Effects of Some Seasonal Vegetables and Fruits on the Growth of Bacteria

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Abstract: A total of sixty eight strains of 19 different species of bacteria viz., Bacillus megaterium, B. brevis, B. firmus, B. subtilis, B. pumilus, B. pasteurii, Staphylococcus aureus, S. haemolyticus, Micrococcus luteus, M. sedenterius, M. varians, M. nishinomiyaensis, M. roseus, Escherichia coli, Neisseria sicca, N. mucosa, N. lactamica, N. denticifeces and N. canis were used to evaluate the antibacterial effects of juices of garlic (Allium sativum), lemon (Citrus limonum), unripe papaya (Carica papaya), pomegranate (Punica granatum) and white radish (Raphanus sativus) by well diffusion method. Juice of garlic exhibited the highest activity against all species with average zone of inhibition 32.66 mm. The juices of lemon, unripe papaya and pomegranate also possess good antibacterial effect with 25.81, 21.25 and 17.75 mm, respectively. Besides, all isolates were found resistant to the juice of white radish.

Key words: Antibacterial effect, garlic, lemon, unripe papaya, pomegranate, well diffusion method

INTRODUCTION

Antimicrobial resistance is a natural biological phenomenon exacerbated by the misuse of drugs (Anonymous, 2006). The problem of microbial resistance is growing and the outlook of the use of antimicrobial drugs in future is still uncertain. Therefore, action must be taken to reduce this problem, for example, to control the use of antibiotics, to develop research to better understanding of the genetic mechanism of resistance and to continue studies to develop new drugs either synthetic or natural (Nascimento et al., 2000).

For a long period of time, plants have been a valuable source of natural products for maintaining human health (Tanaka et al., 2006). Different parts of plants, herbs and spices have been used for many years for the prevention of infections. The use of plants with known antimicrobial properties can be of great significance in treatment of infections (Nascimento et al., 2000; Cowan, 1999).

In view of this, the present study gives an access of the antibacterial effects of juices of some seasonal vegetables and fruits viz., garlic (Allium sativum), lemon (Citrus limonum), papaya (Carica papaya), pomegranate (Punica granatum) and white radish (Raphanus sativus) against Gram-positive and Gram-negative bacteria.

MATERIALS AND METHODS

The study was conducted at the Department of Microbiology, University of Karachi, Karachi, Pakistan during the month of December, 2005.

Isolates: A total of sixty eight isolates belonging to 19 different species of bacteria viz., Bacillus megaterium, B. brevis, B. firmus, B. subtilis, B. pumilus, B. pasteurii, Staphylococcus aureus, S. haemolyticus, Micrococcus luteus, M. sedenterius, M. varians, M. nishinomiyaensis, M. roseus, Escherichia coli, Neisseria sicca, N. mucosa, N. lactamica, N. denticifeces and N. canis were used to evaluate the antibacterial effects of juices of garlic (Allium sativum), lemon (Citrus limonum), unripe papaya (Carica papaya), pomegranate (Punica granatum) and white radish (Raphanus sativus).

Preparation of juice: Garlic, lemon, unripe papaya, pomegranate and white radish were purchased from a local market of Karachi, Pakistan. These were washed separately with tap water followed by sterile distilled water. Juices were prepared separately by juicer machine (Moulinex Juice Extractor, Model No. 864).

Screening of antibacterial activity

Media: Mueller-Hinton agar (MHA) (Merek) and Mueller-Hinton broth (MHB) (Merek) were used as base medium for screening of antibacterial activity and for preparation of inoculum, respectively.

Preparation of McFarland nephelometer standard: McFarland tube number 0.5 was prepared by mixing 9.95 mL 1% Sulfuric acid in MHB and 0.05 mL 1% Barium chloride in distilled water in order to estimate bacterial density (Saeed et al., 2005). The tube was sealed and used for comparison of bacterial suspension with standard whenever required.
Preparation and standardization of inoculum: Four to five colonies from pure growth of each test organism were transferred to 5 mL of MHIB. The broth was incubated at 35-37°C for 18-24 h. The turbidity of the culture was compared to 0.5 McFarland Nephelometer standard to get 150-10^6 CFU mL^-1. The standardized inoculum suspension was inoculated within 15-20 min (Saeed et al., 2005).

