycan synthesis, damage microbial membrane structures, modify bacterial membrane surface hydrophobicity, and modulate quorum sensing, all of which could influence biofilm formation (7).

The current study aimed to test some spices and herbs extracts to inhibit the growth of Streptococcus mutans.

MATERIALS AND METHODS

Bacteria employed in the study

Streptococcus mutans was isolated from dental plaque patient and cultured on blood agar and nutrient agar to obtain single colonies, then identified according to the cultural, morphological and some biochemical tests. The isolate was maintained and preserved on nutrient agar slants. For every experiment, freshly prepared sterile nutrient broth (10ml) was inoculated from the slants and incubated at 37°C for 24 hours.

Plant Extraction

Collection and preparation of plant samples: The plants (Bitter fennel, Black tea, Ginger, Turmeric, Nutmeg, Coriander, Cubeb, Dry black lime and Senna) were obtained from market in Erbil city, then were washed with tap water, then with distill water, then left for air drying until become completely drying, after drying the plants converted into powder form and stored in polyethylene sacks in refrigerator at 4°C for further process.

Extracts preparation: 150 ml of sterilized distilled water was added to 15 g of ground dried plant, heated below the boiling point and stirred for 2-3 h. The extract was filtered by muslin cloth, then by filter paper (Whatman No. 1). Half quantity of prepared extract was evaporated to dryness and, the both states were stored in the refrigerator at 5°C for using (2, 8).

Preparation of inoculums: Two to three colonies from pure growth of each tested organism were transferred to (5) ml of nutrient broth. Broths were incubated overnight at 37°C. The suspension was diluted with sterile distilled water to obtain approximately $1*10^6$ CFU/ml (9).

Well diffusion technique: Screening of antibacterial activity of plant extracts was performed by well diffusion technique (10). The Nutrient agar (NA) plates were seeded with 0.1 ml of the inoculums of tested organism. The inoculums were spread evenly over plate with loop. A standard cork borer of 8 mm diameter was used to cut uniform wells on the surface of the NA and 100µl of each concentration of plant extracts was introduced in the well, the plates were incubated for 24 hours at 37°C, and the zones of inhibition was measured to the nearest millimeter (mm).

TABLE 1: Antibacterial activity of aqueous plant extracts against S. mutans.

Plant Extract	Scientific Name	Concentration v/v *Zone of inhibition/mm				
		100%	75%	50%	25%	12.5%
Coriander	Coriandrum sativum	-	-	-	-	-
Black Tea	Camellia sinensis	22	20	20	20	20
Bitter Fennel	Foeniculum vulgare	-	-	-	-	-
Cubeb	Piper cubeba	21	20	20	15	13
Dry Black Lime	Citrus aurantifolia	35	32	28	22	15
Ginger	Zingiber officinale	9	8.2	-	-	-
Nutmeg	Myristica fragrans	15	10	-	-	-
Turmeric	Curcuma longa	10	10	-	-	-
Senna	Cassia acutifolia	15	-	-	-	-

*: Values calculated as mean of triplicates. -: No inhibition zone or less than 8 mm.

TABLE 2: Antibacterial activity of aqueous plant extracts against S. mutans.

Plant Extract	Scientific Name	Concentration µg/ml *Zone of inhibition/mm				
		5000	2500	1250	625	312.5
Coriander	Coriandrum sativum	-	-	-	-	-
Black Tea	Camellia sinensis	30	21	20	17	15
Bitter Fennel	Foeniculum vulgare	20	-	-	-	-
Cubeb	Piper cubeba	-	-	-	-	-
Dry Black Lime	Citrus aurantifolia	30	25	22	-	-
Ginger	Zingiber officinale	-	-	-	-	-
Nutmeg	Myristica fragrans	24	21	20	20	15
Turmeric	Curcuma longa	-	-	-	-	-
Senna	Cassia acutifolia	-	-	-	-	-

*: Values calculated as mean of triplicates. -: No inhibition zone or less than 8 mm.

