

**Research Article****Phytochemical evaluation and antibacterial activity of four Indian spices**Ravi Datta<sup>1</sup>, Hari Om Saxena<sup>2\*</sup>, Arun Kakkar<sup>1</sup><sup>1</sup>Natural Products Laboratory, Department of Chemistry, Govt. Science College (Autonomous), Jabalpur (M.P.) – 482001, India<sup>2</sup>Tropical Forest Research Institute, Jabalpur (M.P.) – 482021, India

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**Abstract**

**Objective:** The objective of the present work is to evaluate four Indian spices viz. *Cinnamomum verum*, *Curcuma longa*, *Piper nigrum* and *Syzygium aromaticum* for phytochemicals and antibacterial activity. **Material and Methods:** The powdered spices were extracted in ethanol and these ethanolic extracts were evaluated for their phytochemicals using standard methods. The antibacterial activity of ethanolic extracts was studied by agar well diffusion method. **Results:** The extracts of all four spices showed presence of alkaloids, flavonoids, saponins and terpenoids in different quantities. These extracts exhibited positive results against clinically important gram positive bacteria i.e. *Bacillus cereus* and *Staphylococcus aureus* and gram negative bacteria i.e. *Escherichia coli*, *Salmonella typhimurium* and *Pseudomonas aeruginosa*. **Conclusion:** It can be concluded that the ethanolic extracts of tested spices possess therapeutically important phytochemicals and *in-vitro* antibacterial activity.

**Keywords:** Spices, phytochemicals, antibacterial activity, gram positive and gram negative bacteria

**Introduction**

Since time immemorial, spices have been widely used as flavoring agents and natural medicines against a variety of human diseases. Most of the Asian countries have rich diversity of wide range of spices used for various ailments (Pei, 2001). The spices could be the seed, fruit, root, bark, berry, bud or vegetable substances which exhibited antimicrobial, antioxidant and chemo-protective activities due to presence of various secondary metabolites in different amounts (Lampe, 2003; Kapilan, 2015). The medicinal properties of most of the spices have been well supported by modern day science. According to Ayurveda, spices contribute significantly for the treatment of key disorders of human body. Spices such as Clove (toothache, fever, pain), Cinnamon (nervous problem, intestine infection), Turmeric (antimicrobial, wound healing), Garlic (antiseptic, diuretic), Ginger (digestive aid, cold), Black pepper (cough and cold) etc have been reported to possess various medicinal properties (Tapsell et al., 2006). The present study has been planned to evaluate the four Indian spices for phytochemicals and antibacterial activity.

**Materials and methods****Spices selected for the study**

The spices used in the present study were purchased from local market. Dried rhizomes of turmeric, flower buds of clove, bark of cinnamon and fruits of black pepper were selected for the phytochemical analysis and antibacterial activity. The rhizomes 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 through 100 µm sieves. The powdered materials were stored in airtight jars for further use.

**Extraction of powdered spices**

The 10 gm of each spice powder was extracted with ethanol using Soxhlet extractor. The extracts obtained were evaporated *in vacuo* and the residues were re-dissolved in 10 ml of absolute ethanol, filtered and stored under refrigeration.

**Phytochemical Analysis**

The ethanolic extracts of selected spices were quantified for alkaloids, flavonoids, saponins and terpenoids by using standard methods.

**Quantification of Alkaloids**

The alkaloid content of samples was measured using 1, 10 phenanthroline method given by Singh et al. (2004). 0.1 gm

**\*Address for Corresponding Author:**

Dr. Hari Om Saxena,  
Tropical Forest Research Institute, Jabalpur (M.P.) – 482021, India  
Email: hariomsaxena81@gmail.com

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of powdered plant material was weighed and kept in 20 ml of 80% methanol for 24 hrs. It was then centrifuged at 10,000 rpm for 10 minutes and supernatant was taken. 0.1ml of this supernatant was then mixed with 1.9 ml of 80% methanol, 2ml ferric chloride and 2 ml phenanthroline. The tubes were then incubated at 70 °C for ½ hr and the absorbance was measured at 510 nm against blank. Alkaloid content was measured and calculated with the help of standard graph of Colchicine.

$$\text{Percentage of Alkaloids} = \frac{\text{Conc. of standard} \times \text{O. D. of sample} \times \text{Total volume made up}}{\text{O. D. of standard} \times \text{Weight of sample} \times \text{Volume taken}} \times 100$$