Well diffusion method: Screening of antibacterial activity was performed by well diffusion technique (Saeed and Tariq, 2005). The MHA plates were seeded with 0.1 mL of the standardized inoculum of each test organism. The inoculum was spread evenly over plate with loop or sterile glass spreader. The seeded plates were allowed to dry in the incubator at 37°C for 20 min. A standard cork borer of 8 mm diameter was used to cut uniform wells on the surface of the MHA and 0.1 mL of each fresh vegetable juice was introduced in the well.

Incubation: The inoculated plates were incubated at 35-37°C for 24 h and zone of inhibition diameter was measured to the nearest millimeter (mm).

RESULTS AND DISCUSSION

In present study, garlic possessed the highest antibacterial effects against all organisms. Lemon, unripe papaya and pomegranate also possessed good antibacterial effects against all organisms while radish did not exhibited any antibacterial effect (Table 1).

Microbial drug resistance is a difficult problem. A large number of bacterial species have become resistant to antibacterial drugs (Garau, 1994). Thus, there is a need to develop alternate strategies. To evaluate the efficacy of phytochemicals concerning the growth of bacteria, extracts of herbs typically have been used. These are normally obtained by means of steam-distillation, dichloromethane extraction (Laenger et al., 1996), maceration, sonication, soxhlet and supercritical fluid extraction with hexane (Vilegs-Jande et al., 1997). These types of preparations are normally unavailable to person in a domestic setting for purposes of self medication. Instead aqueous solutions (e.g., infusion and decoction) and juices that are prepared in the home are most frequently used. Given these considerations, the effects of juices of garlic (Allium sativum), lemon (Citrus limonum), unripe papaya (Carica papaya), pomegranate (Punica granatum) and white radish (Raphanus sativus) on the growth of bacteria were studied.

Garlic has been used for its medicinal properties for thousands of years to treat many conditions including hypertension, infections and snakebite. Currently, it is used for reducing cholesterol level and cardiovascular risk (Setiawan et al., 2005; Tattelman, 2005). It also has beneficial effects on the immune system (Harris et al., 2001). Garlic has also long been known to have antifungal, antiprotozoal, antiviral and antibacterial properties (Bakri and Douglas, 2005). Louis Pasteur was the first to describe the antibacterial effect of garlic juice and found that it exhibits a broad antibacterial spectrum against both Gram-positive and Gram-negative bacteria (Sivam, 2001). Present results are in fair correlation with the study carried out by Reuter et al. (1996) in which garlic has been reported to inhibit Aerobacter, Aeromonas, Bacillus, Citrella, Citrobacter, Clostridium, Enterobacter, Escherichia, Klebsiella, Lactobacillus, Leuconostoc, Micrococcus, Mycobacterium, Proteus, Providencia, Pseudomonas, Salmonella, Serratia, Staphylococcus, Streptococcus and Vibrio. In another study, crude juice of garlic has been found to be strongly active against Escherichia coli, Pseudomonas pyocyaneus, Salmonella typhi and Bacillus subtilis (Abdou et al., 1972). Garlic extract also inhibits the growth of Streptococcus mutans and Porphyromonas gingivalis (Bakri and Douglas, 2005). Besides, the aqueous extract of garlic has also shown significant antibacterial activity against isolates of multi-drug-resistant Shigella dysenteriae, Sh. flexneri, Sh. sonnei and enterotoxigenic E. coli (Chowdhury et al., 1991). Sasaki et al. (1999) found that the antibacterial activity of garlic was resistant to heat treatment at 100°C for 20 min. They also found its activity against E. coli O157 methicillin-resistant Staphylococcus aureus (MRSA), Salmonella enteritidis and Candida albicans. Garlic extract also possess antibacterial activity against Helicobacter pylori at a fairly moderate concentration (Sivam, 2001; Jonkers et al., 1999). H. pylori is a bacterium implicated in the etiology of stomach cancer and ulcers (Sivam, 2001). Thus, garlic has protective effect against stomach ulcers (Setiawan et al., 2005). Allin is the biologically active compound responsible for the antimicrobial properties of garlic. In another study, pure allin was found effective against 31 clinical isolates of Aspergillus in vitro (Shadkhan et al., 2004).

