ABSTRACT
The present study compares the antibacterial activities of ethanol extracts of four Indian spices, which are used in day-today Indian food with emphasis on their phytochemical composition. The spices included turmeric (Curcuma longa), clove (Syzygium aromaticum), cinnamon (Cinnamomum verum) and black pepper (Piper nigrum). The extracts showed presence of alkaloids, flavonoids, saponins and terpenoids in different quantities. The ethanolic extract of clove and black pepper showed antibacterial activity against all five test human pathogenic bacteria. Ethanolic extract of cinnamon produced higher size of inhibitory zone against Bacillus cereus ATCC 10876 in comparison to positive control; ampicillin.

KEYWORDS: Spices, phytochemicals, antibacterial activity, food.

INTRODUCTION
The traditional Indian cuisine is not only known for its great taste, but also due to the exotic spices used to prepare such food. These spices, apart from adding taste; add distinct flavour and aroma to the food. Most of these spices have been shown to have medicinal properties in ancient Indian literature i.e. Ayurveda and the fact is well supported by the modern day science.

For centuries, Indian spices have made a significant contribution both in the health care system and the food industry. According to Ayurveda, spices contribute a major amount for the treatment of key disorders of the human body. Homeopathic medicine also has been using spices as one of the chief ingredients in most of their preparations. The modern day
medication system also uses natural products from a variety of spices as drugs and pharmaceuticals.

Spices, like other plants, possess a mixture of phytochemicals carrying out various activities. Apart from phytochemicals that add flavour, odour, colour or texture to the food (i.e. isoflavones, anthocyanins and flavonoids), many other phytochemicals are also of great importance. Among such phytochemicals are those who exhibit antibacterial, antifungal, antioxidant and chemoprotective activities.\[1\]

Spices, such as clove (toothache, fever, pain), cinnamon (nervous problems, stomach/intestine infections), turmeric (antimicrobial, wound healing), garlic (antiseptic, diuretic), ginger (digestive aid, cold), black pepper (cough and cold) etc. have been reported to possess very good medicinal properties.\[2\] Although, these spices provide a wide spectrum of medicinally important activities, a comparative account of various species is often not available. Such studies are needed in reference to optimize their use in food as well as in medicine. The present study compares the phytochemical profiles of four traditional Indian spices namely, turmeric (Curcuma longa), clove (Syzygium aromaticum), cinnamon (Cinnamomum verum) and black pepper (Piper nigrum) viz a viz their antibacterial properties against human pathogenic bacteria.

**MATERIALS AND METHODS**

*Extraction of phytochemicals from spices*

The spices used for the present study were purchased from local market. Only the plant part that is used as spice was selected for all the tested spices i.e. dried roots of turmeric, dried flower buds of clove, bark of cinnamon and dried fruit of black pepper. The roots of the turmeric were descaled before use, while the other spices were used as such. The spices were ground using a mixer grinder so as to get the powder able to pass 100 µm sieve. The powders were stored in an airtight jar for further use. Ten grams of each spice powder was extracted with ethanol using Soxhlet extraction. The extracts obtained were evaporated in vacuo and the residue was redissolved in 10 ml of absolute ethanol, filtered and stored under refrigeration.
Phytochemical analysis

Qualitative screening of various secondary metabolites were performed using standard protocols.[3] The methods for quantitative analysis of different phytochemicals are given below-

For alkaloids, 5 g of the each spice sample was taken and 200 ml of 10% diethyl ether in ethanol was added to it. The mixture was allowed to stand for 4 hours, filtered and the filtrate was concentrated on a water bath until it is ¼ of the original volume. Alkaloids were precipitated by adding concentrated NH₄OH and the precipitate collected on a pre-weighed filter paper. After washing with dilute NH₄OH, the precipitated alkaloid was dried and weighed.

For quantification of flavonoids, 10 g of each spice sample was extracted with 100 ml of 80% aqueous methanol. The mixture was then filtered through a filter paper into a pre-weighed beaker and was allowed to evaporate to dryness and weighed. The percentage of flavonoids was calculated as weight of extracted dry matter.