### Quantification of Flavonoids

Total flavanoids were determined by aluminium chloride colorimetric technique reported by Chang et al. (2002). 0.5 gm sample was weighed and kept in 95% ethanol for 24 hrs. It was then filtered and volume was made up to 25 ml with 80% ethanol. 0.5 ml of filtered was then mixed with 1.5 ml of 95% ethanol, 0.1 ml of 10% aluminium chloride, 0.1 ml of potassium acetate and 2.8 ml water. The tubes were then incubated at room temperature for 30 minutes and absorbance was measured at 415 nm. The flavanoid content of the samples was calculated from the standard graph of Quercetin.

$$\text{Percentage of flavonoids} = \frac{\text{Conc. of standard} \times \text{O. D. of sample} \times \text{Total volume made up}}{\text{O. D. of standard} \times \text{Weight of sample} \times \text{Volume taken}} \times 100$$

### Quantification of Saponins

Total saponins were determined by the method reported by Obadoni and Ochuko (2001). The 2 gm of sample was taken into a conical flask and 25 ml of 20% ethanol was added. The samples were heated over a hot water bath for four hours at 55°C. The mixture was filtered and the residue was re-extracted with another 25 ml of 20% ethanol. The combined extracts were reduced to 15 ml over water bath at about 90°C. The concentrate was transferred into a 250 ml separation funnel and 20 ml of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The aqueous layer was further separated by 60 ml (2 x 30) of butanol. The combined butanol extracts were washed twice with 10ml of 5% sodium chloride. The extract was then transferred to a pre-weighed beaker and dried in oven upto a constant weight. Percentage of saponin was calculated from the following formula:

$$\text{Percentage of Saponin} = \frac{(B - A) \times 100}{\text{Weight of sample}}$$

Where,

B = weight of beaker with content

A = weight of empty beaker

### Quantification of Terpenoids

Total terpenoid content in spice powders were determined by the method as described by Ferguson, (1956). 10 gm of each spice powder was taken in a conical flask and soaked in ethyl alcohol for 24 hrs, filtered and filtrate was extracted with petroleum ether. The ether extract was treated as total terpenoid content.

### Evaluation of antibacterial activity

The antibacterial tests were performed by agar well diffusion method using Muller Hinton agar plates (Jahangirian et al., 2013). The antibacterial activity of the different extracts of four spices was evaluated against two Gram-positive i.e. *Bacillus cereus* ATCC 10876 and *Staphylococcus aureus* ATCC 25923 and three Gram-negative i.e. *Escherichia coli* ATCC 35218, *Pseudomonas aeruginosa* ATCC 27853 and *Salmonella typhimurium* ATCC 13311 bacteria. The test organisms were sub-cultured in nutrient broth for 2-8 hrs before performing the test. The solidified plates were swabbed with 0.1 ml of each test organism's broth (turbidity equivalent to 0.5 McFarland) in respective plates. Well 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.

### Statistical analysis

Each experiment was carried out in triplicate and results expressed as Mean ± SD (n=3).

### Results and discussion

Wide range of spices is being used in food preparations by the people living in Asian countries. In the traditional ayurvedic treatments, a number of plant extracts are used to prepare drugs to treat different human ailments (Bonjar et al., 2004). Different studies have been carried out to understand the role of such plant extracts in human body and their anti-microbial properties (Voravuthikunchai et al., 2005; Vaishnavi et al., 2007; Kapilan, 2015). In the present study, four spices were tested for their phytochemicals and antibacterial property against five bacterial pathogens. The results of phytochemicals i.e. alkaloid, flavonoid, saponin and terpenoid contents of ethanolic extracts of spices are summarized in table 1. It can be observed that all spices contained higher alkaloid content than other phytochemicals. The black pepper contained the maximum alkaloid content among all spices while clove was found to contain highest flavonoid and saponin contents. Cinnamon contained the highest terpenoid content.

