A Research Note

Antimicrobial Activity of Some Egyptian Spice Essential Oils

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ABSTRACT

Six spice essential oils (sage, rosemary, caraway, cumin, clove, and thyme) and their basic ingredients were tested for their inhibitory effect against 3 strains of Gram-negative bacteria, 4 strains of Gram-positive bacteria, and one yeast. Preliminary screening of antimicrobial activity of the essential oils was done using the filter paper disc agar diffusion method. The minimum inhibitory concentration for each essential oil against various micro-organisms was also measured. Very low concentrations (0.25 - 12 mg/ml) of the various essential oils were sufficient to prevent microbial growth. The data show that Gram-positive bacteria were more sensitive to the antimicrobial compounds in spices than Gram-negative. The inhibition zones of different microbial growth produced by various essential oils were similar to those produced by their basic compounds. Thyme and cumin oils possessed very strong antimicrobial activity compared with the other essential oils. There was a relationship between the chemical structures of the most abundant compounds in the essential oils under investigation and the antimicrobial activity.

The problem of food preservation has grown to be more complex as new food products are frequently being introduced on the market, requiring longer shelf life and greater assurance of protection from microbial spoilage. There are several chemicals that can be used as antimicrobial agents. For instance, acetic acid and sulfur dioxide are widely used as food preservatives. However, these chemicals require caution in handling since they are corrosive and their vapors can irritate the eyes and respiratory tract. It has been reported that sodium nitrite combines with secondary and tertiary amines, forming nitrosamines which are carcinogenic (7). The most commonly used antioxidants, BHA and BHT, are added to a wide variety of foods to prevent rancidity of lipid-containing products and also have antimicrobial activity (4). These synthetic chemicals convert some ingested materials into toxic substances or carcinogens by the increase of microsomal enzymes (11,15). Consequently, alternative preservatives are needed which possess antimicrobial activity but cause no health problems to the handler and consumer. In this respect, various spice essential oils were tested for their inhibitory activity towards the growth of some micro-organisms. The oregano and thyme were highly toxic to Vibrio parahaemolyticus when present in growth media at a concentration of 0.5% (2). The essential oils of heart wood of Santalum album and of whole part of Glossogyne pinnatifida exhibited antibacterial activity against some pathogenic bacteria such as Bacillus mycoides and Escherichia coli (5). The essential oil from Capillipedium foetidum displayed high antibacterial activity against Gram-positive bacteria and moderate to excellent activity against Gram-negative bacteria (10). Our objectives were to: 1) evaluate the inhibitory potency of some Egyptian spice essential oils on various bacteria representing Gram-positive, Gram-negative bacteria, an acid fast bacterium, and one yeast; and 2) determine whether the antimicrobial effect was due to the major compounds of the oils.

MATERIALS AND METHODS

Micro-organisms

Three microbes representing Gram-negative bacteria (Pseudomonas fluorescens, Escherichia coli, and Serratia marcescens), four Gram-positive bacteria (Staphylococcus aureus, Micrococcus spp., Sarcina spp., and Bacillus subtilis), and acid fast bacterium (Mycobacterium phlei), and one yeast (Saccharomyces cerevisiae) were used in the present study. All strains were grown in a nutrient agar (beef extract, 3 gm; peptone, 5 gm; agar, 15 gm/L) adjusted to pH 7.0. For growing yeast, sucrose (5 gm) was added to the nutrient agar.

