



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

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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

Scolfriendly 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 μ L 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 °C 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 \pm 0.5) mm to (27.0 \pm 1.0) mm for ginger and (20.0 \pm 0.5) mm to (27.0 \pm 0.7) mm for honey, as compared with (18.0 \pm 0.7) mm to (23.0 \pm 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 \pm 1.5) mm but least effective against *L. acidophilus* (21.0 \pm 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 concoctions.

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

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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 °C 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 °C 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 °C 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 °C 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 °C 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 *Veillonella alcaligens* (*V. alcaligens*). *S. mutans*

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