ASSESSING THE ANTIBACTERIAL ACTIVITY OF HONEY AND LEMON JUICE AGAINST BACTERIAL ISOLATED FROM UPPER RESPIRATORY TRACT INFECTIONS

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Abstract: This study aims to assess the antibacterial activity of honey and lemon juice extracts on clinical isolates from upper respiratory tract infections. The antibacterial effect of honey and lemon were investigated, their use separately and in combination against *Staphylococcus aureus, Pseudomonas aeruginosa, Klebsiellapneumoniae, Streptococcus pyogenes* and *Streptococcus pneumoniae*.. Agar diffusion methods were employed to assess degree of susceptibility of the bacteria isolates to honey and lemon juice. Lemon juice together with mixture of honey and lemon juice alone showed a very good antibacterial activity when compared to honey itself. The results indicated that both (lemon and honey) extracts showed considerable antibacterial activity against all clinical isolates from respiratory tract infections. This study justifies the use of honey and lemon juice separately and in mixture as an alternative medicine in the treatment of respiratory tract infections.

Keywords: Honey, lemon juice, synergistic effect, antibacterial activity, zone of inhibition.

I. INTRODUCTION

Honey is a sweet food made by bees using nectar from flowers. The variety produced by honey bees (the genus Apis) is the one most commonly type of honey collected by beekeepers and consumed by humans. The various species of Apis include; Apisandreniformis, Apisflorea, Apisdorsata, Apiscerana, Apiskoschevnikovi, Apismellifera, Apisnigrocincta [22]. Honey bees convert nectar into honey by a process of regurgitation and store it as primary food source in wax honeycombs inside the beehive. The sweet taste of honey is as a result of the monosaccharide; fructose and glucose and has approximately the same relative sweetness as that of granulated sugar [16]. Hydrogen peroxide (H2O2), methylglyoxal (MGO), bee defensing-1, pH and osmotic effect of honey are known to be responsible for the antimicrobial effects [14][13]. Lemon fruit is an inexpensive, easily available citrus fruit, popular for its culinary and medicinal uses [17]. The lemon fruit juice consists of about 5% citric acid that gives a sour (tarty) taste to the lemon. It is a rich source of vitamin C [4].Lemon has been classified scientifically into:

Kingdom: Plantae;
Order: Sapindales;
Family: Rutaceae;
Genus: Citrus;
Species: limon;
Binomial name: Citrus limon.

II. BACKGROUND OF THE STUDY

The respiratory tract extends from the larynx to the nostrils and comprises of oropharynx and the nasopharynx together with the communicating cavities, the sinuses, the middle ear and extends to the lungs. The respiratory tract is the part of the anatomy involved with the process of respiration [10]. The respiratory tract is divided into the upper and the lower respiratory tract. The upper respiratory tract is generally considered to be the airway above the vocal cords or glottis. This includes the nose, sinuses, pharynx, and larynx [18]. While, the lower respiratory tract consists of the trachea (wind pipe), bronchial tubes, the bronchioles, and the lungs [25].Respiratory infections involving this tracts are referred to as respiratory tract infections [8]. Infection of the respiratory tract is one of the most common illness in the general population and results in significant morbidity, which may account for missed days of work and school ,it also contribute to mortality.The respiratory tract infections range from pneumonia (caused by Klebsiellapneumoniae, Haemophilusinfluenzae, Streptococcus pneumonia), eear infection-(otitis media), conjunctivitis, epiglottis (caused by Haemophilus influenza and Pseudomonas aeruginosa), tonsillitis, pharyngitis, laryngitis, sinusitis, and common cold (caused by Staphylococcus aureus, Streptococcus pyogene, Haemophilusinfluenzae, and/or Pseudomonas aeruginosa) which account for number of death in patients especially the immune-compromised, despite improvement in antimicrobial therapy [23]. Up to 15% of acute pharyngitis cases may be caused bybacteria, commonly group A Streptococcus in Streptococcal pharyngitis (Strept-Throat) [11].

