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Evaluation of antibacterial and phytochemical properties of different spice extracts

Suman Upadhyaya*, Divya Yadav, Ram Chandra and Naveen Arora

Department of Environmental Microbiology, Babasaheb Bhimrao Ambedkar University (A Central University), Vidya Vihar, Rae Bareli Road, Lucknow, 226025, Uttar Pradesh, India.

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Now-a-days majority of world population rely on the plant preparations as medicines to cure diseases, as they are considered safe and as effective as allopathic preparations without any side effects. Spices are plant products having aroma, are mainly used during cooking to impart flavor and taste to the dish and also possess medicinal values. The present study was designed to evaluate the antimicrobial activity of four Indian spices: clove *(Syzygium aromaticum),* cinnamon *(Cinnamomum verum*), turmeric *(Curcuma longa),* and black pepper *(Piper nigrum)* against Gram positive and Gram negative pathogenic bacteria viz., *Staphylococcus aureus* (ATCC 25923), *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), and *Klebsiella pneumoniae* (ATCC 70063) using aqueous, 75% ethanol and 75% chloroform extracts. The antibacterial activity of spices extract and spices which are used in powder form was determined by agar well diffusion method and the antimicrobial activity of antibiotics (Clindamycin, Ciprofloxacin, Gentamycin) was measured by disc diffusion method. The clove and cinnamon extracts had exhibited maximum antibacterial property against pathogens; also, turmeric had shown less antimicrobial activity, while black pepper had exhibited moderate activity. Spices (powder) had shown highest antimicrobial activity than spice extracts. Phytochemical screening was carried out on ethanol, chloroform and distilled water extracts of spices for its chemical composition. Qualitative phytochemical analysis of these spice extracts confirm the presence of various phytochemicals like alkaloids, terpanoids, flavonoids, saponins, steroids and tannins.

Key words: Antimicrobial activity, *Piper nigrum,* agar well diffusion method and phytochemicals.

INTRODUCTION

The natural products are more effective with least side effects when compared to commercial antibiotics. Consequently they are used as a substitute medication for curing various infections. Spices are plant substances that are generally used to enhance flavor, which include leaves (coriander or mint), flower bud (clove), fruits (black

*Corresponding author. E-mail: upadhyayasuman@gmail.com. Tel: +91 522 244 0822, 9936302204. Fax: +91 522 244081.

pepper), bark (cinnamon), and rhizomes (turmeric) (Pavithra et al., 2016). Natural antioxidants such as flavonoids, tannins and phenols are increasingly attracting because they are disease preventing, health promoting and anti-aging substances (Tamizhazhagan et al., 2017). Medicinal plants produce bioactive molecules that have both antibacterial and antifungal activities. Spices have been found to reduce inflammation, protect against infection, helps to detoxify the liver and also protect from cell damage that can lead to rheumatoid arthritis, osteoporosis, heart disease and other degenerative diseases. The term spices refer to aromatic or pungent vegetable substances used for flavouring foods and have several commercial uses according to International Organization for Standardization (ISO). Since ancient times people used spices for preventing food deterioration and pathogenic diseases. Spices have become today as an integral part of our daily diet and many of the spices are widely used to flavour food and beverages for food preservations, medicinal preparations, cosmetics, perfumery, bakery goods and various other products (Shiva Rani et al., 2013). Even today spices are used as an ingredient in drug preparations in Unani, Homeopathy and Ayurveda systems of medicine (Bharath et al., 2016). Phytochemicals are bio-active chemicals of plant origin and are regarded as secondary metabolites because the plant that manufactures them they may have little need for them. They are naturally synthesized in all parts of the plant body; bark, leaves, stem, root, flower, fruits, seeds etc. that is, any part of the plant body may contain active components (Tiwari et al*.,* 2011). Homeopathic medicine has been using spices as one of the chief ingredients in most of their preparations. Phytochemical investigations of the aerial parts of the plants have tartaric acid, acetic acid, citric acid, succinic acid, gums, pectin, sugars, tannins, alkaloids, flavonoids, glycosides and sesquiterpenes (Bharath et al., 2016). Although the primary purpose of spices is to impart flavour and piquancy to food, the medicinal, antimicrobial and antioxidant properties of spices have also been exploited. Major antimicrobial components in clove and cinnamon have been reported to be eugenol and cinnamaldehyde, respectively, which have been given special attention to find their antimicrobial activity against micro-organisms. Spice, cinnamon or *Cinnamomum zeylanicum*, found in the inner bark of Cinnamon trees, is commonly used in cooking for its aroma, flavor, and taste. Historically, cinnamon has been used by the Egyptians for embalming, most likely due to its antimicrobial properties. Eugenol and cinnamaldehyde are the two major chemical components in cinnamon that are responsible for its health benefits. Eugenol, a phenol compound, inhibits mold and adds flavor and aroma to bakery items (Mahfuzul et al., 2008). Now micro-organisms have become resistant to many antibiotics due to increased use of drugs, which is decreasing efficiency of conventional medicines. So it has become necessary to find out new antimicrobial agents. *Syzygium aromaticum* (Linn.) cloves the aromatic dried flower buds of a tree in the family Myrtaceae (Srivastava and Malhotra, 1991; Chaieb et al*.,* 2007a) cloves are used in Ayurveda, Chinese medicine and western herbalism. In addition, the cloves are antimutagenic (Miyazawa and Hisama, 2003), antiinflammatory (Kim et al*.,* 1998) antioxidants (Chaieb et al*.,* 2007b), antiulcerogenic (Bae et al*.,* 1998; Li et al*.,* 2005), antithrombotic (Srivastava and Malhotra, 1991) and antiparasitic (Yang et al., 2003).