For saponins, 25 g of each spice powder was mixed with 100 ml of 20% ethanol. The mixture was heated at about 55°C over a hot water bath for 4 hours with continuous stirring, filtered through a Whatman No. 42 filter paper and the residue was re-extracted with another 200 ml of 20% ethanol. The combined extract was concentrated to 40 ml at about 90°C. In a separatory funnel, the concentrated extract was shaken vigorously with 20 ml of diethyl ether. The aqueous layer was recovered from three such extractions and pooled. The saponins were further extracted by 60 ml of n-butanol and was washed twice with 10 ml of 5% NaCl. The remaining solution was dried to a constant weight and the percentage of total saponins was calculated by difference in weight.

For quantification of total terpenoids, 10 g of spice powder was soaked in alcohol for 24 hours, filtered and the filtrate was extracted with petroleum ether; the ether extract was treated as total terpenoids.

For total phenolic content, 1.5 ml of Folin-Ciocalteau reagent (SRL, India) and 1.2 ml of 75% (w/v) sodium carbonate solution was taken in test tubes. To these tubes, 0.3 ml of each spice extract was added, vortexed for 15 sec and allowed to stand for 30 min at room temperature.
Absorbance was measured at 765 nm and results were expressed as milligram of gallic acid equivalent per gram of extract weight using calibration curve of gallic acid (R²=0.952).

**Antibacterial Activity**

The antibacterial activity of the different extracts from four spices was evaluated against five clinically important bacteria, i.e. *Staphylococcus aureus* ATCC 25923, *Bacillus cereus* ATCC 10876, *Escherichia coli* ATCC 35218, *Pseudomonas aeruginosa* ATCC 27853, *Salmonella enteric* serovar *typhimurium* ATCC 13311. The test organisms were sub-cultured in nutrient broth for 2-8 h before the test. The antibacterial tests were performed by agar well diffusion method using Müller Hinton agar plates. The solidified plates were swabbed with 0.1 ml of each test organism’s broth (turbidity equivalent to 0.5 McFarland) in respective plates. Wells of 6 mm were punched using well punching machine at equal distance and the well was loaded with 50 μl extracts of cyanobacteria. On each plate, 50 μl of absolute ethanol served as negative control. Ampicillin (100 μg disc) was used as positive control on separate plates for each tested bacteria.

**RESULTS**

The phytochemical screening program showed that all the four ethanolic extracts of different spices showed presence of major phytochemicals that may be responsible for the antibacterial activity. All four extracts were positive for alkaloids, flavonoids, saponins and terpenoids. the phytochemicals were then quantified to get more insight view. Table 1 shows that alkaloids were higher in black pepper followed by turmeric and cinnamon and least in clove extract. in contrast to alkaloids, flavonoids were higher in clove extract followed by cinnamon. Clove extract showed highest saponins among all while black pepper showed least amount of saponins. The terpenoids were highest in cinnamon followed by turmeric ethanolic extract.

**Table 1: Qualitative estimation of major phytochemicals in ethanolic extracts of spices.**

The data are presented as amount of phytochemical in mg per gram. Values are presented as mean ± standard deviation (n=3).

<table>
<thead>
<tr>
<th>Spices</th>
<th>Phytochemicals (mg g⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Alkaloids</td>
</tr>
<tr>
<td>Clove</td>
<td>82.00 ± 3.4</td>
</tr>
<tr>
<td>Cinnamon</td>
<td>312.00 ± 23.2</td>
</tr>
<tr>
<td>Turmeric</td>
<td>360.00 ± 21.5</td>
</tr>
<tr>
<td>Black pepper</td>
<td>474.00 ± 34.8</td>
</tr>
</tbody>
</table>
Antibacterial activity was measured in vitro against five clinically important human pathogenic bacteria. Table 2 shows that ethanolic extracts of clove and black pepper were effective against all five tested pathogens, while extracts of cinnamon was effective against all but E. coli ATCC 25922. The ethanolic extract of turmeric was effective only against Gram positive bacteria i.e. S. aureus ATCC 25923 and B. cereus ATCC 10876.

Table 2: Effect of ethanolic extract of four spices on human pathogenic bacteria in vitro.
The inhibition zone was measured in millimetres and the values are presented as mean ± standard deviation for three replicates. Ampicillin 100 µg disc served as control.

