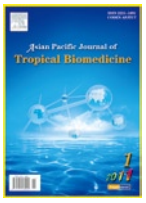
Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Biomedicine

journal homepage:www.elsevier.com/locate/apjtb



Document heading

Antimicrobial activity of ginger and honey on isolates of extracted carious teeth during orthodontic treatment

Roopal V Patel[1*], Vidhi T Thaker[2], VK Patel[2]

[1]*Department of Orthodontic, Narsinhbhai Patel Dental College and Hospital, Visnagar, Gujarat, India*
[2]*Department of Pharmacology, Narsinhbhai Patel Dental College and Hospital, Visnagar, Gujarat, India*

ARTICLE INFO

Article history:
Received 18 July 2011
Received in revised form 7 August 2011
Accepted 25 August 2011
Available online 10 September 2011

Keywords:
Ginger
Honey
Carious teeth
Orthodontic treatment
Antimicrobial activity
Antibiotic resistance
Streptococcus mutans
Susceptibility test
Scofriendly medicine

ABSTRACT

Objective: To evaluate the *in vitro* effects of ginger and honey on micro-organisms on carious teeth by employing antibiotic sensitivity test. **Methods:** Two hundred and fifty (250) extracted, carious teeth were aseptically collected into sterile peptone water. Bacterial species were isolated from the peptone water broth, characterized and identified according to standard methods described in the Manual of Clinical Microbiology. Aqueous ginger extract and honey were employed for sensitivity test. Suspensions of the bacterial isolates were made in sterile normal saline and adjusted to the 0.5 McFarland's standard. Each Mueller Hinton (MH) agar plate was uniformly seeded by means of sterile swab dipped in the suspension and streaked on the agar plate surface, and the plates left on the bench for excess fluid to be absorbed. Approximately 100 μL of the extracts were dropped into each well which filled them respectively to fullness. The setup was allowed to stabilize for 3 h before being incubated at 37 ℃ for 24 h. The mean zones of inhibition were thereafter measured in mm, for all the individual isolates. **Results:** *Streptococcus mutans* (88.0%) and *Lactobacillus acidophilus* (*L. acidophilus*) (39.0%) were most prevalent as compared with other isolates. The diameter of the zone of inhibition ranged from (18.0 ± 0.5) mm to (27.0 ± 1.0) mm for ginger and (20.0 ± 0.5) mm to (27.0 ± 0.7) mm for honey, as compared with (18.0 ± 0.7) mm to (23.0 ± 0.5) mm for gentamycin, at the various concentrations used. Results indicate a considerable antibacterial activity of ginger and honey. The combined extracts were most effective against *Staphylococcus aureus* (30.0.0 ± 1.5) mm but least effective against *L. acidophilus* (21.0 ± 0.7) mm. **Conclusions:** For the prevention of the emergence of resistant microorganisms, use of combination of herbal preparations is more useful.Considering *in vitro* data obtained in this study, there is a significant synergistic effect of antimicrobial activity from the combination of ginger and honey, against isolates from carious teeth.

1. Introduction

Antibiotics provide an invaluable tool for a control of infection in modern dentistry[1]. Development of resistance to various antibiotics makes it necessary to select logically and rationally, a drug for successful gingival therapy during orthodontic treatment. A rekindled interest in the pharmaceutical importance of plants has led to the discovery and adaptation of plant extract which were commonly used in traditional medicine as alternative source of remedy[2].

Moreover, most antimicrobial agents that are currently in use have been rendered ineffective by a wide occurrence of multiple drug resistant strains of microbes[3]. So herbal preparation of honey and ginger are used as an essential ingredient in the preparation of most herbal concortions.

Honey (*Apis mellifera*) has been used as an eco-friendly medicine throughout the ages and recently regarded for its potential in treatment of burns and peptic ulcer, infected wounds, bacterial gastro-enteritis and eye infection. Honey has a potent broad-spectrum antibacterial activity and studies have demonstrated that manuka honey with a high antibacterial activity is likely to be non-cariogenic[4]. Repeated use of antibiotics increases the percentage of resistant micro-organisms to various antibiotics. Honey increases the sensitivity of micro-organisms to antibiotics

*Corresponding author: Roopal V Patel, MDS Professor and Head, Department of Orthodontic Narsinhbhai Patel Dental College and Hospital Visnagar -384 315 (North Gujarat) India.
Tel: + 91 2765 222 271
Fax: + 91 2765 233 008
E-mail: drroopal@yahoo.co.in
Foundation Project: Supported by Narsinhbhai Patel Dental College and Hospital, Visnagar, Gujarat.

and decreases the microbial resistance to antibiotics[5].

