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Investigate the Antimicrobial Activity of Raw Lemon and Honey against Human Enteric Pathogens in Vitro

Somnath De[1], NilanjanaMondal[2], Susmita Bhowmick[3], Puja Giri[4], Snighadha Maji[5], Moumi Das[6], Siddhartha Chakraborty[7].

Department of Biotechnology, Panskura Banamali College. West-Bengal. India.

Corresponding Author :- Somnath De

Address:- Assistant Professor, Department of Biotechnology, Panskura Banamali College. Email id:-

Abstract

Over three-quarter of the world's population is using herbal medicines with increasing trend globally. Plant medicines may be beneficial but are not completely harmless. The aim of this study is to evaluate the antimicrobial activity of raw lemon and honey against different enteric human pathogens.Antimicrobial activity of lime juice is characterized by damage to the cell wall resulting in changes in cell morphology analysis of bacteria. Mixing lime juice with honey was also shown a maximum zone of inhibition against pathogenic fungi *Candida albicans*. Traditionally in Indonesian, lime juice added honey is often used as a cough medicine.

Keywords: Antibacterial activity, honey, lemonjuice.

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 werecommonly used in traditional medicine as alternative sourceof 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 lemon are used as an essentialingredient 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 antibiotic and decreases the microbial resistance to antibiotics[5].

Honey produced by *Apis mellifera* is one of the oldest traditional medicines considered to be important in the treatment of several human ailments [6]; as herbal medicine has been widely employed in the treatment of diseases since the origin of mankind. Honey is the sweet substance; produced when the nectar and deposit from plant are gathered, modified and stored in the honey comb by honey bees [7]. According to [8], honey has been extensively used as healing agent throughout the human history in addition to its widespread usage as popular food. Different cultures have extensively used honey as a medicine for many health issues and disorders.

Honey is an excellent source of flavonoids, a natural antioxidant which have the ability to protect against allergens, viruses and carcinogens [9]. Also as related by [10], honey includes hydrogen-peroxide, flavonoids and phenolic acids plus many other unidentified properties. Also the chemical composition of honey is said to comprise of seven tetracycline, fatty acids, lipids, amylase, ascorbic acid, peroxidase and fructose all of which are attributed to its antimicrobial activity together with high osmolarity, low pH (3.6-3.7), content of phenol (inhibine), peroxidase, glucose and fructose in honey and the presence of tetracycline derivatives of fatty acids [11]. The antibacterial activity of honey is highly complex due to the involvement of multiple compounds and due to the large variation in the concentrations of these compounds among honeys [12]. Acidity is also said to be one of the factors that contributes to the antibacterial property of honey [13].

In some bee products, the antibacterial activity of honey is attributed to the presence of "inhibin", which acts as an antibacterial factor other than hydrogen peroxide [14], more recently, methylglyoxal and the antimicrobial peptide bee defensin-1 were identified as important antibacterial compounds in honey. Honey has been used as a medicine throughout the human history [15]. Also antibacterial properties of honey were recognized more than a century ago and have subsequently been extensively studied [16]. In modern medicine, particularly in the last three decades, interest in the application of honey for the treatment of infections has increased. Recently, the use of honey as a therapeutic agent has been re-discovered especially in the field of medicine thus gaining acceptance as an antibacterial agent. Many studies demonstrated the use of honey when antibiotic treatments had failed to clear the infection [15]. The control of infection by honey is said to be attributed to the high osmolarity while its hydrogen peroxide contents, lysozyme and other unidentified substances from certain flora sources are responsible for its antibacterial properties [17]

Citrus is one ofthe most important commercial fruit crops grownin all continents of the world. Citrus fruits belong to six genera (*Fortunella*, *Eremocitrus*, *Clymendia*, *Poncirus*, *Microcitrus* and *Citrus*), which are native to the tropical and subtropical regions of Asia, but the major commercial fruits belong to genus *Citrus*.The genus *Citrus* includes several important fruits such as oranges, mandarins, lime, lemons and grape fruits [18]. Citrus fruits is reported for enormous number of biological activities such as anti-cancer, anti-diarrheal, antibacterial, antifungal, antiviral insecticidal and antioxidant, Some oils have been used in cancer treatment [19,20,21] .

In the present investigation attempts have been made to find out the antimicrobial activity study of raw lemon and honey against human enteric pathogens.