In present study, the antimicrobial effect of lemon was found next to garlic. Lemon is used in cookery and confectionary. It is also used in medicines to correct the taste and augment the power of bitter infusions and tinctures. Lemon is mostly valued for its juice which contains sugars and fruit acids mainly citric acid. The active chemical constituents of lemon are limonene, citral, citronellal, α-terpineol, linalyl, geranylacetate, α-terpinene, β-bisabolene, trans-α-bergamotene, nerol and neral. Lemon has been found to be antifungal and antibacterial. Its action also include antiseptic, antiscorbutic,
Table 1: Antibacterial activity of garlic, lemon, unripe papaya, pomegranate and radish

<table>
<thead>
<tr>
<th>Organisms</th>
<th>No. of isolates</th>
<th>Garlic (mm)</th>
<th>Lemon (mm)</th>
<th>Unripe papaya (mm)</th>
<th>Pomegranate (mm)</th>
<th>Radish (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>B. subtilis</em></td>
<td>3</td>
<td>36.00</td>
<td>24.00</td>
<td>19.67</td>
<td>-</td>
<td>-</td>
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<tr>
<td><em>B. pumilus</em></td>
<td>3</td>
<td>33.67</td>
<td>25.33</td>
<td>17.00</td>
<td>17.00</td>
<td>-</td>
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<tr>
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<td>44.00</td>
<td>19.00</td>
<td>19.00</td>
<td>16.00</td>
<td>-</td>
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<td>32.40</td>
<td>20.80</td>
<td>24.40</td>
<td>15.80</td>
<td>-</td>
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<tr>
<td>*S. haemolyticus</td>
<td>8</td>
<td>35.00</td>
<td>24.50</td>
<td>20.38</td>
<td>18.25</td>
<td>-</td>
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<tr>
<td><em>M. luteus</em></td>
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<td>35.00</td>
<td>31.50</td>
<td>22.00</td>
<td>17.00</td>
<td>-</td>
</tr>
<tr>
<td><em>M. sedentarius</em></td>
<td>2</td>
<td>35.00</td>
<td>32.00</td>
<td>30.00</td>
<td>18.50</td>
<td>-</td>
</tr>
<tr>
<td><em>M. varians</em></td>
<td>3</td>
<td>36.00</td>
<td>29.00</td>
<td>23.00</td>
<td>20.67</td>
<td>-</td>
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<tr>
<td><em>M. miskinomiyaensis</em></td>
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<td>42.00</td>
<td>31.00</td>
<td>22.00</td>
<td>22.00</td>
<td>-</td>
</tr>
<tr>
<td><em>M. roseus</em></td>
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<td>43.00</td>
<td>30.67</td>
<td>27.33</td>
<td>17.33</td>
<td>-</td>
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<tr>
<td><em>E. coli</em></td>
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<td>17.00</td>
<td>-</td>
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<tr>
<td><em>N. stec</em></td>
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<td>33.00</td>
<td>22.50</td>
<td>18.00</td>
<td>15.00</td>
<td>-</td>
</tr>
<tr>
<td><em>N. mucosa</em></td>
<td>4</td>
<td>25.25</td>
<td>25.00</td>
<td>19.50</td>
<td>19.75</td>
<td>-</td>
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<tr>
<td><em>N. lactamica</em></td>
<td>1</td>
<td>32.00</td>
<td>18.00</td>
<td>15.00</td>
<td>17.00</td>
<td>-</td>
</tr>
<tr>
<td><em>N. denitrificans</em></td>
<td>1</td>
<td>32.00</td>
<td>23.00</td>
<td>18.00</td>
<td>14.00</td>
<td>-</td>
</tr>
<tr>
<td><em>N. cinii</em></td>
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<td>32.00</td>
<td>28.00</td>
<td>16.00</td>
<td>25.00</td>
<td>-</td>
</tr>
<tr>
<td>Total</td>
<td>68</td>
<td>32.66</td>
<td>25.81</td>
<td>21.25</td>
<td>170.75</td>
<td>-</td>
</tr>
</tbody>
</table>