Ginger (*Zingiber officinale* Roscoe, Zingiberaceae) is a medicinal plant that has been widely used in Chinese, Ayurvedic and Tibb-Unani herbal medicines all over the world, since antiquity, for a wide array of unrelated ailments including arthritis, rheumatism, sprains, muscular aches, pains, sore throats, cramps, constipation, indigestion, vomiting, hypertension, dementia, fever, infectious diseases and helminthiasis[6]. The antimicrobial activity of ginger has been described and studied by Onyeagba *et al*[7].

The need to identify a common and cheap herbal remedy for the prevention and treatment of sore-throat, mouth sore and dental caries, especially in a developing nation, prompted us to investigate the therapeutic potentials of ginger and honey.

2. Materials and methods

2.1. Isolation and identification of isolates

Two hundred and fifty (250) extracted, carious teeth were aseptically collected, each into a wide-mouthed screw-capped universal bottle containing 10 mL of sterile peptone water, from City Dental Clinics, Ahmedabad, India. Samples were immediately transported in ice-packed containers to the Microbiology Laboratory of National Institute of Occupational Health at Ahmedabad and incubated aerobically at 37 ℃ for 24 h. Bacterial species were isolated from the peptone water broth, characterized and identified according to standard methods described in the Manual of Clinical Microbiology[8].

2.2. Preparation of extracts

Aqueous ginger extract was prepared according to methods previously reported by Onyeagba *et al*[7]. 100 g of fresh, washed ginger cloves was macerated in a sterile, ceramic mortar. The homogenate was then filtered off with a sterile, muslin cloth and used directly for the sensitivity test.

Similarly, the study of the antimicrobial activity of honey (Dabar India Ltd., Ghaziabad, India) on inflamed gingiva was carried out by employing sensitivity test by disc diffusion method as described by Cruickshank[1]. To ensure aseptic conditions, sterile gloves and face masks were worn and the entire experiment was carried out in a media room of the Microbiology Laboratory, of National Institute of Occupational Health at Ahmedabad.

2.3. Standardization of isolates

A standard stock of the bacteria isolates were prepared by suspending a loop full of each microbial growth in about 10 mL of nutrient broth. After incubation at 37 ℃ for 12 h, the turbidity was adjusted to be visually comparable with a 0.5 McFarland's standard giving a bacterial load of about $1-2 \times 10^8$ cfu/mL[8].

2.4. Susceptibility test

The agar-well diffusion method prescribed by NCCLS (2000) was employed in the susceptibility testing[9]. Suspensions of the bacterial isolates were made in sterile normal saline and adjusted to the 0.5 McFarland's standard. Each Mueller Hinton (MH) agar plate was uniformly seeded by means of sterile swab dipped in the suspension and streaked on the agar plate surface, and the plates left on the bench for excess fluid to be absorbed. Wells of 5 mm in diameter, 4 mm deep and about 2 cm apart were punched in the MH agar with a sterile cork-borer. Approximately 100 μL of the extracts were dropped into each well which filled them respectively to fullness. The setup were allowed to stabilize for 3 h before being incubated at 37 ℃ for 24 h as described previously by Shahidi and Aibinu *et al*[10,11]. The mean zones of inhibition were thereafter measured in mm, for all the individual isolates. A positive control well was equally filled with gentamycin (32 μg/mL) while sterile, distilled water served as negative control.

2.5. Determination of MIC and MBC

The minimum inhibitory concentration (MIC) of the extracts was determined according to methods described by Shahidi[10] and Kabir *et al*[2]. Extracts were diluted to concentrations ranging from 7.82 mg/mL to 500 mg/mL (for honey and a mixture of honey with ginger), and 1:16 to 1:1 (v/v), for honey. To each dilution of honey, ginger and a mixture of both, in nutrient broth tubes were seeded 0.1 mL of the standard bacterial inoculum. Negative control tubes with no bacterial inoculation, were simultaneously maintained. Tubes were incubated aerobically at 37 ℃ for 24 h. The lowest concentration of the extract that produced no visible bacterial growth (turbidity) was recorded as the MIC. Dilutions showing no visible growth for the MIC was sub-cultured onto a fresh MH agar plate and incubated at 37 ℃ for 24 h. The lowest concentration of the extracts yielding no growth on the MH plate was recorded as the minimal bactericidal concentration (MBC).