III. MATERIAL AND PROCEDURE

3.1. Materials

Equipments: Incubator (Chines company,IB-9272A), autoclave(Bellstone,HI-TECH), Fume hood (BIOBASE), electronic weighing balance (NAPCO,JA-500), refrigerator (Panasonic model), hot air oven (Astral Oven: OOG STD 42) and wire loop.

Glasswares:Test-tubes (Witeg,Germany), beakers (witeg,Germany) conical flask (Witeg,Germany), petri-dishes (Brandon,Malaysia) and measuring cylinder (Witeg,Germany).

Culture Media:Mueller-Hinton agar (SRL), Nutrient broth (NM 012,SRL), Blood agar (SRL) and Nutrient agar (NM 012,SRL).

Test Organisms (Clinical Isolates):Staphylococcusaureus, Streptococcus pneumoniae, Streptococcus pyogene, Klebsiella pneumonia, Pseudomonas aeruginosa

3.2 Methodology

3.2. 1 Collection of Materials: The research was conducted in the Microbiology Laboratory, Department of Biotechnology, Lincoln University College, Kelana Jaya, Malaysia.The strains were obtained from isolated cultures from respiratory tract infected patients at HUKM. Pure colonies were subculture on blood agar and nutrient agar.

Pure Honey (farm honey) was obtained from Taman Tun Market and the lemon was obtained from the giant grocery shop,kotadamansara.

The honey and lemon juice were used in their raw concentration without dilution and the mixture of honey with lemon juice were combined in 50:50 volume to volume ratio.

3.2.2 Preliminary test for the confirmation of the pure honey

Dissolution Test: This is a process of adding some portion of honey into water. If the honey is impure, it will dissolve in the water at the top. But if the honey is pure, it will stick together and sink to the bottom of the glass.

Crystallization Test: This involves subjecting the honey to low temperature. At temperature even below 5oC, pure honey will not crystallize. Therefore, the original texture and flavor is preserved indefinitely[24].

3.2.3 Culture Media Preparation:

The media were prepared according to the manufacturer's direction and sterilized at 121oC for 15minutes. The media were allowed to cool to about 47oC and poured into sterile plates.

3.2.5 Purification of Isolates:

Pure colonies were inoculated into nutrient broth (5ml), and incubated at 37oC for 24hours. All organisms uses were further purified by sequentially streaking a loop full of the resulting overnight broth culture on Blood agar and Nutrient agar to give well distinct isolated colonies after incubation at 37oC for 24-48hours.

The distinct colonies were then picked with the aid of a wire loop and inoculated on nutrient agar slants for subsequent usage.

3.3 Antibacterial Activity Testing

3.3.1 Standardization of inoculum

Overnight cultures of the test organisms were diluted in sterile normal saline to match 0.5 McFarland turbidity as used by [19]. At this point, the organisms should be at a concentration of approximately $10^5$to 10^6cfu/ml.

3.4 Susceptibility Testing of The Bacteria Isolates to Honey and Lemon juice.

The lemon was washed with water (to remove sand and other particles) and then washed with sterile distilled water and then cut using sterile knife before the juice was squeezed out and sieved. The sieving was done to remove the seeds and other particles.

Agar well diffusion technique as used by [1] and [2] was used to determine the antibacterial activities of the honey, lemon and the combinations of the two agents.

Mueller-Hinton agar were prepared and poured into sterile petri-dishes, and then allowed to set.

The prepared inoculum was then spread thinly with sterile swab stick on the surface of the agar. Thereafter, holes were bored using sterile cork-borer to make uniform wells on the inoculated agar.

The bottom of the hole was then sealed with 2 drops of molten sterile Mueller Hinton agar and then 10µl of the test antibacterial agent (honey, lemon, and honey/lemon) was use to fill up the wells.

Care was taken to ensure that the correct measure of the agent was added into each of the holes and to avoid spillage. After which the petri-dishes were incubated at 37oC for 18-24 hrs.

After the incubation period, the diameters of the zones of inhibition were measured in millimeters(mm) with the aid of a meter rule and recorded accordingly.

Table.1.Antibacterial activity of honey against clinical isolates in (mm).