*No activity

Antineuralgic, antirheumatic, antispasmodic, cieartisant, coagulant, cooling, depurative, detoxifying, disinfectant, emollient, escharotic, stimulant, insecticide, laxative, stomachic, styptic and vermifuge (Grieve, 2005). The results of present study are in accordance with another study carried out by Valnet (2006) who reported that lemon oil kills meningococi, typhoid bacilli, pneumococci, diphtheria bacteria and *S. aureus*. Literature about the antibacterial activity of its juice is lacking.

Another fruit used in the present study was unripe papaya. Present results are in harmony with the study in which ripe and unripe papaya produced significant antibacterial activity on *S. aureus*, *B. cereus*, *E. coli*, *P. aeruginosa* and *S. flexneri*. While extract of leaves of papaya did not show antibacterial activity (Emeruva, 1982). In another study, ripe and unripe papaya also inhibit the growth of *B. cereus*, *E. coli*, *S. faecalis*, *S. aureus*, *P. vulgaris* and *S. flexneri*. The active ingredients of papaya is flavonoid (Hsu, 2005), which is responsible for antibacterial activity of papaya. Besides, cysteine proteinases, papain, chymopapain, caracian and glyceryl endopeptidase are other bioactive compounds of papaya (Moussaoui et al., 2001). It also has remarkable antiparasitic (Houzanghe-Adote et al., 2005), hepatoprotective (Rajkappaor et al., 2002), antioxidant and wound-healing effect (Mikhalehik et al., 2004). Its fruit is also used in cosmetics for healthy skin complexion. The unripe fruit is also laxative and diuretic (Halimbawa, 2005).

In present study, antibacterial effect of juice of pomegranate was also evaluated. Pomegranate possesses strong antioxidant and anti-inflammatory properties. Pomegranate juice has cancer-chemo-preventive as well as cancer-chemo-therapeutic effects against prostate cancer in humans (Malik et al., 2005). Daily consumption of pomegranate juice improves stress-induced myocardial ischemia in patients who have coronary heart disease (Huang et al., 2005). It also prevents and reduces the symptoms of hypoxic-ischemic brain damage (Loren et al., 2005). New research indicates that pomegranate may offer protection from heart diseases and blood pressure (Aviram et al., 2004). Pomegranate also has antioxidant and antibacterial properties (Negi and Jayaprakasha, 2003). Present findings are contrary to the study in which extract of pomegranate inhibited only *P. aeruginosa* and *B. subtilis* while *S. aureus*, *Salmonella choleraesuis*, *Proteus mirabilis*, *K. pneumoniae*, *Shigella spp.*, *Enterobacter aerogenes* and *E. coli* were found totally resistant to it (Nascimento et al., 2006). On the other hand in another study, it has been reported to inhibit the growth of *S. aureus* (Braga et al., 2005). Its bioactivity is due to the presence of polyphenol (Loren et al., 2005), tannins, flavonoids and ellagic acid (Gil et al., 2000; Sumner et al., 2005). In addition, pomegranate offers antiviral (Li et al., 2004) and antifungal properties and inhibits the growth of *Candida* associated with denture stomatitis (Vasconcelos et al., 2003).

In present study, the antibacterial activity of white radish was also evaluated. Present findings are in fair correlation with a study carried out by Tirrnan et al. (2001), who found that radish has no effect on bacterial growth. In contrary, some workers found that crude juice of radish has been found to be strongly active against *E. coli*, *P. pyocyaneus*, *S. typhi* and *B. subtilis* (Abdou et al., 1972). It was also found that radish...
contains raphanin which is antibacterial and antifungal and inhibits the growth of *S. aureus, S. pneumoniae* and *E. coli* (Anonymous, 2005). It has been used for medicinal purposes by the Egyptians, Greeks, Romans and Chinese. Leaves and roots of radish have been used in various parts of the world to treat cancer. Consuming radish generally results in improved digestion, but some people are sensitive to its acidity and strong action (Prez and Lule, 2004).

**REFERENCES**


