ANTIBACTERIAL EFFECTS VARIOUS TYPES OF HONEY AND CITRUS JUICE ON STREPTOCOCCUS PYOGENES: A SYSTEMATIC REVIEW

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ABSTRACT: The home remedy of taking honey along with a citrus juice of lemon, lime or calamansi to soothe sore throat has long been practiced in many cultures across the world, since ancient times. This paper aims to systematically review the antibacterial effect of honey and citrus juice on Streptococcus pyogenes by means of a systematic search in EBSCOhost, Medline, Scopus and ISI Web of Science databases for reports of studies investigating the antibacterial effects of honey and citrus fruit juice on S. pyogenes. A total of 415 publications were initially identified, out of which, 20 were finally chosen and reviewed by looking at the titles, abstracts and full paper using pre-determined inclusion and exclusion criteria in relation to honey (n=16) and citrus (n=4). The majority of the studies showed that both honey and citrus have significant antimicrobial effect on S. pyogenes. There are still not many available data though on the combined effect of honey and citrus on the bacterium. This knowledge gap offers an opportunity to investigate those effects with the purpose of supporting traditional practice with scientific evidences.

KEY WORDS: Antimicrobial activity, Citrus, Honey, Streptococcus pyogenes, Systematic review

INTRODUCTION

Acute pharyngitis or inflammation of the pharynx ranks within the top 20 most common reasons for clinic visits. It accounts for approximately 12 million clinic-visits annually, in the United States alone (Bata, 2014). Most cases are caused by viruses such as rhinovirus, coronavirus and adenovirus. However, about 15 to 30% of pharyngitis in children and 5 to 10% of pharyngitis in adults are caused by Streptococcus pyogenes (Alcaide et al., 2007). The most common clinical presentation of viral and bacterial pharyngitis is sore throat, even though it is difficult to distinguish between viral and bacterial causes of pharyngitis, on the basis of history and physical examination alone. Viral pharyngitis is self-limiting and requires no antibiotic treatment, whereas bacterial pharyngitis warrants antibiotic treatment to reduce complications. Bacterial invasion of nearby structures may cause otitis media, sinusitis, peri-tonsillar abscess, retropharyngeal abscess, and cervical adenitis.

Clinical guidelines advise against the routine use of antibiotics in patients with upper respiratory tract infection, sore throat, and otitis media (Smith et al., 2004). Recommendations for no antibiotic prescription are based on concerns about the development of antimicrobial resistance and evidence from meta-analyses of randomized controlled trials that have shown minimal benefits (Petersen et al., 2007).

A study conducted among general practitioners in Malaysia showed that antibiotic prescription rates for upper respiratory tract infection was 33.8%. Antibiotic prescribing rates were higher in private clinics compared to public clinics (Teng et al., 2011). According to guideline, treatment of S. pyogenes pharyngitis should be initiated only after confirmation with a rapid test or throat culture (Shulman et al., 2012). Facilities
for identification of S. pyogenes are not available in the general practitioner’s clinical setting. Thus, antibiotics are prescribed based on symptoms, doctor preferences and/or demand from patients. A Malaysian study showed that parents often have inadequate knowledge and misconceptions on antibiotic use for acute upper respiratory tract infection (Chan et al., 2012).

In order to avoid under-treatment of bacterial pharyngitis or over-treatment of viral pharyngitis, alternative medicine should be sought. Taking honey with citrus juice (lemon/lime/calamansi) to soothe sore throat has been practiced in many parts of the world for decades. Various combinations of honey with different types of citrus juice (e.g. lime, lemon and calamansi to name a few) have been traditionally used worldwide for the treatment of sore throat. The amount of honey and citrus juice used also varies from different cultures and places. As aptly stated in a review, “Honey has been used as a medicine for thousands of years and its curative properties are well documented. However, modern medicine turned its back on honey and it is only now, with the advent of multi-resistant bacteria, that the antibiotic properties of honey are being rediscovered” (Molan, 1999).

The present review aims to examine available data on the \textit{in vitro} effect of honey and/or citrus juice against S. pyogenes.