The objective of this research is to evaluate the antimicrobial activity of clove (*S. aromaticum*), cinnamon (*C. zeylanicum),* Turmeric (*Curcuma longa)* and black pepper (*Piper nigrum*) powder extracts (ethanol, aqueous and chloroform) against *Pseudomonas aeruginosa* (ATCC 27853), *Staphylococcus aureus* (ATCC 25923), *Escherichia coli* (ATCC 2592), *Klebsiella pneumonia* (ATCC 70063). Antibiotics (*clindamycin, ciprofloxacin and gentamycin*) were used to determine the sensitivity of bacterial species. Phytochemical screening was carried out on ethanol, chloroform and distilled water extracts of spices for its chemical composition.

MATERIALS AND METHODS

The spices namely cinnamon (*Cinnamomum verum*), black pepper (*P. nigrum),* clove (*S. aromaticum*) and Turmeric (*C. longa*) used in the study were collected from the local market in Lucknow.

Bacterial strains: The micro-organisms were obtained from the research laboratory of S.G.P.G.I., Lucknow. The microbes used in the study includes:

i) *E. coli* (ATCC 25922)
ii) *P. aeruginosa* (ATCC 27853)
iii) *K. pneumoniae* (ATCC 70063)
iv) *S. aureus* (ATCC 25923).

Antibiotics: Gentamycin for *K. pneumoniae*, Ciprofloxacin for *E. coli* and *P. aeruginosa,* and Clindamycin for *S. aureus.*

Solvents: 75% Chloroform, Distilled water and 75% Ethanol.

Maintenance of bacterial cultures and preparation of inoculum

Pure cultures were subcultured and maintained on nutrient agar plates regularly. The cultures were inoculated on sterile agar plates and placed in an incubator at 37°C for 24 h and was stored at 4°C. Bacterial cultures were subcultured after every 3-4 days to avoid contamination. Inoculum was prepared by inoculating the pure culture in nutrient broth and incubated overnight at 37°C (Pavithra Sivakumar et al., 2016).

Preparation of spice extracts

Three solvents distilled water, 75% ethanol and 75% chloroform were used to extract phytochemical of clove, cinnamon, turmeric and black pepper.

Table 1. Antibacterial activity of standard discs.

S/N	Micro-organisms	Antibiotics	Zone of inhibition (mm)
1	*E. coli*	Ciprofloxacin	28
2	*S. aureus*	Clindamycin	32
3	*P. aeruginosa*	Ciprofloxacin	24
4	*K .pneumoniae*	Gentamycin	20

Extraction with distilled water: 10 g of powdered plant material was dissolved in sterile distilled water to make 40 ml of aqueous extract (25% w/v). The mixture was placed undisturbed at room temperature for 24 h in a sterile flask and it was filtered through sterilized Whatman no.1 filter paper. After filtration, the extract was evaporated in water bath until 25 ml extract was left in the container (Barreto et al*.,* 2002).

Extraction with 75% ethanol: 10 g of powdered plant material was dissolved in enough ethanol to make 40 ml of ethanolic extract (25% w/v). The extraction procedure followed was the same as that used for aqueous extract (Barreto, et al*.,* 2002).

Extraction with 75% chloroform: 10 g of powdered plant material was dissolved in methanol to make 40 ml of methanolic extract (25% w/v). The extraction procedure was similar to that used for aqueous extract. Extracts thus obtained were evaluated for antibacterial activities (Barreto et al*.,* 2002).