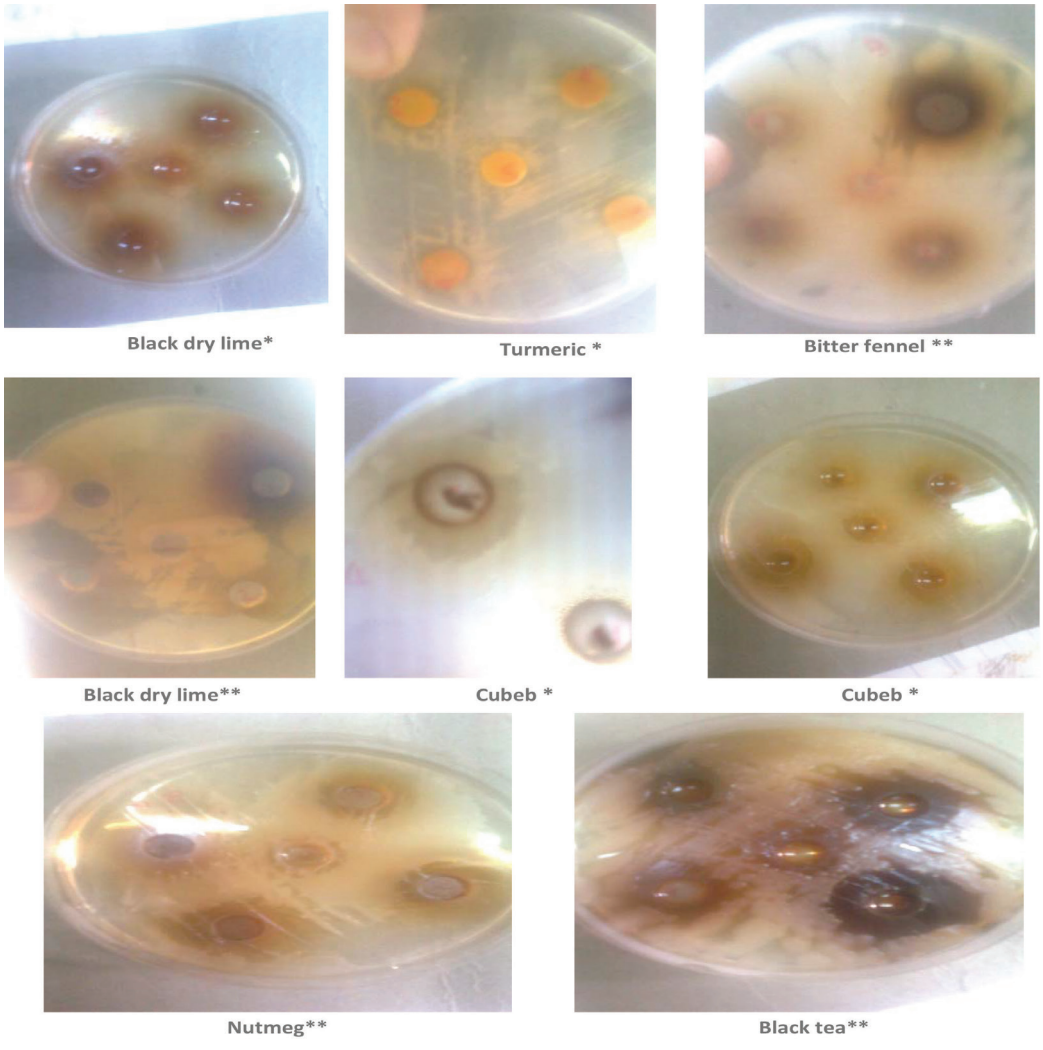

FIGURE 1: Inhibition zone of S. mutans with different concentrations of plant extracts.

*: Used extract without evaporation **: used extract after evaporation

MIC determination: The minimum inhibitory concentration (MIC) of medicinal plant extracts were determined by turbidity method (spectrophotometeric method) at 600 nm, and serial dilutions were prepared for each extract 500-9000 µg/ml (11). In addition to the control sample that consists of 10 ml of nutrient broth and 0.1ml ($1*10^6$ CFU/ml) of overnight culture of bacterial suspension then incubated at 37°C for 24 hours.

All experiments were applied in triplicates.