**METHODS**

Full published articles were retrieved using four databases of medical literature which were EBSCOhost (1835 to date), MEDLINE (1879 to date), Scopus (1960 to date) and Thomson Reuters Web of Science formerly known as ISI Web of Knowledge (1966 to date). The search keywords of Honey, Manuka, Tualang, Citrus, Lemon, Lime fruit, \textit{Citrus aurantifolia}, and Calamansi lime were individually searched. Then, combinations of the individual keywords groups of honey (i.e. Manuka, Tualang) and Citrus (i.e. Lemon, Lime fruit, \textit{Citrus aurantifolia}, and Calamansi lime) were then correlated with the combination of \textit{Streptococcus} and \textit{pyogenes}. Wide free search of relevant scholar journals of honey and citrus juice related to \textit{S. pyogenes} was undertaken qualitatively. Furthermore, in order to consider the relevant literature,
Google Scholar search was used to screen publications based on the title and full text. All searches were directed toward papers published until February 2016. An EndNote library was created to compile and manage all titles and abstracts (EndNote X7.4).

By using determined inclusion and exclusion criteria, all the searches were independently screened through the abstracts to identify relevant studies. The inclusion criteria applied for both honey and citrus groups were; i) studies using *S. pyogenes*, ii) studies related to assessment of antimicrobial properties of honey and citrus, and iii) studies that involved antibacterial effect and Minimum Inhibitory Concentration (MIC) / Minimum Bactericidal Concentration (MBC). For honey, studies of all types of honey were included. Similarly, studies which used all types of citrus fruits, and all parts of citrus fruits were also included. Studies that combined other herbs or natural products with honey and citrus were also taken into consideration. This review was limited only to scholarly articles published in the English language. Articles not related to in vitro studies were excluded. Studies involving propolis of honey were also excluded. Newspaper and magazine articles were excluded from the selection.

The primary literature searches were done after applying all the inclusion and exclusion criteria by a single author. All articles were screened and re-evaluated by two authors where unrelated articles were removed. All papers were specifically focused on honey or citrus fruits and their antimicrobial effect against *S. pyogenes*. The protocols for all the final articles were also taken into account as important criteria, since they involve inhibition effect and MIC/MBC. The bacterial source for each study was documented. The methodological quality of systematic reviews was evaluated by Assessment of Multiple Systematic Reviews (AMSTAR) tool (Shea et al., 2007). Each of the studies was assessed to determine any potential bias among the studies chosen. The protocol for each study was evaluated by the species of bacteria used for the assessment of antimicrobial properties involving MIC/MBC of honey or citrus against the bacteria.

Comprehensive data about the first author, year of publication, type of treatment used, methodological aspects, and the outcomes from each of the study were tabulated in Table 1. All the articles were independently screened. Data from each article were tabulated to ensure that the credibility of the extracted lists can be easily assessed. Report of this systematic review was done according to Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines (Moher et al., 2009). PRISMA is an evidence-based tool which consists of a 27 points checklist which helped in reporting the reviews gained.

**RESULTS**

All studies included in the qualitative synthesis were experimental studies. Selected studies involved screening of antimicrobial properties and inhibitory effect of honey and citrus fruit on *S. pyogenes*. Studies involving all types of honey and citrus fruits were included for this review. A total of 421 articles were obtained from all four databases. Of these, 353 references were from the honey group and were obtained from databases EBSCOhost (15), MEDLINE (102), Scopus (227) and Thomson Reuters Web of Science (9). On the other hand, 62 references were from citrus group and were obtained from
# TABLE 1. Summary of the studies on honey included in this review.