Essential oils

Sage leaves, rosemary leaves, caraway fruits, clove flower buds, cumin fruits, and thyme leaves were collected from the Pharmacy Farm, Cairo University. The plant materials, cut into small pieces (ca 100 gm) were placed in a flask (2 L) together with double distilled water (1.5 L). A continuous steam distillation extraction head was attached to the flask. After steam distillation
Authentic volatile compounds

A set of 24 standard materials with a stated purity of 99% by GLC was obtained from Dragoco company (Holzminden, West Germany). The standard materials were: cyclic terpenes (α-pinene, B-pinene, camphene, limonene, α-terpinene, terpinolene, and phellandrene); aliphatic hydrocarbon (myrcene); aromatic hydrocarbon (p-cymene); sesquiterpene (caryophyllene); phenol and phenol ethers (eugenol, thymol, and methyl chavicol); cyclic terpene ketones (carvone, dihydrocarvone, and thujaone); aromatic aldehyde (cumin aldehyde); aliphatic terpene alcohols (linalool and geranial); cyclic terpene alcohols (t-carvol, α-terpineol, and borneol) and terpene esters (linalyl acetate and terpinyl acetate).

Identification and determination of essential oil composition

The essential oils were analyzed by a GCV Pye Unicam gas chromatograph equipped with dual flame ionization detectors. The chromatograph was fitted with a coiled glass column (1.5 m X 4 mm) packed with Diatomite C (100-120 mesh) and coated with 10% PEGA. The oven temperature was programmed at 4°C/min from 60°C to 180°C and isothermal operation at 180°C for 15 min. Detector and injector temperatures were 220°C and 300°C, respectively. Gas flow rates for N₂, H₂ and air were 30, 33 and 330 ml/min, respectively. Peak identification was performed by comparing the relative retention times of each peak with those of known compounds. Also, the essential oils were mixed with their major compounds and injected into GLC to verify the peak identity. The relative retention times for thymol, cumin aldehyde, camphene, eugenol, α-pinene, and thujaone are given a value of 1.00 depending on essential oil origin. The peak areas were measured by triangulation, and percentage of each oil component was calculated as the ratio of the peak area to the total chromatographic area. All samples were analyzed in triplicates and the values agreed within 2%.

Antibacterial activity

Disc-diffusion method.

The disc-diffusion method was used to measure the antimicrobial activity (14). The essential oils were added at a concentration of 1 mg/disk (1 cm diameter) in triplicates while their basic compounds were added according to their presence in the neat oil. Inoculated plates were incubated at 30°C for 24-48 h and the inhibition zones of the microbial growth produced by different essential oils were measured.

Minimum inhibitory concentration (MIC) method.

Different concentrations of the essential oils up to 20 mg/ml were thoroughly mixed with sterilized nutrient agar (10 ml) using a Julabo ultrasonic bath at 35 KHz, then poured into petri dishes and inoculated by streaking with suspension of each microorganism. The plates were incubated at 30°C for 1 to 3 d and the lowest concentration of the essential oil required to inhibit the growth of the test microorganisms was designated as the MIC.

RESULTS AND DISCUSSION

Preliminary screening of the antibacterial activity in vitro of the essential oils from sage, rosemary, cumin, caraway, clove, and thyme was studied against some bacteria and one yeast using the filter paper disk agar diffusion technique. The data for the inhibition zones (mm) of various microorganisms indicate that sage, cumin, rosemary oils and their basic components had no or very little effect against the Gram-negative bacteria. The oil of caraway (2-2.5 mg/ml) showed moderate effect against Gram-negative bacteria, whereas, the oils from clove and thyme (0.75-1.5 mg/ml) were highly active. The trend for the activity towards the Gram-negative bacteria by clove and thyme oils was the same for the Gram-positive bacteria. Generally, the Gram-positive bacteria were more sensitive towards all the oils under study than the Gram-negative ones. For instance, thyme and clove oils showed inhibition zones of >2.5 and >2 mm for Staphylococcus aureus and Micrococcus spp., while >2 and >1.5 mm inhibition zones for the Gram-negative bacteria, respectively. The values for the inhibition zones for M. phlei using all the oils under investigation were generally higher than the Gram-negative and positive bacteria. In general, the essential oils under study displayed activity against Saccharomyces cerevisiae and this influence was similar to that found with Gram-positive bacteria. Generally speaking, the inhibition zones of different microbial growth produced by various essential oils were similar to those produced by their basic components. Consequently, the activity of these oils can be explained by reference to their basic components.