Determination of antibacterial activity

Agar well diffusion method

The antimicrobial activity of the extracts was performed by well diffusion method (Parez et al*.,* 1990). The bacterial suspension was spread on Muller Hinton Agar (MHA) medium. Sterile 8 mm diameter cork borer was used to make well in the nutrient agar. Each well was filled with 0.1 ml of spice extract. Negative control was prepared using the same solvents employed to prepare the plant extracts. The inoculated plates were incubated at 37°C for 24 h and the clear zone of growth inhibition around the well was measured.

Antibiotic sensitivity test

Disc diffusion method

A single colony of the purified isolates was inoculated in 5 ml sterile peptone water and incubated at 37°C overnight. Thereafter, a loopfull culture was diluted in 5 ml sterile phosphate buffered saline and seeded into Muller Hinton agar. Antibiotic disc (Hi-Media) was placed on the surface of agar and incubated overnight at 37°C. Zone of inhibition was recorded and a control sensitive culture was included in the experiment (Bauer et al*.,* 1966).

Phytochemical screening of spices

Screening for alkaloids

To 5 ml each of the spice extracts, 5 ml of aqueous hydrochloric acid was added on a steam bath at 60°C for 5 min. The spice extract was filtered with a 3 layered muslin cloth. In one ml of the filtrate, few drops of Draggendoff's reagent was added. Appearance of Blue black turbidity was positive for alkaloids (Omoya and Akharaiyi, 2012).

Screening for steroids

1 ml of extract was dissolved in 10 ml of chloroform and equal volume of concentrated sulphuric acid was added by the sides of the test tube. The upper layer turns red and sulphuric acid layer showed yellow with green fluorescence. This indicates the presence of steroids (Jyothiprabha and Venkatachalam, 2016).

Screening for tannins

5 ml each of the extracts were stirred separately with 100 ml distilled water and filtered. One millilitre ferric chloride reagent was added to the filtrate. A blue-black or blue green precipitate was an indication of the presence of tannins (Trease and Evans, 1989).

Screening for terpenoids

5 ml of extract was taken in a test tube and 2 ml of chloroform was added to it followed by the addition of 3 ml of concentrated sulphuric acid. Formation of reddish brown layer at the junction of two solutions confirms the presence of terpanoids (Jyothiprabha and Venkatachalam, 2016).

Screening for flavonoids

5 ml of diluted ammonia solution was added to aqueous extract followed by the addition of 1 ml concentrated H_2SO_4. Appearance of yellow colour indicated the presence of flavonoids (Harborne and Williams, 2000).

Screening for saponins

5 ml each of the extracts were mixed with distilled water and shaken separately in a test tube. Frothing, which persists on warm heating was taken as preliminary evidence of the presence of the saponins (Omoya and Akharaiyi, 2012).

RESULTS AND DISCUSSION

The findings of this research are as shown in Tables 1 to 9 and Figures 1 to 8. In India, spices are ethnically used as active ingredients in ayurvedic medicines and reported

Table 2. Antibacterial activity of negative control.

S/N	Micro-organisms	Ethanol solution (zone of inhibition)	Chloroform solution (zone of inhibition)	Distill water (zone of inhibition)
1	*E. coli*	-	-	-
2	*S. aureus*	-	-	-
3	*P. aeruginosa*	-	-	-
4	*K .pneumoniae*	-	-	-

Table 3. Antimicrobial activity of chloroform extract of spices against micro-organisms.

S/N	Spices	Micro-organisms with zone of inhibition (mm)			
		K. pneumoniae	*S. aureus*	*E. coli*	*P. aeruginosa*
1	Cinnamon	6	7	6	6
2	Clove	12	12	12	16
3	Black pepper	-	-	7	-
4	Turmeric	-	-	-	-

Table 4. Antimicrobial activity of aqueous extract of spices against micro-organisms.

S/N	Spices	Micro-organisms with zone of inhibition (mm)			
		K. pneumoniae	*S. aureus*	*E. coli*	*P. aeruginosa*
1	Cinnamon	9	10	8	8
2	Clove	12	14	10	14
3	Black pepper	-	-	7	-
4	Turmeric	-	-	-	-

Table 5. Antimicrobial activity of ethanol extract of spices against micro-organisms.