RESULTS AND DISCUSSION

Recently there has been a renewed interest in improving health and fitness through the use of more natural products. Herbs and spices are an important part of the human diet. They have been used for thousands of years to enhance the flavor, color and aroma of food. In addition to boosting flavor, herbs and spices are also known for their preservative and medicinal value which forms one of the oldest sciences. Yet it is only in recent years that modern science has started paying attention to the properties of spices (12).

The inhibition effect of plant extracts used in this study was shown in Tables 1 and 2 and Figure 1; the results showed that the antibacterial effect of these extracts was better in case of adding the extract to the medium than in case of evaporating the extract and then adding it to the medium except in case of Black tea and Nutmeg were better in case two than in case one. These results may return to when the extract is evaporated may some constitutes of plant loss their activity.

The results of the well diffusion test indicated that crude aqueous extracts of plants under study showed different degrees of growth inhibition. The aqueous extract of Black dry lime showed the broadest antibacterial activity by inhibiting growth of isolate tested and in both cases, followed it Black tea and Cubeb.

While the aqueous extract of Nutmeg, showed moderate antibacterial activity against the tested bacteria, followed it Ginger, Turmeric and Senna. The aqueous extract of Coriander did not show inhibition activity against S. mutans, while Bitter fennel showed only inhibiting growth of S. mutans in case two at the concentration 5000 µg/ml.

TABLE 3: MIC determination of aqueous plant extracts (µg/ ml against S. mutans

Plant extract	MIC
Coriandrum sativum	4000
Camellia sinensis	3000
Foeniculum vulgare	5000
Piper cubeba	6000
Citrus aurantifolia	2000
Zingiber officinale	6000
Myristica fragrans	3000
Curcuma longa	6000
Cassia acutifolia	5000

Similar finding were obtained from other researchers (1, 13-16) found that Ginger and Nutmeg, Lime, Turmeric, Cubeb and Fennel respectively have antibacterial activity against tested bacteria in each study.

The MIC was determined for all extracts against tested bacteria, where the lowest value of MIC was (2000 µg/ml) for Black dry Lime and the highest value was (6000 µg/ml) for Cubeb, Ginger and Turmeric.

Plants contain multiple organic components including Phenols like Black tea, quinones, flavones, tannins, terpenoids, and alkaloids like Cubeb all of which are known to have bactericidal effects. These substances are also water-soluble and therefore very likely to be present in the plant extracts produced in this study, and are likely candidates responsible for the killing effect of the extracts on S mutans (1, 17,18).

Reversible findings found that Streptococcal disease problems and control-a review have been reported by (19) Ginger, Cubeb and Turmeric extracts did not show inhibitory activity. It was observed that the antimicrobial effect of plant extract varies from one plant to another in different researches carried out in different regions of the world. This may be due to many factors such as, the effect of climate, soil composition, age and vegetation cycle stage, on the quality, quantity and composition of extracted product, different bacterial strains. Moreover, different studies found that the type of solvent has an important role in the process of extracting.

CONCLUSION

It concluded that antibacterial property of studied plant extracts varied from strong, moderate and with no inhibiting action; black dry lime, black tea and cubeb are good bacterial inhibitor extracts. It recommended to mixing those plant extracts having potent antibacterial (anti-S. mutans) with tooth pastes.

REFERENCES

1. Aneja KR, Joshi R, Sharma C, Aneja A. Antimicrobial Efficacy of Fruit Extracts of Two Piper Species against Selected Bacterial and Oral Fungal Pathogens. Brazilian J of Oral Sciences 2010; 9(4): 421-426.

2. Babpour ES, Angaji A, Angaji SM. Antimicrobial Effects of Four Medicinal Plants on Dental Plaque. J of Medicinal Plants Research 2009; 3(3): 132-137.

3. Castro P, Tovar JA, Jaramillo L. Adhesion of Streptococcus Mutans to Salivary Proteins in Caries-Free and Caries-Susceptible Individuals. Acta Odontol Latinoam 2006; 19(2): 59-66.

4. Wen ZT, Yates D, Ahn S-Joon, Burne RA. Biofilm Formation and Virulence Expression by Streptococcus mutans Are Altered When Grown in Dual-Species Model. BMC Microbiology 2010; 10 (111): 1-9.