The minimum inhibitory concentrations (mg oil/ml medium) of some essential oils against various microorganisms are shown in Table 1. The data show that Gram-negative bacteria were more resistant to various essential oils than Gram-positives. Generally, E. coli was less resistant compared to other Gram-negative bacteria. Ps.fluorescens and Serratia marcescens had the same efficacy power against various essential oils. Very low concentrations (0.25-12 mg/ml) of the essential oils were quite sufficient to prevent the growth of all tested Gram-positive

<table>
<thead>
<tr>
<th>Essential oil</th>
<th>Gram-negative bacteria</th>
<th>Gram-positive</th>
<th>Acid-fast bacteria</th>
<th>Yeast</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pseudomonas</td>
<td>Serratia</td>
<td>Micrococcus</td>
<td>Bacillus</td>
</tr>
<tr>
<td></td>
<td>fluorescens</td>
<td>marcescens</td>
<td>spp.</td>
<td>subtilis</td>
</tr>
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<td>Sage oil</td>
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<td>3.50</td>
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<td>0.75</td>
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<tr>
<td>Rosemary oil</td>
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<td>1.25</td>
<td>1.75</td>
</tr>
<tr>
<td>Cumin oil</td>
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<td>0.50</td>
</tr>
<tr>
<td>Caraway oil</td>
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<td>2.00</td>
<td>1.25</td>
<td>1.00</td>
</tr>
<tr>
<td>Clove oil</td>
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<td>1.25</td>
<td>0.75</td>
<td>0.75</td>
</tr>
<tr>
<td>Thyme oil</td>
<td>1.00</td>
<td>0.75</td>
<td>0.125</td>
<td>0.25</td>
</tr>
</tbody>
</table>

TABLE 1. Minimum inhibitory concentrations (mg/ml medium) of some essential oils.
bacteria and *S. cerevisiae*. In general, the oils of thyme and clove had strong potent antibacterial efficacy against the micro-organisms under investigation. Consequently, these two oils provide a powerful means for inhibiting the growth of micro-organisms. Similar findings have been reported by others (3,6,13).

The volatile matter composition of sage, rosemary, caraway, clove, cumin, and thyme oils was determined by gas-liquid chromatography. Thyme oil containing thymol (42.7%) and p-cymene (36.0%) as the most prevalent compounds. The major substances for cumin were cumin aldehyde (55.7%), B-pinene (20.6%) and terpinolene (12.0%). Caraway oil was characterized by the presence of carvone and p-cymene (15.8%) as major materials. Eugenol occurred, as a major component only in clove oil (85.3%). The most abundant substances in rosemary oil were borneol (26.5%), α-terpinene (15.6%) and α-pinene (12.7%). Sage oil had the highest concentration of thujone (41.5%) and limonene (14.7%). Similar results were in accordance with the data of others (1,9,12).

It appears that there is a relationship between the chemical structures of the most abundant compounds in the tested essential oils and the antimicrobial activity. Generally, the extent of the inhibitory effect of the oils could be attributed to the presence of aromatic nucleus containing a polar functional group. The wide spread use of phenol and chlorophenols and related compounds as disinfectants is well established. Borneol and thujone had little inhibitory effect compared to thymol due to the absence of an aromatic moiety. The higher inhibitory action of thymol might be due to the presence of phenolic OH groups. It is well known that the OH group is quite reactive and easily forms hydrogen bonds with active sites of target enzymes. The inductive effect of the isopropyl group must also be taken into consideration besides the aromaticity of the molecule.

Our data suggest that the essential oils under study and in particular thyme and clove oils can be applied practically as anti-microbial agents and as food treatments which will prevent the deterioration of stored foods by bacteria. Recently, thyme and cumin oils were used in an attempt to prevent butter deterioration during storage at room temperature (8). Their results showed that these oils remarkably decreased both the total bacterial and lipolytic bacterial counts.

REFERENCES