S/N	Spices	Micro-organisms with zone of inhibition (mm)			
		K. pneumoniae	*S. aureus*	*E. coli*	*P. aeruginosa*
1	Cinnamon	8	14	11	8
2	Clove	11	11	12	12
3	Black pepper	-	8	10	-
4	Turmeric	-	6	9	12

to possess a number of pharmacological effects to treat different human ailments (Bonjar et al., 2004). Several investigations have been directed towards their anti-microbial properties (Voravuthikunchai et al*.,* 2005; Vaishnavi et al., 2007). In the present work, among the four spices (clove, black pepper, cinnamon and turmeric) tested against four bacterial pathogens (*P. aeruginosa, S. aureus, K. pneumoniae, E. coli*), all the spices showed antibacterial activity. The results of antibacterial activity of these spices against pathogens tested revealed that of the three types of extracts tested, ethanolic and aqueous extracts of spices had shown better antibacterial activity against pathogens when compared to chloroform extracts of the spices. This may be due to the better solubility of active ingredients of spices in alcoholic and distilled water solvents than chloroform. Ahmad et al (1998) also reported that alcoholic extracts of medicinal plants had greater activity than their aqueous extracts. Earlier studies have demonstrated that the extracts of cinnamon, clove, turmeric and black pepper had shown maximum

Table 6. Antimicrobial activity of spices (powder) against micro-organisms.

S/N	Spices	Micro-organisms with zone of inhibition (mm)			
		K. pneumoniae	***S. aureus***	***E. coli***	***P. aeruginosa***
1	Cinnamon	14	14	12	8
2	Clove	12	12	14	10
3	Black pepper	7	8	7	7
4	Turmeric	7	7	7	-

Table 7. Phytochemical screening of chloroform extract of spices.

S/N	Phytochemicals	Chloroform extracts			
		Cinnamon	**Clove**	**Black pepper**	**Turmeric**
1	Alkaloids	-	-	+	+
2	Flavonoids	-	-	-	-
3	Tannins	-	-	-	-
4	Saponins	+	+	+	+
5	Steroids	-	-	-	-
6	Terpenoids	+	+	+	-

Table 8. Phytochemical screening of aqueous extract of spices.

S/N	Phytochemicals	Aqueous extracts			
		Cinnamon	**Clove**	**Black pepper**	**Turmeric**
1	Alkaloids	+	-	+	-
2	Flavonoids	+	+	-	+
3	Tannins	+	+	+	+
4	Saponins	+	-	+	-
5	Steroids	+	+	-	-
6	Terpenoids	+	+	+	+

Table 9. Phytochemical screening of ethanol extract of spices.

S/N	Phytochemicals	Ethanol extracts			
		Cinnamon	**Clove**	**Black pepper**	**Turmeric**
1	Alkaloids	-	-	+	-
2	Flavonoids	-	-	-	-
3	Tannins	-	+	-	+
4	Saponins	+	-	-	-
5	Steroids	+	+	+	+
6	Terpenoids	+	+	+	-

antimicrobial activity against pathogens than other spices. In the present study it was found that turmeric had shown less antimicrobial activity, while black pepper had exhibited moderate activity. Spices (Powder) had shown highest antimicrobial activity than spice extracts. It was observed that cloves and cinnamon had shown highest antimicrobial property against gram negative bacteria. Shihabudeen et al. (2010) and Ceylan and Fung

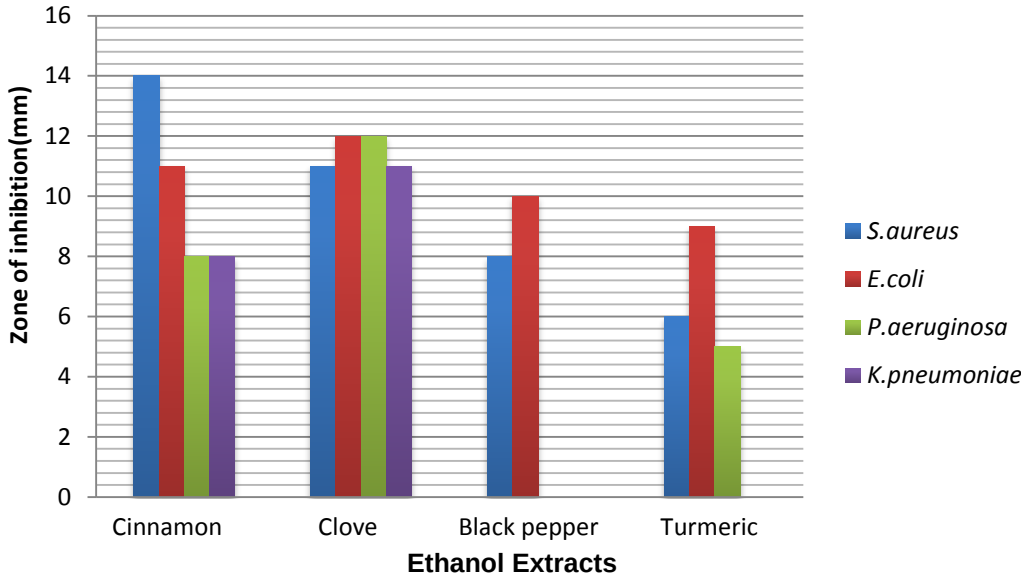

Figure 1. Graph of antimicrobial activity of ethanol extracts against micro-organisms.