**Table 1.** Phytochemical contents in ethanolic extracts of spices

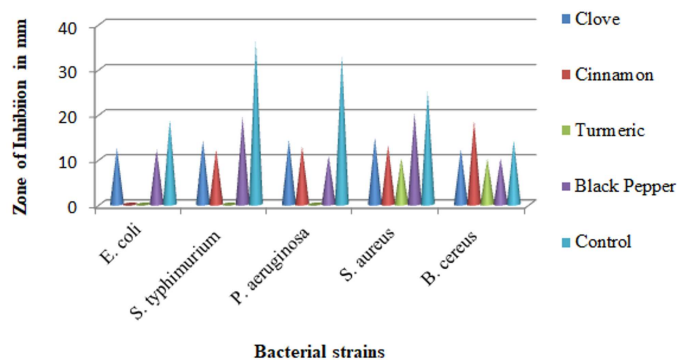
Spices	Phytochemicals (mg gm <sup>-1</sup> ) (Mean ± SD)			
	Alkaloids	Flavonoids	Saponins	Terpenoids
Clove	82.00 ± 3.4	17.75 ± 1.4	7.82 ± 1.2	0.18 ± 0.07
Cinnamon	312.00 ± 23.2	10.78 ± 2.6	2.12 ± 1.1	16.12 ± 2.1
Turmeric	360.00 ± 21.5	7.53 ± 1.8	6.36 ± 1.8	12.36 ± 1.6
Black Pepper	474.00 ± 34.8	7.66 ± 1.1	0.14 ± 0.04	0.10 ± 0.06

**Table 2.** *In-vitro* anti-bacterial activity of ethanolic extracts of spices

Ethanolic extracts	Zone of inhibition in mm (Mean ± SD)				
	<i>E. Coli</i> ATCC 25922	<i>P. aeruginosa</i> ATCC 27853	<i>S. typhimurium</i> ATCC 13311	<i>B. cereus</i> ATCC 10876	<i>S. aureus</i> ATCC 25923
Clove	12.5 ± 1.2	14.3 ± 0.9	14.2 ± 1.3	12.2 ± 1.1	14.8 ± 0.97
Cinnamon	-	12.8 ± 1.1	12.1 ± 0.8	18.5 ± 2.1	13.1 ± 1.2
Turmeric	-	-	-	10.1 ± 1.1	10.2 ± 0.9
Black Pepper	12.2 ± 0.6	10.8 ± 1.2	19.3 ± 2.1	10.2 ± 1.1	20.2 ± 2.1
Control	18.7 ± 2.3	32.9 ± 2.1	36.2 ± 1.2	14.2 ± 0.8	25.1 ± 1.3

Antibacterial activity of spices was measured *in vitro* and the results are represented in table 2 and figure 1 which showed that ethanolic extracts of clove and black pepper were found effective against all five tested pathogens, while extract of cinnamon was effective against all except *E. coli* ATCC 25922. The ethanolic extract of turmeric was seen effective only against Gram-positive bacteria i.e. *S. aureus* ATCC 25923 and *B. cereus* ATCC 10876.

In the present study, all four spices were found to contain alkaloids, flavonoids, saponins and terpenoids in different concentrations, therefore, it can be speculated that these phytochemicals may be responsible for their antibacterial activities which is supported by the study of Aboaba and Efuwape (2001) who reported the antimicrobial potency of the spices due to the presence of secondary metabolites i.e. tannins, saponins, essential oils, phenolic compounds and flavonoids.

**Figure 1.** *In-vitro* anti-bacterial activity of ethanolic extracts of spices

The effectiveness of crude extracts of spices has also been reported against the multidrug resistant bacterial strains where modern antibiotic therapy has limited impact. Table 2 showed *in vitro* antibacterial activity only, however, for accurate results, detailed *in vivo* study will be needed to ensure their action with diverse bacterial species and this would provide scientific evidence for their efficacies. Mishra and Behal (2010) reported that practical approach using disk plate assays and the size of inhibition zone to indicate relative antibacterial activity is not adequate to determine the antibacterial nature of the spices. Recently, Pandey et al. (2014) also studied antibacterial activity of five common Indian spices namely clove, turmeric, cinnamon, ajwain and black pepper against *Klebsiella pneumoniae* and *Staphylococcus aureus*. The results revealed that the methanolic extracts of spices showed low MIC values as compared to their acetone extracts in the same concentration. Ghosh et al. (2015) reported that the spices have different degree of bacterial growth inhibition depending on the strains and combination of different solvent extracts used. Thus, in continuation of previous studies on spices carried out by various authors the present study is also an effort to establish the therapeutic potential of spices.

### Conclusion

The antibacterial activity of spices has always been the center of attraction due to the various ailments caused by pathogenic bacteria. No wonder, this has made the necessity

of spices in daily food. The spices are the integral part of the human diet and long known for their pharmacological potentials. The present study estimated the important secondary metabolites i.e. alkaloids, flavonoids, saponins and terpenoids in these four Indian spices along with their antibacterial activities which will be useful in upgrading the knowledge on spices and their usefulness as supplementary or alternative medicines against bacterial infections.

#### Conflicts of interest

Authors do not have any conflicts.

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