Citrus limon

b) *Apis mellifera*

Materials and Methods

Preparation Juice Lime-Honey

Fresh lime juice that has been cleaned, halved, and squeezed with a manual juicer utility in order to obtain juice and then filtered using a tea strainer. Lemon honey were then added at a ratio of 1:1 (v /v).

Bacterial and Fungal strains

The following bacteria and fungi used for antimicrobial assay respectively, *Klebsiella pneumonia (K. pneumonia* MTCC 109), *Salmonela typhi (S. typhi* MTCC 890), *Proteus vulgaris (P.vulgaris* MTCC 1429), *Aspergillus niger* (*A. niger*MTCC 281) and *Candidaalbicans* (*C.albicans* MTCC 3017) were provided byfrom Microbial Type Culture Collection (MTCC) IMTECH, Chandigarh,India.

Antibacterial activities

The screening of antimicrobial activities of each crude raw lemon and mixture of lemon and honey (1:1) on the tested bacteria used in this investigation was determined on Muller Hinton agar media (all tested organism grow on Muller Hinton agar media), by the using agar well diffusion method. Wells of 6 mm diameter and 5 mm depth were made on the solid agar using a sterile glass borer [22]. Approximately 20µl of each extract was inoculated onto wells were made in the spread plate culture of each microbial isolates. (The plates were performed in triplicates). All plate of the tested organisms was then allowed to incubate at 37°C for overnight. After 24 h of incubation, each extract was noted for zone of inhibition for all isolates. The diameters of the zone of inhibitions were measured by measuring scale in millimetre (mm).

Antifungal activity

For testing antifungal activity the standard test cultures of *Aspergillus niger* (MTCC 281), *Candidaalbicans* (MTCC 3017) were used. The cultures were obtained from Microbial Type Culture Collection (MTCC) IMTECH, Chandigarh. Antifungal activity was determined by agar well diffusion method using Sabouraud‘s dextrose agar (SDA). Test fungus was inoculated by streaking the swab over the entire sterile agar surface to get almost confluent lawn of growth after incubation. A sterile stainless steel borer was used to prepare two cups in the agar media. To each plate, one bore was filled with 100 µl raw lemon and lemon and honey mixture marked accordingly. Petri dishes were then incubated at 25^0C for 24 - 48 h. The diameters of the zone of inhibitions were measured by measuring scale in millimetre (mm)[23].

Results and Discussion

The present study showed varying degree of *in vitro* growth inhibition activity of lemon and mountain honeys against the tested organisms. Results of antimicrobial activity of raw lemon and hone against different human pathogenic isolates by the agar well diffusion method were shown on Table (1) and (2) and Figure(1) and (2) respectively. From this results the maximum zone of inhibition was shown of lemon extract on *K.pneumonia* (21mm) and lemon extract and honey on *Candida albicans* (31mm). But both these extracts are completely fails to inhibit the *Proteus vulgaris.*The results of our study showed that raw lemon extract and honey had broad spectrum of antibacterial and antifungal activity , and it could be used as alternative of antibiotics ,therefore pharmacological tests are necessary to isolate and characterize its active compounds and it should be investigated in-vivo to better understanding of its safety efficiency and properties.

Table 1. Antibacterial activity of raw lemon and honey extracts against human enteric pathogens.

Sl. No.	Zone of inhibition (mm) against different microorganisms		
Extracts	*K. pneumonia* (MTCC 109)	*S. typhi* (MTCC 890)	*P. vulgaris* (MTTC 1429)
Lemon juice	21	15	**
Lemon juice + Honey	16	16	**

Values are mean of three replicates, ± values represent SD, ** = No zone of inhibition.