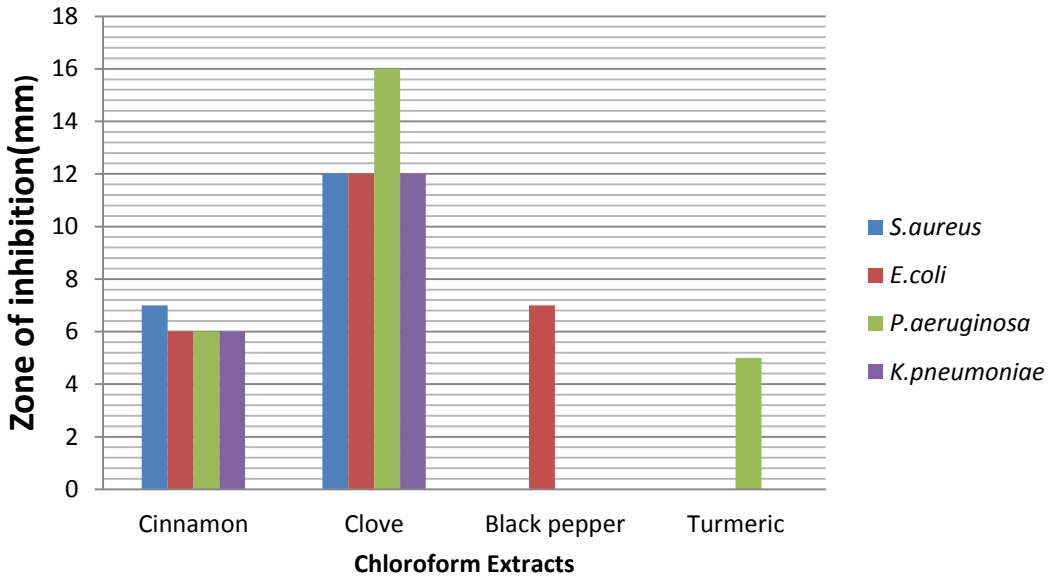

Figure 2. Graph of antimicrobial activity of chloroform extracts against micro-organisms.

(2004) demonstrated that the gram positive bacteria were more sensitive to spices than gram negative bacteria due to the difference in there cell wall structure. In the present study, it was observed that chloroform extract of clove had shown highest antimicrobial zones against *S. aureus, E. coli, P. aeruginosa, K. pneumonia,* whereas chloroform extract of cinnamon had shown maximum zones against all test micro-organisms; also, chloroform extract of black pepper had shown zone against *E. coli* only. In case of chloroform extract of turmeric, it was observed that there is no zone of inhibition observed. The antimicrobial activities of the plants extracted in different solvents varied greatly because there are many factors that influence the active principle present in the plant (Parekh and Chanda, 2006). Aqueous extract of clove had shown highest antimicrobial zone against all test micro-organisms, while aqueous extract of cinnamon exhibited maximum zones against pathogens. Also, aqueous