<table>
<thead>
<tr>
<th>First author, years</th>
<th>Type of treatment used</th>
<th>Method</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Efem et al., 1992</td>
<td>Honey</td>
<td>MIC</td>
<td>Unprocessed honey inhibited <em>Streptococcus pyogenes</em> but not golden syrup which has similar physical properties as honey.</td>
</tr>
<tr>
<td>Al-Waili et al., 2005</td>
<td>Honey collected from United Arab Emirates</td>
<td>MIC</td>
<td>Honey prevents growth of the <em>Streptococcus pyogenes</em> and inhibits their growth when honey was added to growing culture.</td>
</tr>
<tr>
<td>Tan et al., 2009</td>
<td>Tualang (<em>Koompassia excelsa</em>) and Manuka honey</td>
<td>Broth dilution method</td>
<td>Malaysian Tualang honey exhibited variable activities against <em>Streptococcus pyogenes</em>, and within the same range as manuka honey. This result suggests that tualang honey could potentially be used as an alternative therapeutic agent.</td>
</tr>
<tr>
<td>Oyeleke et al., 2010</td>
<td>Honey samples from Iyale, Dekina Local Government area in Kogi state.</td>
<td>Well diffusion assay</td>
<td>The antibacterial activity of undiluted and diluted honey was effective against <em>Streptococcus pyogenes</em>.</td>
</tr>
<tr>
<td>Voidarou et al., 2011</td>
<td>15 of coniferous origin, 15 of citrus, 15 of thyme origin and finally 15 polyfloral.</td>
<td>Agar well and disk diffusion method</td>
<td>All honeys showed antibacterial activity against pathogenic and ref. strain of <em>Streptococcus pyogenes</em>.</td>
</tr>
<tr>
<td>Halawani et al., 2011</td>
<td>Saudi Arabia (Yemeni Sidr, Taify Sidr, Kashmri Sidr, Shaoka, Somra, Black Seed, Black Forest, and Clover honeys), and Manuka honey</td>
<td>MIC</td>
<td>Shaoka and Sidr honeys surpass other honeys in their antibacterial activity against <em>Streptococcus pyogenes</em>.</td>
</tr>
<tr>
<td>Hegazi et al., 2011</td>
<td>Acacia honey, citrus honey, clover honey, coriander honey, cotton honey, palm honey and sesame honey and saudi honey sample (sider honey)</td>
<td>MIC</td>
<td>The honey samples with different Egyptian honeys and Saudi honey sample (sider honey), were effective antibacterial against <em>S. pyogenes</em>.</td>
</tr>
<tr>
<td>Hegazi et al., 2012</td>
<td>Sidr honey, Tabah honey, Rok honey, spring Lena honey, Harbingers honey, Valley offense (Q tad) honey, Abu roses honey, spring hospitality honey, Agaa mount honey, spring honey pride, Sair honey, Hegaz Spring honey and Shamar mount honey.</td>
<td>MIC</td>
<td>The findings indicated that samples with different Saudi honeys were effective antibacterial agents <em>Streptococcus pyogenes</em> as they inhibited their growth when honey was added to their growing culture.</td>
</tr>
<tr>
<td>Maddocks et al., 2012</td>
<td>Manuka honey</td>
<td>MIC &amp; MBC</td>
<td>In the absence of manuka honey, <em>Streptococcus pyogenes</em> is strongly aggregated but in the presence of 10% (w/v) honey, aggregation was completely inhibited.</td>
</tr>
<tr>
<td>Al Masaudi et al., 2012</td>
<td>Red Egyptian onion, honey alone (Langaneza honey, Black Forest) and honey-onion mixture</td>
<td>Broth dilution method</td>
<td>Mixture (1/1) had a very noticeable effect on <em>Streptococcus pyogenes</em>. Honey-onion mixture was significantly more effective comparing with onion or honey alone.</td>
</tr>
<tr>
<td>Moussa et al., 2012</td>
<td>4 honey samples from 2 areas different from the Algeria west.</td>
<td>Well and disc diffusions method</td>
<td>Algeria honey, in vitro possess antibacterial activity against <em>Streptococcus pyogenes</em>.</td>
</tr>
<tr>
<td>Suntiparapop et al., 2012</td>
<td><em>Tetragonula leaviceps</em></td>
<td>Well diffusion method</td>
<td>The honey was found to inhibit the growth of <em>Streptococcus pyogenes</em>.</td>
</tr>
</tbody>
</table>

Table 1: continued
Manuka honey (Medihoney 2013) studied the antimicrobial activities of the medical grade honey (Molan et al., 1992). Maddocks et al. (2012, 2013) used four different honey samples from Algeria in his study. However, Efem et al. (1992) did not mention honey’s origin for his research. Manuka honey contains high level of hydrogen peroxide which contribute to a great antibacterial activity in this honey (Molan et al., 1992). Maddocks et al. (2012, 2013) studied the antimicrobial activities of the medical grade Manuka honey (Medihoney™). On the other hand, Halawani et al. (2011) and Tan et al. (2009) used Manuka honey for comparison with local honey.

Citrus studies included in this review investigated mainly the antibacterial activity of the fruit juice (Eveline et al., 2014; Kadhim Hindi et al., 2013; Okeke et al., 2015), or fruit peel (Mehmood et al., 2015), whole dried fruit (Kadhim Hindi et al., 2013; Okeke et al., 2015), or fruit peel and juice (Eveline et al., 2014; Mehmood et al., 2015), or extracted essential oil from the zest. Citrus citrus species studied in the latter were lime (Farag et al., 1989; Kadhim Hindi., 2013; Okeke et al., 2015), limetta (El Asbahani et al., 2015; Kadhim Hindi et al., 2013), Citrus sinensis (Mehmood et al., 2015) and Limonia aurantifolia (Eveline et al., 2014).