3. Results

Culture of the extracted, carious teeth investigated implicated 6 bacterial species to be associated with the various degrees of dental caries observed in this study. These include *Streptococcus mutans* (*S. mutans*), *Lactobacillus acidophilus* (*L. acidophilus*), *Norcadia asteroides*, *Pseudomonas aeruginosa* (*P. aeruginosa*), *Actinomyces viscosus* (*A. viscosus*), *Staphylococcus aureus* (*S. aureus*) and *Veilonella alcaligens* (*V. alcaligens*). *S. mutans*

(88.0%) and *L. acidophilus* (39.0%) were most prevalent as compared with other isolates (Table 1). This predominance is in agreement with findings of Hedge *et al* where a prevalence of 87.4% and 36.7% were recorded for *S. mutans* and *L. acidophilus*, respectively. *L. acidophilus* is the major culprit usually associated with carious teeth and this is not unconnected with its elaborate acid production[12]. Since these bacteria convert sugars into acid such as lactic acid through the glycoltic process of fermentation[13], their role in demineralization and ultimately, formation of cavities, is not in doubt.

Table 1
Percentage prevalence of isolates in extracted carious teeth (n= 250).

Isolates	Number (% of occurrence)
S. mutans	206 (88.0)
L. acidophilus	94 (39.0)
A. viscosus	50 (22.0)
P. aeruginosa	38 (18.0)
V. alcaligens	29 (16.6)
S .aureus	27 (15.0)

Previous authors have described the prevalence of *S. mutans* and *Lactobacillus* in carious teeth[14]. However, Aas *et al*[15] used polymerase chain reaction (PCR) amplified 16S rRNA genes to identify bacteria isolates from the plaque of carious teeth. They concluded that bacterial species other than *S. mutans*, *e.g.*, species of the genera *Veillonella*, *Lactobacillus*, *Bifidobacterium* and *Propionibacterium*, low pH non- *S. mutans* streptococci, *Actinomyces* sp., and *Atopobium* sp., likely play important roles in caries progression.

The diameter of the zone of inhibition ranged from (18.0 ± 0.5) mm to (27.0 ± 1.0) mm for ginger and (20.0 ± 0.5) mm to (27.0 ± 1.0) mm for honey, as compared with (18.0 ± 0.7) mm to (23.0 ± 0.5) mm for gentamycin (Table 2), at the various concentrations used. Results indicate a considerable antibacterial activity of ginger and honey. The combined extracts were most effective against *S. aureus* (30.0 ± 1.5) mm but least effective against *L. acidophilus* (21.0 ± 0.7) mm (Table 2). *L. acidophilus* is the major culprit implicated with dental caries[16] with susceptibility equally comparable

Table 2
Mean ± SD zones of inhibition of extracts on isolates

Isolates	Gentamycin (32 μg/mL)	Water	Ginger (500 mg/mL)	Honey (undiluted)	Ginger + Honey
S. mutans (n = 20)	20.0 ± 0.0	–	20.0 ± 0.5	23.0 ± 1.0	23.0 ± 1.0
L.acidophilus (n = 96)	18.0 ± 0.7	–	20.0 ± 0.7	21.0 ± 1.0	21.0 ± 0.7
A. viscosus (n = 48)	23.0 ± 0.5	–	26.0 ± 0.5	27.0 ± 1.0	27.0 ± 1.0
P.aeruginosa (n = 36)	21.0 ± 0.7	–	19.0 ± 1.0	22.0 ± 0.7	23.0 ± 1.0
V.alcaligens (n = 28)	20.0 ± 1.0	–	18.0 ± 0.5	20.0 ± 0.5	22.0 ± 1.0
S. aureus (n = 24)	22.0 ± 0.0	–	27.0 ± 1.0	27.0 ± 0.7	30.0 ± 0.5

– Means no zone of inhibition.

with that of gentamycin with ZI of (18.0 ± 0.0) mm. Previous researchers have described the antibacterial activity of ginger against multi drug resistant (MDR) *S. mutans*[17-26], as well as methicillin resistant *S. aureus* and MDR *P. aeruginosa*[27].