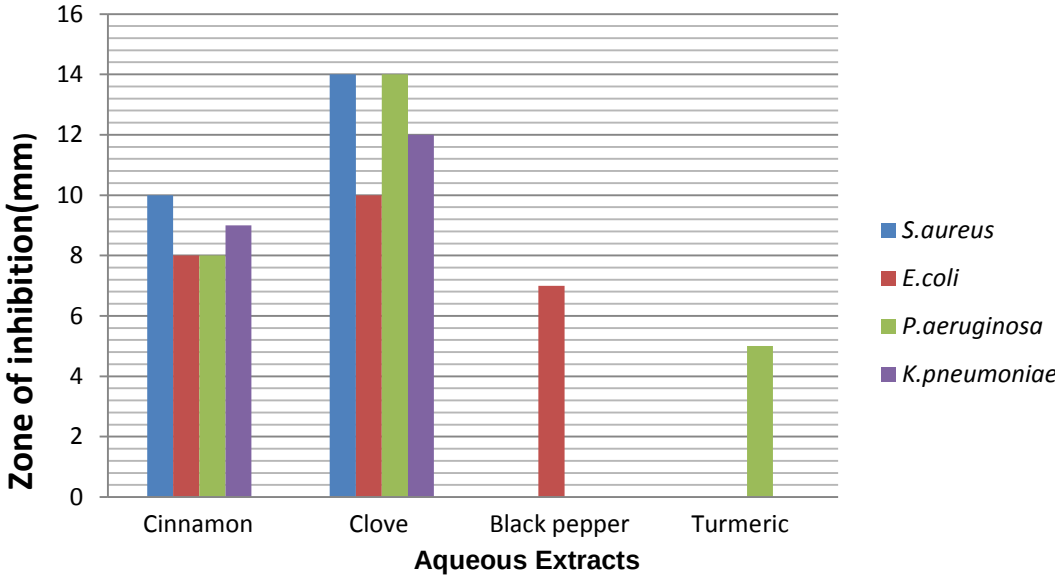

Figure 3. Graph of antimicrobial activity of aqueous extracts against micro-organisms.

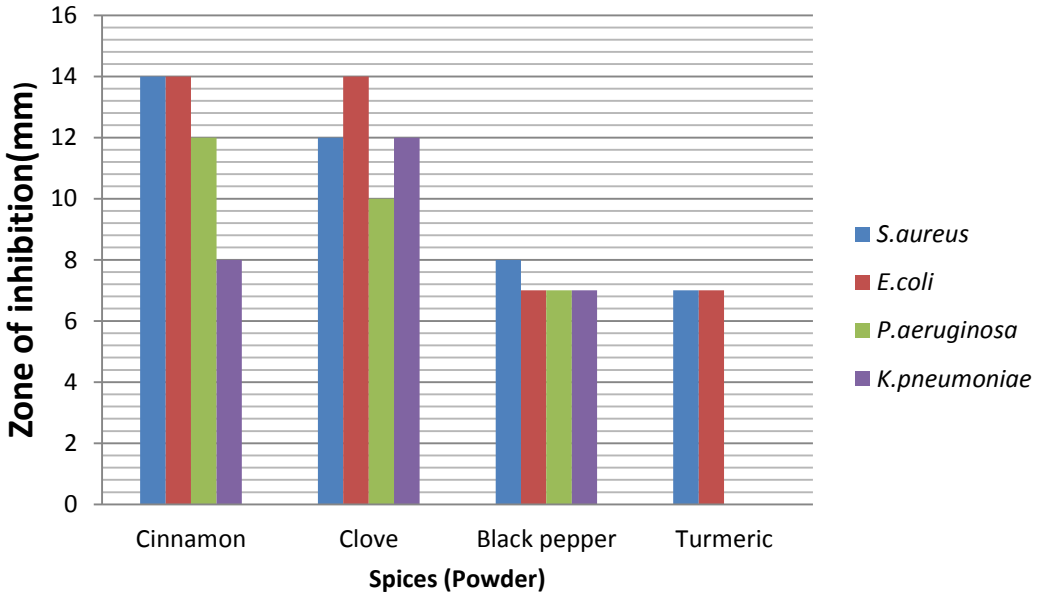

Figure 4. Graph of antimicrobial activity of Spices (Powder) against miro-organisms.

extract of black pepper had shown zone against *E. coli.* In case of aqueous extract of turmeric, no zone of inhibition was observed. Several researchers (Martin, 1995; Paz et al., 1995; Vlientinck et al., 1995) have generally reported that water extracts of plants do not have much activity against bacteria.

Ethanol extract of clove had shown highest antimicrobial zones against all test pathogens, while ethanol extract of cinnamon exhibited maximum zones against micro-organisms. Also, ethanol extract of black pepper had shown antimicrobial zones against *S. aureus* and *E. coli.* Moreso, ethanol extract of turmeric had shown antimicrobial zones against *S. aureus, E. coli* and *P. aeruginosa.*

Cinnamon and clove which was used as powder form had shown highest antimicrobial zones against all test

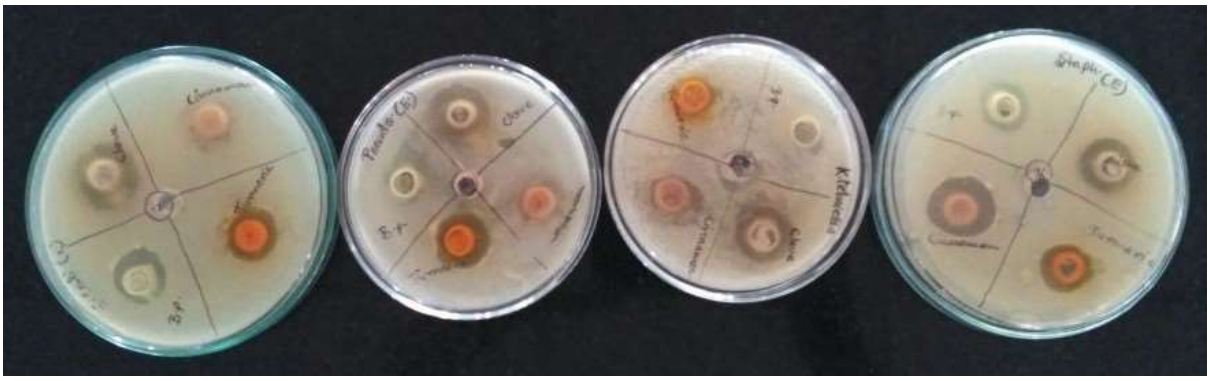

Figure 5. Antimicrobial activity of ethanol extract of spices against micro-organisms.