All selected studies on both honey and citrus showed positive antibacterial activity against S. pyogenes. Table 1 shows methods used to evaluate this. Susceptibility of S. pyogenes towards honey and citrus was tested either using both agar well diffusion and/or disk diffusion methods. On the other hand, the antimicrobial test used was Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC).

### Table 1: Information of the included studies for honey.

<table>
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<th>First author, years</th>
<th>Type of therapy used</th>
<th>Method</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Huttunen et al., 2013</td>
<td>Citrus aurantifolia Swingle</td>
<td>Broth dilution method MIC &amp; MBC</td>
<td>S. pyogenes were susceptible to willow herb, heather and buckwheat honeys.</td>
</tr>
<tr>
<td>Maddocks et al., 2013</td>
<td>Manuka honey</td>
<td>Minimum inhibitory concentration (MIC)</td>
<td>Clinical isolates of Streptococcus pyogenes did not show higher or lower resistance to manuka honey compared with the laboratory strains.</td>
</tr>
<tr>
<td>Al-Waili et al., 2014</td>
<td>Honey was collected from United Arab Emirates</td>
<td>MIC</td>
<td>Honey prevents and inhibits growth of Streptococcus pyogenes either singly or in polymicrobial culture.</td>
</tr>
<tr>
<td>Mahendran et al., 2015</td>
<td>Honey samples were collected from Thani District Western Ghats, India.</td>
<td>Disc diffusion method</td>
<td>Six winter honey samples were more effective in inhibiting the pathogenic bacteria of Streptococcus pyogenes than the summer honey samples</td>
</tr>
</tbody>
</table>

### Table 2: Information of the included studies for citrus fruits.

<table>
<thead>
<tr>
<th>First author, years</th>
<th>Type of therapy used</th>
<th>Method</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kadhim Hindi., 2013</td>
<td>Citrus limon L. (peel, juice and dried fruit) Citrus limetta (peel and juice)</td>
<td>Well diffusion method</td>
<td>The juice of C. limon has antimicrobial activities more than other types of extracts. Streptococcus pyogenes showed resistance to these extracts.</td>
</tr>
<tr>
<td>Eveline et al., 2014</td>
<td>Citrus aurantifolia Swingle</td>
<td>Well diffusion method MIC &amp; MBC</td>
<td>The lime juice conc. at 50% has a strong antibacterial activity for S.pyogenes. Sweet soy sauce and honey addition does not affect the antibacterial activity.</td>
</tr>
<tr>
<td>Mehmood et al., 2015</td>
<td>Citrus sinensis</td>
<td>Agar well and disc diffusion method</td>
<td>Moderate bacterial growth inhibition was recorded with diethyl ether Citrus sinensis extract against Streptococcus pyogenes</td>
</tr>
<tr>
<td>El Asbahani et al., 2015</td>
<td>Thymus leptobotrys, Thymus pallidus and Thymus satureioiodes, Citrus limon, Mentha piperita, Mentha pulegium, Mentha spicata, Pelargonium graveolens and Rosmarinus officinalis.</td>
<td>MIC</td>
<td>Thymus leptobotrys, P. graveolens and T. satureioiodes showed interesting antimicrobial properties. Streptococcus pyogenes shows the highest inhibition against Citrus limon compared to other essential oils tested.</td>
</tr>
</tbody>
</table>

databases EBSCOhost (9), MEDLINE (29), Scopus (16) and Thomson Reuters Web of Science (8). In addition, six other articles were selected through hand picking; four for honey and another 2 for citrus groups.

Upon implementation of inclusion and exclusion criteria, a total of 16 and 4 studies were included in the honey (Figure 1) and citrus (Figure 2) groups, respectively. For studies using honey, a total of six studies studied involved honey obtained from Middle-Eastern regions, namely United Arab Emirates (Al-Waili et al., 2005; Al-Waili et al., 2014), Saudi Arabia (Al Masaudi et al., 2012; Hegazi et al., 2012) while Hegazi (2011) used both Saudi Arabia and Egyptian honey. Moussa (2012) used four different honey samples from Algeria in his study. However, Efem et al. (1992) did not mention honey’s origin for his research. Manuka honey contains high level of hydrogen peroxide which contribute to a great antibacterial activity in this honey (Molan et al., 1992). Maddocks et al. (2012, 2013) studied the antimicrobial activities of the medical grade Manuka honey (Medihoney™). On the other hand, Halawani et al. (2011) and Tan et al. (2009) used Manuka honey for comparison with local honey.