<table>
<thead>
<tr>
<th>Extract</th>
<th>E.coli ATCC 25922</th>
<th>Salmonella typhimurium ATCC 13311</th>
<th>Pseudomonas aeruginosa ATCC 27853</th>
<th>Staphylococcus aureus ATCC 25923</th>
<th>Bacillus cereus ATCC 10876</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clove</td>
<td>12.5 ± 1.2</td>
<td>14.2 ± 1.3</td>
<td>14.3 ± 0.9</td>
<td>14.8 ± 0.97</td>
<td>12.2 ± 1.1</td>
</tr>
<tr>
<td>Cinnamon</td>
<td>-</td>
<td>12.1 ± 0.8</td>
<td>12.8 ± 1.1</td>
<td>13.1 ± 1.2</td>
<td>18.5 ± 2.1</td>
</tr>
<tr>
<td>Turmeric</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>10.2 ± 0.9</td>
<td>10.1 ± 1.1</td>
</tr>
<tr>
<td>Black pepper</td>
<td>12.2 ± 0.6</td>
<td>19.3 ± 2.1</td>
<td>10.8 ± 1.2</td>
<td>20.2 ± 2.1</td>
<td>10.2 ± 1.1</td>
</tr>
<tr>
<td>Control</td>
<td>18.7 ± 2.3</td>
<td>36.2 ± 1.2</td>
<td>32.9 ± 2.1</td>
<td>25.1 ± 1.3</td>
<td>14.2 ± 0.8</td>
</tr>
</tbody>
</table>

DISCUSSION

Since time immemorial, spices have been known to exert synergistic effects on human health by employing one or more types of biological activities apart from providing flavour and aroma to the food. The antibacterial activities of spices have always been the centre of attraction due to the various ailments caused by pathogenic bacteria and a regular need to counter such harmful bacteria during living. No wonder, this has made the necessity of spices in daily food.

The plant secondary metabolites (phytochemicals) are mainly responsible for the biological activities exerted by the plants or plant part. These plant compounds, including glucosides, saponins, tannins, alkaloids, essential oils, organic acids and others, are present as parts of the original plant defense system against microbial infection.[4] The antimicrobial activity of saponins and flavonoids in plants like oats (Avena sativa) and anthraquinones, carbohydrates and alkaloids derived from plants like Bersama engleri (Melianthaceae) have been proven.[5,6,7,8,9] Essential oils, constituted mainly of terpenoids, may exert antimicrobial effects such as components in oregano, clove, cinnamon, garlic, coriander, rosemary, parsley, lemongrass, sage and vanillin.[10] In the present study also, the compounds that have potential to act as antimicrobial compounds i.e. alkaloids, terpenoids, saponins and flavonoids have
been detected in varying concentrations. All four spices used in present study were rich in alkaloids and flavonoids and hence it can be speculated that these are the prime phytochemicals responsible for antibacterial activity.

Although, a variety of reports deal with antibacterial activity of spices, only few of them shown greater antibacterial activities. Joe et al. showed the antimicrobial activity of ethanolic extracts of Allium sativum (garlic), Zingiber officinale (ginger) and Piper nigrum (pepper) extracts against Klebsiella pneumoniae, Staphylococcus aureus, Morganella morgani, Candida albicans, Escherichia coli and Proteus vulgaris.\(^{[11]}\) Only garlic extract showed good antibacterial activity against P. vulgaris and M. morgani. The ginger extract showed moderate antimicrobial activity against S. aureus, whereas the pepper extract showed the least activity against the test organisms. Our study showed that black pepper extracts showed antibacterial activity against five tested pathogens. The discrepancy is because of different bacterial strains used in the study as well as the concentrations of the extract. Recently, Pandey et al. studied antibacterial activity of five common Indian spices namely clove, ajwain, turmeric, dalchini and black pepper against Klebsiella pneumoniae and Staphylococcus aureus. The results revealed that the methanol extracts of spices showed low MIC values as compared to the acetone extracts of spices in the same concentration.\(^{[12]}\)

Ghosh et al. concluded the spices have different degrees of bacterial growth inhibition, depending on the strains and combination of solvent extract used. The present study selected the test pathogens based on the guidelines of clinical microbiology and in this way presents the more authentic record of antimicrobial activity of spices.\(^{[13]}\) Further, the authentic cultures of test bacteria were used in the study and in this way the data reflected the usefulness of the study against human pathogenic bacteria.

REFERENCES