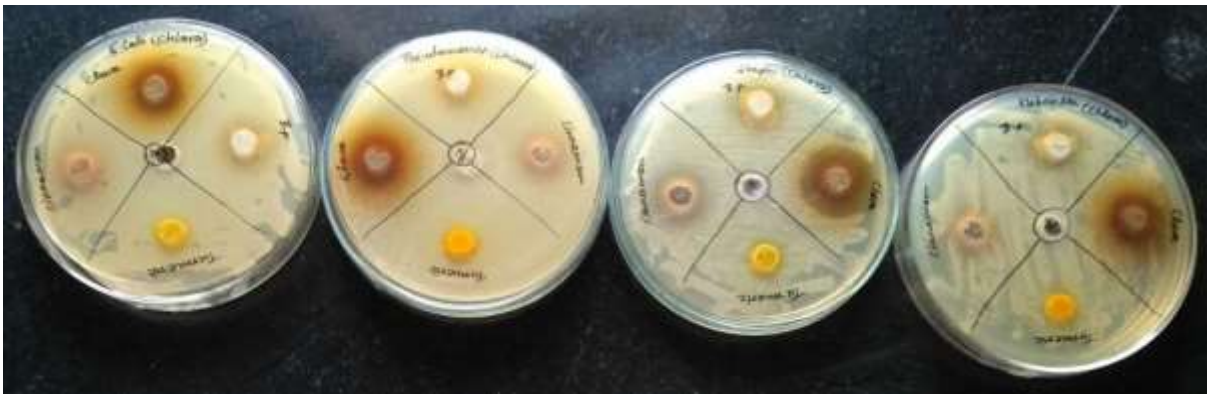

Figure 6. Antimicrobial activity of chloroform extract of spices against micro-organisms.

Figure 7. Antimicrobial activity of aqueous extract of spices against micro-organisms.

pathogens whereas black pepper (Powder) exhibited maximum antimicrobial zones against micro-organisms. In case of turmeric (Powder), antimicrobial zones were shown against *K. pneumoniae, S. aureus* and *E. coli.*

Solvents (75% chloroform, distilled water and 75% ethanol) were used as negative control. The antibacterial activity of spices was compared with the standard antibiotics (Ciprofloxacin, Clindamycin, and Gentamycin). Antibiotics used as positive control showed highest antimicrobial zones than ethanol, chloroform, distilled water extracts and spices (Powder). It is established in this study that the spices reduce and inhibit the growth of