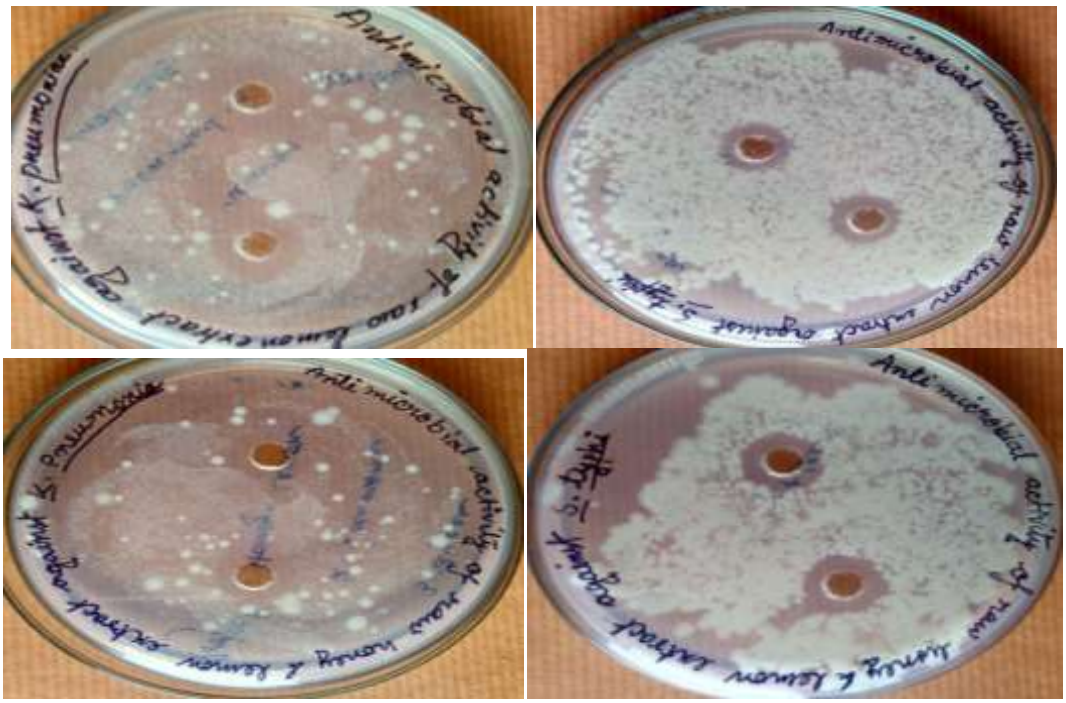
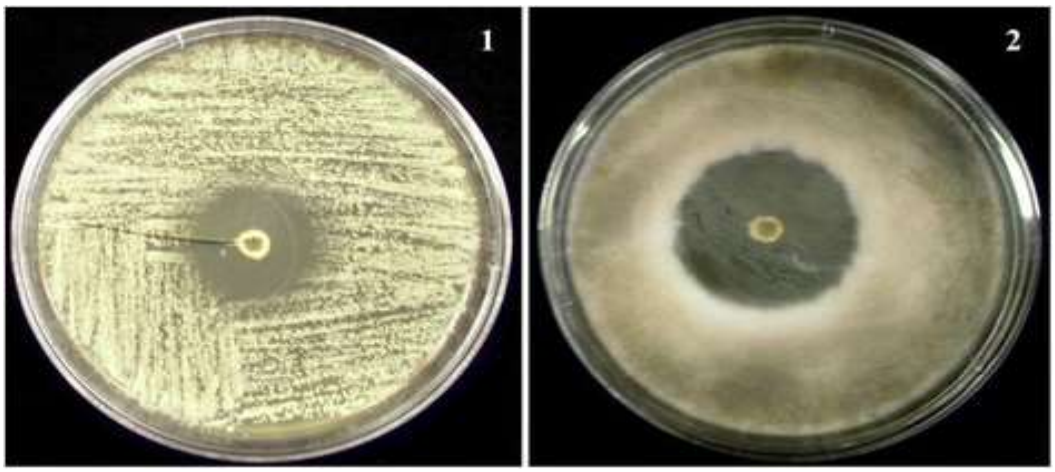


Figure 1. Antibacterial activity of raw lemon and honey extracts against human enteric pathogens.

Table 2. Antifungal activity of raw lemon and honey extracts against pathogenic fungi.

Sl. No.	Zone of inhibition (mm) against different microorganisms	
Extracts	*Aspergillus niger* (MTCC 281)	*Candidaalbicans* (MTCC 3017)
Lemon juice	25	27
Lemon juice + Honey	28	31

Values are mean of three replicates, ± values represent SD



Aspergillus niger (MTCC 281) *Candidaalbicans* (MTCC 3017)

Figure 2. Antifungal activity of raw lemon and honey extracts of against pathogenic fungi.

Conclusion

Lemon juice have an important role as antimicrobial agents against microorganisms. They are natural, cheap, safe, and due to increase antibiotic resistance among bacteria.The present study reveals that mountain honey were effective in inhibiting the *in vitro* growth of human pathogens.

Conflict of interest statement

We declare that we have no conflict of interest.

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