The MIC of garlic against the test isolates ranged from 31.25 – 62.5 mg/mL (Table 3). This is slightly higher compared with the MIC of garlic extract against *S. mutans* which ranged from 4 – 32 mg/mL as previously reported by Fani *et al*[17-26]. This difference in value could be largely attributed to the extraction process. Generally, a synergistic effect was observed for a combination of lime and garlic that shows MIC decrease to a range of 15.63 – 31.25 mg/mL (Table 3).

Table 3
MICs of extracts on isolates.

Isolates	Ginger (mg/mL)	Honey (%, v/v)	Ginger + Honey (mg/mL)
S. mutans	31.25	1:2	15.63
L. acidophilus	31.25	1:2	15.63
A. viscosus	31.25	1:2	15.63
P. aeruginosa	31.25	1:2	15.63
V. alcaligens	62.50	1:2	31.25
S. aureus	31.25	1:2	31.25

4. Discussion

Antibacterial action of honey on the principle of MIC and its synergism with antibiotics are due to hydrogen peroxide which is produced enzymatically in honey[4]. The glucose oxidase enzyme is secreted from the hypopharyngeal gland of the bee into the nectar to assist in the formation of honey from the nectar. The hydrogen peroxide and acidity produced by two reactions (Glucose + O_2 → gluconic acid; gluconic acid + H_2O_2 → honey) has antimicrobial potential[4].

Apart from the recommended use of fluoride toothpaste and some natural antimicrobial agents, the use of mixture of ginger and honey for the treatment and control of dental caries is highly recommended.

For the prevention of the emergence of resistant microorganisms, use of combination of herbal preparations is more useful[28].

Considering *in vitro* data obtained in this study, there is a synergistic effect of antimicrobial activity from the combination of ginger and honey, against isolates from carious teeth. The result of this investigation suggests that a paste made by blending ginger and honey could be used as a mouth wash in the treatment of dental caries, mouth sore, sore throat and also, be incorporated into toothpaste to prevent dental caries. Further studies on toxicity tests are recommended. Also, dental health education and caries

preventive programme will minimize caries in children and even in adults.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgements

Authors are grateful to the study subjects who volunteered to participate in this study. We are thankful to the Superintendent, City Dental Clinic, Ahmedabad, Gujarat and Dean, Narsinhbhai Patel Dental College and Hospital, Visnagar, India for providing the facilities for conducting the study.

References

[1] Cruickshank R. *Medical Microbiology: a guide to diagnosis and control of infection*. 11th ed. Edinburgh and London: E & S Livingstone Ltd. 1968, p. 50-97.

[2] Akinyemi KO, Oladapo O, Okwara CE, Ibe CC, Fasure KA. Screening of crude extracts of six medicinal plants used in Southwest Nigerian orthodox medicine for anti-methicillin resistant *Staphylococcus aureus* activity. *BMC Complement Altern Med* 2005; **5**: 6.

[3] Owhe-Ureghe UB, Ehwarieme DA, Eboh DO. Antibacterial activity of garlic and lime on isolates of extracted carious teeth. *Afr J Biotech* 2010; **9**(21): 3163-3166.

[4] Patel RV, Thaker VT, Patel VK, Shukla P, Bhatnagar P, Patel A. *In-vitro* study of changing antibiotic sensitivity and resistance by honey on gingival inflammation during orthodontic treatment - a preliminary report. *Orthodontic CYBER journal* 2010: 3-8.

[5] Mondal S, Mirdha BR, Mahapatra SC. The science behind sacredness of Tulsi (*Ocimum sanctum* Linn.). *Indian J Physiol Pharmacol* 2009; **53**(4): 291-306.

[6] Ali BH, Blunden G, Tanira MO, Nemmar A. Some phytochemical, pharmacological and toxicological properties of ginger (*Zingiber officinale* Roscoe): A review of recent research. *Food Chem Toxicol* 2008; **46**(2): 409-420.

[7] 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). *Afr J Biotechnol* 2004; **3**(10): 552-554.

[8] Murray PR, Baron EJ, Jorgensen JH, Landry ML, Pfaller MA. *Manual Clinical Microbiology*. 9th ed. Washington DC: ASM Press; 2004, p. 2260.

[9] National Committee for Clinical Laboratory Standards (NCCLS). *Methods for dilution: antimicrobial susceptibility test for bacteria that grow aerobically*. 5th ed. 2000, p. 20.