5. Al-Hussaini R, Mahasneh AM. Antibacterial and Antifungal Activity of Ethanol Extract of Different Parts of Medicinal Plants in Jordan. Jordan J of Pharmaceutical Sciences 2011; 4(1).

6. Genena AK Hense H, Smânia JA, de Souza SM. Rosemary (Rosmarinus officinalis) – A study of the Composition, Antioxidant and Antimicrobial Activities of Extracts Obtained with Supercritical Carbon Dioxide. Ciênc. Tecnol. Aliment., Campinas 2008; 28(2): 463-469.

7. Mousavi NS, Owlia P, Moein NL, Rasooli I, Saderi H, Salari MH. Effects of Sub-inhibitory Concentrations of Essential Oils of Mentha spicata and Cumminum cyminum on Virulence Factors of Pseudomonas aeruginosa. J of Medicinal Plants 2010; 9 (6).

8. Al-Neemy UMSM, AL-Jebury SHKh. The Inhibitory Effect of Some Medicinal Plant Extracts to Streptococcus pyogenes. Duhok Zanko J 2006; 11(1): 45-51. Proceedings of 1st Kurdistan Conference on Biological Science, Duhok.

9. Alhaj NA, Shamsudin MN, Zamri HF, Abdullah R. Extraction of Essential Oil from Nigella sativa Using Supercritical Carbon Dioxide: Study of Antibacterial Activity. American J of Pharmacology and Toxicology 2008; 3(4): 225-228.

10. Kivanc M, Kunduhoglu B. Antimicrobial Activity of Fresh Plant Juice on the Growth of Bacteria and Yeasts. J of Qafqaz University 1997; 1: 26-53.

11. Cruickshank R, Duguid JD, Marmion BP, Swain RHA. Medical Microbiology, the Practice of Medical Microbiology. 12th ed., vol. 1 Churchill Livingstone, London and New York, 1975.

12. Chaudhry NMA, Tariq P. Bactericidal Activity of Black Pepper, Bay Leaf,Aniseed and Coriander Against Oral Isolates. Pakistan J of Pharmaceutical Sciences 2006; 19(3): 214-218.

13. Ibrahim TA, Opawale BO, Oyinloye JMA. Antibacterial Activity of Herbal Extracts against Multi Drug Resistant Strains of Bacteria from Clinical Origin. Life sciences Leaflets 2011; 15:490 – 498.

14. Lawhavinit O, Kongkathip N, Kongkathip B. Antimicrobial Activity of Curcuminoids from Curcuma longa L. on Pathogenic Bacteria of Shrimp and Chicken. Kasetsart. J of Natural Sciences 2010; 44: 364 – 371.

15. El-Adly AA, Abada EA, Gharib FA, Antibacterial Effects of Low Power Laser Light and Volatile Oil of Fennel (Foeniculum vulgare var. dulce) on Gram-Positive and Gram- Negative Bacteria. International J of Agriculture and Biology 2007; 9(1):22-26.

16. Onyeagba RA, Ugbogu OC, Okeke CU, Iroakasi O.Studies on the Antimicrobial Effects of Garlic (Allium sativum Linn), Ginger (Zingiber officinale Roscoe) and Lime (Citrus aurantifolia Linn). African J of Biotechnology 2004; 3 (10): 552-554.

17. Indu MN, Hatha AAM, Abirosh C, Harsha U, Vivekanandan G. Antimicrobial Activity of Some of The South-Indian Spices against Serotypes of Escherichia coli, Salmonella, Listeria monocytogenes And Aeromonas hydrophila. Brazilian J of Microbiology 2006; 37:153-158.

18. Tahir A, Moeen R. Comparison of Antibacterial Activity of Water and Ethanol Extracts of Camellia sinensis (L.) Kuntze against Dental Caries and Detection of Antibacterial Components. J of Medicinal Plants Research 2011; 5(18): 4504-4510.

19. Alsaid M, Daud H, Bejo SK, Abuseliana A. Antimicrobial Activities of Some Culinary Spice Extracts against Streptococcus agalactiae and Its Prophylactic Uses to Prevent Streptococcal Infection in Red Hybrid Tilapia (Oreochromis sp.). World J of Fish and Marine Sciences 2010; 2 (6): 532-538.