Antimicrobial activity of selected natural products against Gram-positive, Gram-negative and Acid-fast bacterial pathogens

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Abstract

Recurring epidemics of drug resistant bacterial diseases such as those caused by mycobacteria (tuberculosis and non-tuberculous infections), staphylococci (methicillin-resistant Staphylococcus aureus or MRSA infections) and various Gram-negative enterobacteria (enterobacterial infections) have reinforced the need to search for alternative antimicrobials. In this context, we investigated the antibacterial potential of nine different natural products and compared them with the antibiotic controls, using three test bacterial species, representing the Gram-negative (Escherichia coli), Gram-positive (Staphylococcus epidermidis), and Acid-fast (Mycobacterium smegmati) pathogen groups. Six of the nine products showed detectable but variable zones of inhibition (mm²). The antibacterial activity (mm² per 100 mg) of the extracts from the four solid natural products was in the following order for all three pathogen groups: Mint (Mentha arvensis) leaf extract, 264-930>Mushroom (Agaricus bisporus) cap extract, 112-241>Turmeric (Curcuma longa) root extract, 4-10>Ginger (Zingiber officinale) root extract, 3-9. For the liquid products, the activity measured on 100 µL aliquots was in the following order: Eucalyptus (Eucalyptus globules) oil, 264-1044>Mustard (Brassica campestris L. var. brown sarson) oil, 45-96. Taken together, these results indicated the highest activity in Mint extract and Eucalyptus oil against all three test organisms. However, the individual test strains showed the following variable order of susceptibility: Mint extract (M. smegmatis>E. coli>S. epidermidis) and Eucalyptus oil (M. smegmatis>S. epidermidis>E. coli). Based on these results it can be concluded that Mint leaves and Eucalyptus oil have an unusually broad spectrum activity and may, therefore, be promising sources of new broad spectrum antimicrobials.

Introduction

Since the discovery of the first antibiotic, penicillin, in 1929, antibiotics have revolutionized modern medicine. However, in the past few decades, there have been increasing global incidences of microbial infections showing resistance to the existing antimicrobial agents.1,2 The ensuing challenges in the treatment of infectious diseases caused by the drug resistant and emerging pathogens have intensified in the 21st century.3,4 This phenomenon is of great concern and has global public health significance. Our ability to effectively combat resistant and emerging pathogenic strains is dependent on the development of novel antimicrobials. This emphasizes the continuing need for discovering new antimicrobials. In this context, as a part of the latest Public Health Action Plan To Combat Antimicrobial Resistance, an Interagency Task Force on Antimicrobial Resistance (co-chaired by the United States federal agencies CDC, FDA, NIH) has presented a proposal to support development of novel broad spectrum antimicrobials (http://www.cdc.gov/drugresistance/pdf/2010/Interagency-Action-Plan-PreClearance-03-2011.pdf)

For centuries, ancient human civilizations and peoples of various continents have been using natural plant-derived products to heal various ailments, including infections.5-10 In certain Asian and African countries such as India, use of medicinal plants or their derived products still forms a significant part of current medical treatment and practice following traditional medicinal systems, such as the Ayurveda in the Indian subcontinent.11 An estimated 80% of the population in developing nations use medications that are based on components originally derived from medicinal plants.12 A number of traditional medicinal plants have been used for their multifaceted therapeutic properties, including anti-infective effects.13-15 Therefore, one viable alternative could be to identify specific promising natural products that could serve as sources of new broad spectrum antimicrobials. Such antimicrobials could be harnessed to design new antibiotics,16,17 bioactive supplements to antibiotics,18 or for use as disinfectants or preservatives for preventing the spread of resistant microbial strains.

Globally, the bacterial pathogens are responsible for the majority of the infectious diseases such as tuberculosis, enteric diseases, and septic infections. The causative bacterial pathogens for these diseases fall into one or more of the three groups based on their cell wall staining characteristics (http://visualsunlimited.photoshelter.com/image/I0000yfHHPqEytal): Gram-positive, Gram-negative, and Acid-fast bacteria. Specific antibiotics are effective against specific bacterial groups with some exceptions that are designed to possess broad spectrum antimicrobial activity. Novel broad spectrum antimicrobials could be the choice for the 21st-century to combat emerging strains and mixed infections. In light of this, the current study was aimed at investigating the potential of selected natural products as sources of such broad spectrum antimicrobials. In this study, the selection of the test natural products was motivated by their reported historical use for various ailments in traditional Indian medicine (Table 1).