Bacterial Isolates	R1	R2	R3	Mean ± SD
Staphlococcusaureas	20	21	19	20.0 ± 0.816
Klebsiellapneamoniae	8	12	13	11.0 ± 2.160
Streptococcus pneamoniae	14	16	16	15.3 ± 0.942
Streptococcus pyogene	38	35	31	34.6 ± 2.867
Pseudomonas aeruginosa	18	18	20	18.6 ± 0.942

Table .2: Antibacterial activity of lemon juice against clinical isolates in (mm)

Bacterial Isolates	R1	R2	R3	Mean ± SD
Staphlococcusaureas	18	19	19	18.6 ± 0.471
Klebsiellapneamoniae	7	11	6	8.0 ± 2.160
Streptococcus pneamoniae	12	13	13	12.6 ± 0.471
Streptococcus pyogene	31	31	32	31.3 ± 0.471
Pseudomonas aeruginosa	15	15	19	16.3 ± 1.885

Table.3:Antibacterial activity of honey/lemon juice mixture against clinical isolates in (mm)

Bacterial Isolates	R1	R2	R3	Mean ± SD
Staphlococcusaureas	*11*	*10*	*9*	*10.0 ± 0.816*
Klebsiellapneamoniae	*9*	*14*	*9*	*10.6 ± 2.357*
Streptococcus pneamoniae	*10*	*12*	*12*	*11.3 ± 0.942*
Streptococcus pyogene	*21*	*20*	*23*	*21.3 ± 1.247*
Pseudomonas aeruginosa	*11*	*12*	*10*	*11.0 ± 0.816*

IV. RESULTS

In this study, there were three replicates for all the results and the Mean and Standard deviation were also determined. The combination of honey and lemon juice has significant antibacterial activity against bacteria isolated from the respiratory tract infection and also when used separately.

From this study, the clinical bacteria isolates; Klebsiella pneumonia, Staphylococcus aureus, Pseudomonas aeruginosa, Streptococcus pyogenes and Streptococcus pneumoniae show significant susceptibility to the honey, lemon juice and the mixture of both agents (Table 1.2 & 3).

No inhibition zone was observed when water was used as a negative control in any isolates.

Staphylococcus aureus shows susceptibility to lemon juice and mixture of honey and lemon juice compared to honey only. *Streptococcus pneamoniae* also shows susceptibility to honey alone compared to lemon juice and combination of both. *Pseudomonas aeruginosa* shows susceptibility to lemon juice and mixture of honey/lemon juice compared to honey alone. Streptococcus pyogenes shows susceptibility to mixture of honey/lemon juice and lemon juice compared to honey only

V. DISCUSSION

In this study, the clinical bacteria isolates were *Streptococcus pneumoniae*, *Staphylococcus aureus*, *Klebsiellapneumoniae*, *Streptococcus pyogenes* and *Pseudomonas aeruginosa*. In a study by [7], various Gram negative and Gram positive organisms similar to those reported in this work, were isolated from patients presented with respiratory infections.

The common antibiotics used in this study for positive susceptibility testing include: Tetracycline(TE),Streptomycin(S),Vancomycin(VA), Gentamicin(CN), Ampicillin(AM), and Penicillin (P) From the records *Streptococcus pneumoniae* were found to be mostly susceptible to GM, S, and TE but mostly resistant to VA ; *Staphylococcus aureus* were found to be sensitive to GM, and S but showed resistance to AM, VA and TE;

Pseudomonas aeruginosa were sensitive to GM but showed resistance to VA, AM, and TE; Klebsiellapneumoniae were sensitive to GM, TE and S but showed resistance to VA and AM; and Streptococcus pyogenes were mostly sensitive to GM, S , TE and VA but showed resistance to AM and P. The inhibitory or susceptibility of the isolates to the antibiotics is probably due to less abuse of these drugs.

The tested bacterial isolates were susceptible to the crude concentrations of Honey, Lemon juice and the mixture of honey and lemon juice. Some were also moderately susceptible to honey and lemon juice. This is in agreement to works done by[12] and [9] who reported that the stock solution of the honey samples inhibited the growth of all the bacterial isolates.This activity of honey is attributed to its antibacterial property with regards to its high osmolarity, acidity (low pH) and content of hydrogen peroxide (H2O2) and the presence of phytochemical components like methylglyoxal (MGO), non-peroxide component[15]. The antimicrobial agents in honey are predominantly hydrogen peroxide, which is formed by the action of the enzyme glucose oxidase which produces gluconic acid and hydrogen peroxide from glucose [5].