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DISCUSSION

From this review, evidence of in vitro antibacterial activities was documented for all types of honey and citrus fruits tested against *S. pyogenes*. In each study, different strain of *S. pyogenes* was used. The bacteria used were either clinical isolates or reference strain (American Type Culture Collection, ATCC). Nevertheless, some of the studies did not mention origin of the strain (El Asbahani et al., 2015; Farag et al., 1989; Hegazi, 2011; Hegazi et al., 2012).

There are several tests used to assess the antimicrobial activity of an extract against pathogenetic bacteria such as *S. pyogenes*. They differ in their effectiveness and sensitivity depending on the strain used. For screening purposes, the available methods are disc diffusion and agar well diffusion (Farag et al., 1989). Although these methods are not crucial to be implemented in every case, the results from these tests can be used for clarification so as to proceed to the next stage of antibacterial assay (Turnidge et al., 2007). Nevertheless, it is difficult to compare and conclude if honey and citrus extracts have significant antibacterial effects against *S. pyogenes*. This is due to the fact that in clinical practice, in vitro parameter should classify the microorganism as either clinically susceptible, intermediate or resistant (Oyeleke et al., 2010). The interpretive standards for the mentioned classification of the tested microorganism are published by different organization such as CLSI (Clinical and Laboratory Standard Institute) in USA and EUCAST (European Committee on Antimicrobial Susceptibility Testing). Since minimum inhibitory concentration (MIC) is used to measure antibacterial activity in vitro, it has become reference measuring tool for susceptibility testing (Oyeleke et al., 2010). However, some of the studies did not proceed to this stage (Mahendran et al., 2015; Mehmood et al., 2015; Okeke et al., 2015; Suntiparapop et al., 2012; Voidarou et al., 2011; Wiegand et al., 2008).

In order to monitor the level of susceptibility of the *Streptococcus pyogenes*, agar and broth dilution are the most common methods used to determine the MIC and in this way definitive results are also given in cases of unusual resistance and borderline result (Andrews, 2001). Al-Waili et al. (2014) reported for the first time the effect of honey against mixed microbial cultures. The results show that honey can prevent and inhibit growth of single microbe inoculated separately in broth containing honey and also the polymicrobial pathogenic cultures inoculated together in broth containing honey (Al-Waili et al., 2014). However, this finding does not state any standardization in bacterial cell number used for this susceptibility test. This is an important criterion to obtain an accurate and reproducible result. The recommended final inoculum size for broth dilution is $5 \times 10^8$ colony-forming units (CFU) ml$^{-1}$. Thus, it is best to measure the bacteria by dilution or optic density (Sutton, 2011).

Studies by Al Masaudi et al. (2102) and Eveline et al. (2014) investigated the combination of two or more antibacterial agents in the treatment of *S. pyogenes* (Al Masaudi et al., 2012; Eveline et al., 2014). Both these studies have successfully explained the methods employed, the outcomes and the results obtained. However, in order to verify the accuracy of susceptibility test results, at least one control organism should be included with every batch of MIC determination (Andrews, 2001). The collection of studies and obtained evidence show that there are excellent antimicrobial properties possessed by honey and citrus fruits against *S. pyogenes*, which may provide valuable information on alternative eco-friendly ways on treating bacterial infection.

In summary, this review provides evidence for the efficacy of honey and citrus fruits as a good inhibitor against pathogenic bacteria, *Streptococcus pyogenes*. Summaries and results of all studies were shown in Table 1.

CONCLUSION

From all studies included in this review, all types of honey and citrus showed positive in vitro antibacterial activities against *S. pyogenes*. Both of these natural products; honey and citrus, provide possibilities to discover new and effective antibacterial agents. The combination of two or more antibacterial agents has long accepted in the treatment of some microorganisms. In this essence, there is a need for further studies to investigate the combined antimicrobial effect of both honey and citrus against *S. pyogenes*.

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Honey and citrus as antibacterials

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