Extracts from nine natural products were tested against the three bacterial types (Gram-positive, Gram-negative, and Acid-fast) using model test species representing the three categories of pathogenic bacteria commonly associated with infectious diseases. To our knowledge, such a broad spectrum antimicrobial activity of these selected products, particularly against mycobacteria, has not been reported.

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Materials and Methods

Natural products and their ethyl acetate extracts

A total of 9 natural products comprising 8 plant-derived products and one dairy product Indian yogurt were investigated. The scientific and common names of the products along with their traditional medicinal use are listed in Table 1. The plant-derived natural products were obtained from the local Indian American (Bombay Grocers, Cincinnati, OH, USA) and International (Jungle Jims, Cincinnati, OH, USA) grocery stores and included four products in liquid form (oil or aqueous extract/juice) and four as solids; the latter were converted into liquid organic extracts. All products except the mushroom caps and the Indian yogurt were imported products from India. Among the oils used in this study, Eucalyptus oil is an essential oil obtained by steam distillation of leaves and twigs of the source plant species. Mustard oil and Neem oil are fixed oils obtained by expression of the source plant species. Mustard oil and Neem oil were imported products from Bombay Grocers (Bombay Grocers, Cincinnati, OH, USA) and used as the test suspension in the antibacterial activity assays. The original preparations (4 oz size each) of the liquid natural products, namely Neem oil, Mustard oil, Eucalyptus oil, Basil juice and Indian yogurt-liquid part (home-made) were used directly in the assay without further concentration or dilution.

Table 1. List of natural products used in the study.

<table>
<thead>
<tr>
<th>Product name (English)</th>
<th>Common name (Hindi)</th>
<th>Botanical name</th>
<th>Family</th>
<th>Plant part/form used</th>
<th>Medicinal applications</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ginger</td>
<td>Adrak</td>
<td>Zingiber officinale</td>
<td>Zingiberaceae</td>
<td>Root</td>
<td>Treat diarrhea, colic infections, nausea, arthritis</td>
</tr>
<tr>
<td>Turmeric</td>
<td>Haldi</td>
<td>Curcuma longa</td>
<td>Zingiberaceae</td>
<td>Root</td>
<td>Indigestion, ulcerative colitis, osteoarthritis, atherosclerosis in some animals</td>
</tr>
<tr>
<td>Mint</td>
<td>Pudina</td>
<td>Mentha arvensis</td>
<td>Lamiaceae</td>
<td>Leaf</td>
<td>Treat stomach ache and other digestive disorders, chest pain, teeth whitening, diuretic</td>
</tr>
<tr>
<td>Mushroom</td>
<td>Mushroom</td>
<td>Agaricus bisporus</td>
<td>Agaricaceae</td>
<td>Cap of mushroom</td>
<td>Cholesterol regulation, some species possess antifungal, antibacterial, antiviral, or antimicrobial properties as a defense mechanism</td>
</tr>
<tr>
<td>Neem oil</td>
<td>Neem</td>
<td>Azadirachta indica</td>
<td>Meliaceae</td>
<td>Seed</td>
<td>Treat various skin disorders (i.e. leprosy, skin ulcers, chicken pox, acne), pest repellant</td>
</tr>
<tr>
<td>Mustard oil</td>
<td>Sarson</td>
<td>Brassica campestris L var. brown sarson</td>
<td>Brassicaceae</td>
<td>Seed</td>
<td>Treat skin disorders, Internal congestion, sore-throat, pneumonia</td>
</tr>
<tr>
<td>Eucalyptus oil</td>
<td>Nilgiri</td>
<td>Eucalyptus globules</td>
<td>Myrtaceae</td>
<td>Leaf/twig</td>
<td>Treat diabetes, coughs and the common cold, congestion, bronchitis, bad breath; can be used to revive someone who has fainted; insect repellant</td>
</tr>
<tr>
<td>Basil juice</td>
<td>Tulsi</td>
<td>Ocimum sanctum</td>
<td>Lamiaceae</td>
<td>Leaf</td>
<td>Used to clear the bronchial tube, treatment for fever and the common cold, coughs, sore throat, respiratory disorder, kidney stones, heart disorder, diarrhea, stress, mouth infections, skin disorders, teeth disorders, insect bites, headaches, eye disorders</td>
</tr>
<tr>
<td>Indian yogurt</td>
<td>Dahi</td>
<td>-</td>
<td>-</td>
<td>Liquid part of the yogurt</td>
<td>Strengthens immune system and digestive health, adequate consumption can minimize bladder and vaginal infections in women, remedy for yeast infections</td>
</tr>
</tbody>
</table>
Preparation of inocula
The three test strains were freshly cultured in their respective broth media at 37°C using 200 rpm until mid-log phase to obtain 120 reading on the Klett-Summmerson photometer. A working stock of the cell suspension (1×10^7 colony forming units/mL) was prepared by centrifugation (10,000g for 15 min) and resuspension in 1×PBS (0.01 M), pH 7.4 (Sigma, St. Louis, MO, USA). The cells were monodispersed by repeated passing through 26 Gauge BD syringe needle (Becton Dickinson & Co., Franklin Lakes, NJ, USA), and the cell suspensions stored at 4°C till use. The cell viability of the working stock was verified based on standard plate count method using the respective agar media.