[10] Shahidi GA. Evaluation of antimicrobial properties of Iranian medicinal plants against *Micrococcus luteus*, *Serratia marceceans*, *Klebsiella pneumonia* and *Bordetella branchoseptica*. *Asian J Plant Sci* 2004; **3**(1): 82-86.

[11] Aibinu I, Adenipekun T, Adelowotan T, Ogunsanya T, Odugbemi T. Evaluation of the antimicrobial properties of different parts of *Citrus aurantifolia* (lime fruit) as used locally. *Afr J Tradit Complement Altern Med* 2006; **4**(2): 185-195.

[12] Rogers AH, editor. *Molecular Oral Microbiology*. 4th ed. London: Caister Academic Press; 2008.

[13] Holloway PJ. The role of sugars in the etiology of dental caries. *J Dent* 1983; **11**: 189-213.

[14] Nishikawara F, Katsumura S, Ando A, Tamaki Y, Nakamura Y, Sato K, et al. Correlation of cariogenic bacteria and dental caries in adults. *J Oral Sci* 2006; **48**(4): 245-251.

[15] Aas JA, Griffen AL, Dardis SR, Lee AM, Olsen I, Dewhirst FE, et al. Bacteria of dental caries in primary and permanent teeth in children and young adults. *J Clin Microbiol* 2008; **46**(4): 1407-1417.

[16] Hardie JM. The microbiology of dental caries. *Dental Uptake* 1982; **9**: 199-208.

[17] Fani MM, Kohanteb J, Dayaghi M. Inhibitory activity of garlic *Allium sativum* extract on multi drug resistant *S. mutans*. *J Indian Soc Pedod Prev Dent* 2007; **25**(4): 164-168.

[18] Saad S, Taher M, Susanti D, Qaralleh H, Rahim NABA. Antimicrobial activity of mangrove plant (*Lumnitzera littorea*). *Asian Pac J Trop Med* 2011; **4**(7): 523-525.

[19] Raja RDA, Jeeva S, Prakash JW, Antonisamy JM, Irudayaraj V. Antibacterial activity of selected ethnomedicinal plants from South India. *Asian Pac J Trop Med* 2011; **4**(5): 375-378.

[20] Bragadeeswaran S, Priyadharshini S, Prabhu K, Rani SRS. Antimicrobial and hemolytic activity of fish epidermal mucus *Cynoglossus arel* and *Arius caelatus*. *Asian Pac J Trop Med* 2011; **4**(4): 305-309.

[21] Madhumitha G, Saral AM. Preliminary phytochemical analysis, antibacterial, antifungal and anticandidal activities of successive extracts of *Crossandra infundibuliformis*. *Asian Pac J Trop Med* 2011; **4**(3): 192-195.

[22] Johnson M, Wesely EG, Kavitha MS, Uma V. Antibacterial activity of leaves and inter-nodal callus extracts of *Mentha arvensis* L. *Asian Pac J Trop Med* 2011; **4**(3): 196-200.

[23] Peixoto JRO, Silva GC, Costa RA, de Sousa Fontenelle JL, Vieira GHF, Filho AAF, et al. *In vitro* antibacterial effect of aqueous and ethanolic *Moringa* leaf extracts. *Asian Pac J Trop Med* 2011; **4**(3): 201-204.

[24] Kumar SC, Bhattacharjee I, Chandra G. Isolation and identification of bioactive antibacterial components in leaf extracts of *Vangueria spinosa* (Rubiaceae). *Asian Pac J Trop Med* 2011; **4**(1): 35-40.

[25] Irudayaraj V, Janaky M, Johnson M, Selvan N. Preliminary phytochemical and antimicrobial studies on a spike-moss *Selaginella inaequalifolia* (hook. & grev.) Spring. *Asian Pac J Trop Med* 2010; **3**(12): 957-960.

[26] Mandal S, DebMandal M, Kumar NP, Saha K. Antibacterial activity of honey against clinical isolates of *Escherichia coli*, *Pseudomonas aeruginosa* and *Salmonella enterica* serovar Typhi. *Asian Pac J Trop Med* 2010; **3**(12): 961-964.

[27] Tao SM, Yim MC. *In vitro* antimicrobial activity of four diallyl sulphides occurring naturally in garlic and Chinese leek oil. *J Med Microbiol* 2001; **50**: 646-649.

[28] Patel VK. Chemotherapy. In: *Textbook of general and dental pharmacology with MCQs*. 6th ed. Ahmedabad: B.S Shah Publishers; 2010, p. 232.