Apart from this study, other researchers has also found antibacterial activity of honey against other bacteria ; for example, research conducted on manuka (L. scoparium) honey, demonstrated effectiveness against several human pathogens, including *Escherichia coli*, *Enterobacteraerogenes*, *Salmonella typhimurium*, *Staphylococcus aureus* [26]. Laboratory studies have revealed that honey is effective against methicillin-resistant *Staphylococcus aureus* (MRSA), β-haemolytic Streptococci and vancomycin resistant Enterococci (VRE) [3].

For *Streptococcus pyogenes* , honey and lemon juice mixture effected a better decrease in the bacterial cell populations than the lemon juice and honey separately .This is due to the highly acidic pH of the honey and lemon juice mixture .

Therefore, as demonstrated in this research, honey, lemon juice and combination of honey/lemon juice were found to possess antibacterial activity, but to varying degrees. The mixture gave a better activity compared to honey alone, lemon juice alone in some of the organisms and relative activity to lemon in other organisms.

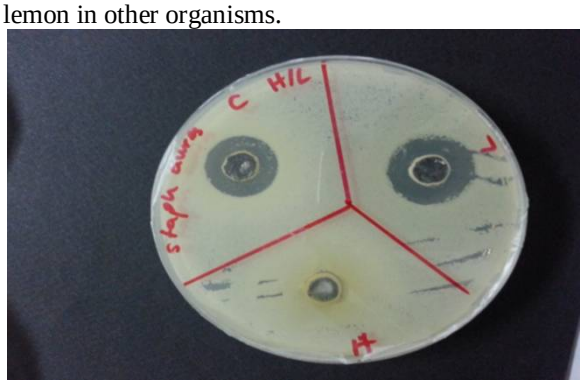

Fig.1. Inhibition zones of *Staphylococcusaureus*

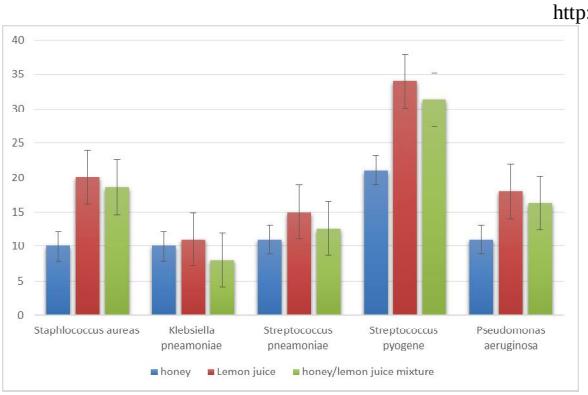

Fig.2.Graph showing the antibacterial activity of honey, lemon juice and mixture of both (in mm) against clinical isolates

CONCLUSIONS

Honey and lemon juice exerted significant antibacterial activity (in vitro) against the various organisms. Honey and lemon mixture gave a better antibacterial activity against the test bacterial isolates, followed by the Lemon and then the Honey. Bacteria isolates associated with respiratory tract infections were found to be Streptococcus pneumoniae, Streptococcus pyogene, Klebsiellapneumoniae, Staphylococcus aureus, and Pseudomonas aeruginosa. Honey and lemon juice has promising antibacterial activity against bacteria that causes respiratory tract infections with relatively higher sensitivity to Staphylococcus aureus and streptococcus pyogenes. Respiratory tract infections are commonly encountered and in this era of ever-increasing antibiotic resistance, use of these non-toxic and inexpensive natural antibacterial agents should be encouraged and further researched upon.

ACKNOWLEDGMENTS

It is of necessity that further investigations be undertaken to discover the possible ways of the clinical effectiveness of honey and lemon in RTIs. Honey samples that have been assayed in the laboratory for antimicrobial activity should be selected for use as an antimicrobial agent.

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