Antibacterial activity assay
Antibacterial activity of the products/extracts was measured in terms of zone of inhibition of growth in an agar well diffusion assay using each of the three test bacterial species: E. coli, S. epidermidis, and M. smegmatis. A negative control (saline for the liquids and ethyl acetate for the extracts) and a positive control (an appropriate antibiotic) were used for comparison. The antibiotics effective against the test species were obtained from Sigma and used as the following working stocks: amikacin (5 µg/mL) for E. coli, cefoxitin (5 µg/mL) for S. epidermidis, and doxycycline (1 µg/mL) for M. smegmatis. Agar plates for the assay were prepared using the appropriate agar medium, namely trypticase soy agar for E. coli and S. epidermidis; Middlebrook 7H10 agar (MBA) for M. smegmatis, reconstituted according to the manufacturer’s instructions. A defined amount of the cell suspension (100 µL of the 1×10^7 CFU/mL working stock) of E. coli, S. epidermidis, or M. smegmatis was spread on the assay plates using sterile cell spreaders. Uniform defined size wells (8 mm) were made in the agar plates. An aliquot (100 µL) of the extract or liquid product or the antibiotic stock solution was placed in the test well, filling it to two-thirds of its capacity. The actual mg amounts per 100 µL volume of the natural product extracts are shown in Table 2. The final antibiotic amounts used in the positive control wells were amikacin 0.5 µg (E. coli), cefoxitin 0.5 µg (S. epidermidis) and doxycycline 0.1 µg (M. smegmatis). The negative control used was either ethyl acetate (assay plates for solid product extracts) or saline (assay plates for liquid products). The plates were incubated at 37°C for 24 h (or 48 h for M. smegmatis). The assay was repeated three times for each bacterial species using all 9 products/extracts and the two controls.

Calculations
For calculating the net area of the zone of clearance, the following measurements were taken: well diameter in millimeter (mm); total diameter of the zone of clearance (including the well) in mm. These diameters were converted first into radii and then into areas using the formula: area=3.14×r² where r is the radius of the zone (r=half of the diameter). The net area of the zone of clearance was calculated by subtracting the area of the well from the total area.

Results and Discussion
The antimicrobial activity measured as area of zone of inhibition varied with the natural product and with the test bacterial species (Figure 1; Table 2). The results showed that Eucalyptus oil and Mint extract possess the highest activities (Figure 1; Table 2) against all three test strains, albeit to a varying extent. The assay also allowed quantitative comparison of the antibacterial activity of some of the crude extracts (extracts with known mg concentrations) with those of the respective pure test antibiotic controls (Table 2; Figure 1). For instance, Mint extract (10 mg) had approximately 3.5-fold activity against M. smegmatis as compared to 100 ng of doxycycline, 2.7-fold activity against S. epidermidis compared to 500 ng cefoxitin and 5.2-fold activity against E. coli compared to 500 ng of amikacin. The other solid products at their test concentrations showed relatively lower activity than the antibiotic controls.