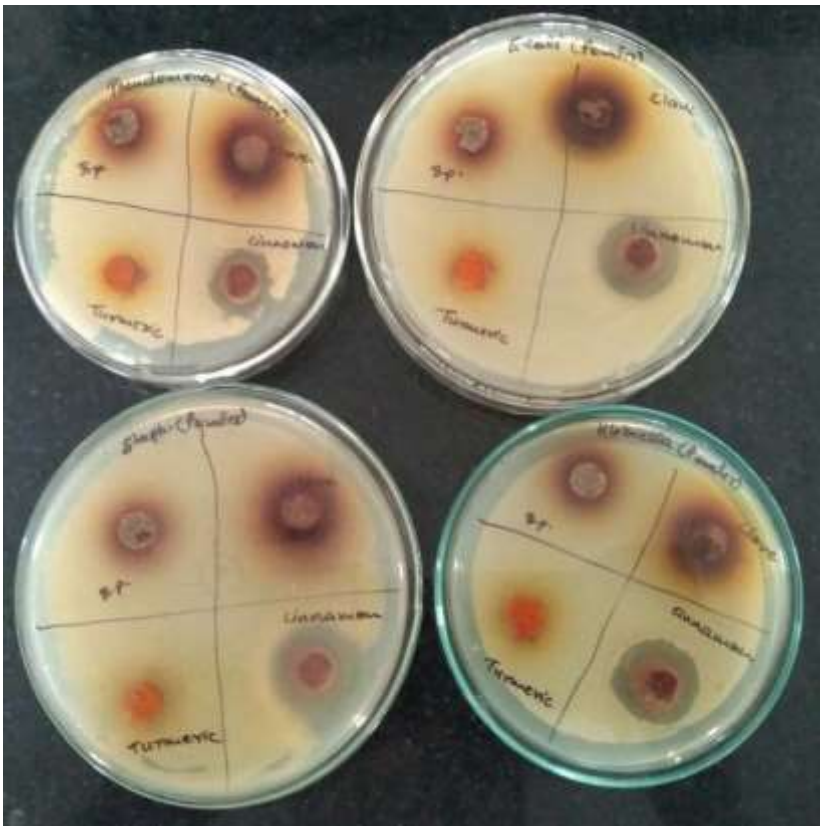

Figure 8. Antimicrobial activity of spices (powder) against micro-organisms.

pathogens.

In the present study, phytochemical screening of four spices clove, cinnamon, turmeric and black pepper were done. The result reveals that some of the phytochemicals analysed were present in the extracts of all the spices. Tannins, flavonoids, steroids, terpenoids were present in aqueous extract of clove; saponins and terpenoids werepresent in chloroform extract of clove; whereas tannins, steroids and terpenoids were present in the ethanolic extract of clove. Aqueous extract of turmeric had tannins, terpenoids and flavonoids; chloroform extract of turmeric had alkaloids and saponins; while tannins and steroids were present in ethanol extract of turmeric. Alkaloids, saponins, tannins, flavonoids, steroids, terpenoids were present in aqueous extract of cinnamon; chloroform extract of cinnamon had saponins and terpenoids; and saponins, steroids, terpenoids were present in ethanolic extract of cinnamon. Aqueous extract of black pepper had alkaloids saponins, tannins and terpenoids; chloroform extract of black pepper had alkaloids, saponins and terpenoids; while alkaloids, steroids and terpenoids were present in ethanol extract of black pepper. The importance of alkaloids, saponins andtannins in various antibiotics used in treating common pathogenic strains has recently been reported by Kubmarawa (2007) and Mensah (2008).

Medicinal plants continue to be an important therapeutic aid for alleviating the ailments of humankind. Today, there is a renewed interest in traditional medicine and an increasing demand for more drugs from plant sources. This revival of interest in plant-derived drugs is mainly due to the current widespread belief that “green medicine” is safe and more dependable than the costly synthetic drugs, many of which have adverse side effects (Al lafi and Ababneh, 1995).

Spices have been added to foods since ancient times as flavoring agent, also as food preservatives and folk medicines. Basically when spices are used for medicinal purpose, their value is dependent on the phytochemicals they possess (Okwu, 2001). The spices, herbs, plant extract and their phytoconstituents have been reported for anti-inflammatory, antidiarrheal, antimicrobial, antioxidant and insecticidal activities (Chouhan and Singh, 2011). Alkaloid has important biological property like cytotoxicity and are used in allophatic systems (Trease and Evans, 2005). Steroids and Sterols are greatly important in pharmacy as they possess compounds like sex hormones and can be used for drug

production (Okwu, 2001). Saponins protect against hypercholesterolemia and antibiotics properties (Amin et al., 2013). The growth of many fungi, yeast, bacteria and viruses was inhibited by tannins (Chung et al., 1998). Phenols and tannins acts as antioxidants (Han et al*.,* 2005). The potential of plants as a source of drugs is still to be vastly explored. Multiple drug resistance has become a critical problem in pharmacotherapeutics as there are increasing numbers of diseases exhibiting various levels of drug resistance, including bacterial infections (Mahesh and Satish, 2008). Herbal medications and phytochemical screening of various plant species for medicinal leads are now receiving much attention (Saleha et al., 2015).

Conclusion

Natural spices (clove, cinnamon, turmeric and Black pepper) possess effective anti-bacterial activity against micro-organisms and can be used for prevention of drug resistant microbial diseases and further evaluation is necessary. The phytochemicals (alkaloids, flavonoids, saponins, terpenoids, tannins and steroids) were present commonly in all the studied spices. The spices have been screened for phytochemical constituents and seemed to have the potential to act as a source of useful drugs and also to improve the health status of the consumers as a result of the presence of various compounds that are vital for good health. The present study showed that, the degree of antibacterial activity of spices tested can be put in the following order: Clove > Cinnamon > Black pepper >Turmeric. The results of the present study are quite encouraging as almost all spices exhibited antimicrobial activity against most of the pathogens, but the antimicrobial activity varies widely, depending on the type of spices, test medium and microorganism. This study opens up the possibility for the search of new antimicrobials as an alternative to the antibiotics.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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