There have been past reports in the literature documenting the medicinal properties of the tested products or their source plants, including antimicrobial effects. For example, Eucalyptus oil is used by traditional medical practitioners in

| Table 2. Concentrations of the natural product extracts (mother suspensions) and their relative antibacterial activity per unit mass. |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| **Natural product** | **Name** | **Original weight used for extraction (g)** | **Dry weight of the extract (g)** | **Solvent volume used for resuspension (mL)** | **Concentration (mg/mL)** | **Test concentration of the mother suspension per well (µL)** | **Amount (mg)** | **Clearance zone area per mg extract in mother suspension (mm²)** |
| **Mint leaves (dry)** | 2.5 | 0.10 | 1 | 100 | 100 | 10 | 62.5 | 26.4 | 93.0 |
| **Ginger root powder** | 50 | 1.21 | 1 | 1210 | 100 | 121 | 0.37 | 0.28 | 0.86 |
| **Turmeric root powder** | 50 | 1.15 | 1 | 1150 | 100 | 115 | 0.44 | 0.49 | 0.97 |
| **Mushroom (wet)** | 200 | 0.04 | 1 | 40 | 100 | 4 | 11.19 | 12.65 | 24.10 |
Africa to treat tuberculosis and related respiratory diseases. An inhalational therapy involving Eucalyptus oil vapors is administered to treat these ailments. However, according to a recent survey,16 no laboratory studies on its activity toward the tuberculosis pathogen (Mycobacterium tuberculosis) have been reported. In this context, it is promising that the current study provides laboratory evidence of in vitro antimicrobial activity of Eucalyptus oil against a related model Mycobacterium species. Likewise, other natural products such as Mint, Ginger, Turmeric and Mushroom have been in use in traditional Asian medical practice and have been documented to have a range of therapeutic properties.9,15,17-19 Our results provide the scientific basis of the antimicrobial property of these medicinal products and in particular identify the specific broad spectrum antibacterial potential in these products. Lack of considerable antibacterial activity of certain test products such as Neem seed oil and Basil juice in this study may be attributed to the nature or concentration of the specific preparations used. In this context, it has been well documented that the antimicrobial property of plant products depends on the plant part used to obtain the product and the method of extraction.14,23,24

The antimicrobial property of the plant products is due to the presence of specific active chemical components. Such specific antimicrobial chemicals are acquired by plants as a self-defense mechanism to combat microbial attacks and infections in general. These same plant chemicals could possess antimicrobial activities against human pathogens. This hypothesis may possibly explain the reported broad spectrum activities of the tested natural plant products such as Eucalyptus and Mint toward the three major groups of human pathogens. This study has some limitations. For instance, future efforts may be needed to characterize the toxicity aspects of these products. Certain products may have possible toxic properties in their native form, particularly when used at high concentrations. Nonetheless, such possible adverse side effects of the otherwise promising antimicrobial natural products may be circumvented by identification and use of specific active chemical components. This aspect requires further investigation.

Conclusions

In conclusion, the tested natural products varied in their antimicrobial activity against the test bacterial agents as compared to antibiotics. In particular, the Eucalyptus oil and Mint extract showed the highest levels of antimicrobial property against all three test bacterial groups. Considering the fact that they showed a broad spectrum antimicrobial activity, these products provide a promising option for the treatment of respiratory infections. Further studies are needed to validate their therapeutic potential in clinical settings and to identify the active chemical components responsible for their antimicrobial activity.
products may be expected to possess a broad species-independent mechanism to inhibit a bacterial cell. This implies that these products may find potential applications in providing the antimicrobial leads active against the resistant and polymicrobial infections, or in disinfection or preservative applications in clinical and veterinary practice. Future efforts to identify the specific active chemical components with the determination of safe levels for biological use could lead to the development or design of broad spectrum antimicrobials from these promising natural products.

References