CODEN [USA]: IAJPBB ISSN: 2349-7750

INDO AMERICAN JOURNAL OF
PHARMACEUTICAL SCIENCES

Available online at: http://www.iajps.com ***Research Article***

EFFECT OF SPICE EXTRACTS AS ANTIMICROBIAL AND ANTI-OXIDATIVE AGENT AGAINST COMMON BACTERIAL AND FUNGAL INFECTIONS STRAINS

[1]Komal Siddiqui, [2]Sania Sahar, [3]Noor-e-Saba, [4]Arsalan Ahmed Uqaili

[1]Assistant Professor, Institute of biotechnology and genetic engineering, University of Sindh, Jamshoro, [2]Institute of biotechnology and genetic engineering, University of Sindh, Jamshoro, [3]Institute of biotechnology and genetic engineering, University of Sindh, Jamshoro, [4]Assistant Professor, Department of Physiology, Isra University Hyderabad.

Abstract:

Various cultures of microbes; including gram positive bacteria, gram negative bacteria and fungi were grown in presence of spice extracts to test antimicrobial properties. Various spice extracts were analyzed for antimicrobial properties. Cumin and Chili inhibited the growth of Enterobacter aerogens. Also cumin extract controlled soil bacterial growth. No, any extract inhibited Staphylococcus aureus multiplication. The extract of the Cinnamon positively controlled growth of Aspergillus Niger. Some standard antibiotics were also checked against these microbes. The results revealed that for Enterobacter aerogens bacterial strain, ceftriaxone antibiotic was found effective, whereas for soil bacteria and Staphylococcus aureus gentamicin was having inhibitory effects. No, any antibiotic was effective for the fungal strain. Antioxidant activity was found higher in cinnamon (0.57 mg/ml). In methanol extract, Cumin, Cinnamon, and Red chili showed higher phenolic contents (0.098 mg/ml, 0.096 mg/ml and 0.094 mg/ml respectively). Flavonoid data showed that Cumin (0.63 mg/ml) had more flavonoid content in water extract. In methanol extract, Red chili and Cinnamon had more flavonoid content (1.98 mg/ml and 0.959 mg/ml). This biochemical analysis showed that antioxidant activity, phenolic content, and flavonoid content was higher in Cinnamon, Cumin, and Red Chili. This data indicates that these spices have strong inhibitory effect on microbial population.

Corresponding author:

Komal Siddiqui,

siddiqui.komal@gmail.com

Assistant Professor, Institute of biotechnology and genetic engineering, University of Sindh, Jamshoro.

QR code

Please cite this article in press Komal Siddiqui et al., ***Effect Of Spice Extracts As Antimicrobial And Anti-Oxidative Agent Against Common Bacterial And Fungal Infections Strains.,*** *Indo Am. J. P. Sci, 2019; 06(02).*

INTRODUCTION:

From the beginning of world history till today, different extracts of the plants have been used to treat various diseases and to inhibit microbial growth. The plant extracts contain various chemicals, which can be explored to test against harmful activities of microbes. Nowadays, in many parts of the world, there is growing interest in studying bioactive compounds from seeds, peels, leaves and flowers as to promote health due to their antioxidant and antimicrobial properties (Geetha *et al*., 2014). Many new medicines have been developed from plant products to treat infectious diseases caused by fungi, bacteria, viruses and parasites which are a major issue to human health in the present scenario of climate change (Arya *et al*., 2010).

Increasing use of antibiotics in worldwide has created resistance in pathogen against antibiotics. It has become common knowledge that the excessive and unnecessary use of antibiotics and the antifungal drug has, on one hand, controlled the diseases but also caused uneven eradication of diseases due to the development of many resistant strains. Multi-drug resistant bacterial infections and the existence of numerous more strains of causative agents like bacteria and fungi are of great danger to millions of life being affected by these and moreover such infectious diseases and new bacterial strains are leading to multi-drug resistance and limited efficacy of the common available (Hancok, 2005). Scientific community worldwide has realized the importance of developing new chemistry drugs and to explore more chemicals to overcome emerging issue of available drug resistance. More recently (Vijayakumar, *et al.,* 2012) antimicrobial effects of hexane, ethyl acetate and methanol extracts of seeds and fruits of *Illicium griffithii* were tested against gram positive bacterial and gram negative bacterial strains and fungi. They found that *Staphylococcus aureus* was inhibited by Ethyl acetate extract of these fruits. In other study conducted by Pritam *et.al*, (2013), a comparison of the antibacterial activity of two essential oils from cinnamon spices, *Cinnamo- mum zeylanicum* and *Cinnamomum cassia* was analyzed and was used to prepare economic food spoilage resistant spray. *Staphylococcus aureus* and *Escherichia coli;* the spoilage bacteria, were tested against these oil extracts. Both spoilage bacteria were sensitive towards the essential oil of *Cinnamomum zeylanicum* and *Cinnamomum cassia*. *Cinnamomum cassia* essential oil was more potent to antimicrobial activity and maximum efficient against *E. coli* (Nimje *et al*., 2013). Use of spices in kitchen products and in meat in warmer climate is common in the household to save them from spoilage but its use in drug preparation has been an ignored area. Many spices possess antimicrobial properties (Thomas *et al*., 2012). Therefore, the present study was carried out to test water and methanol extract of various spices against microbes and to determine biochemical properties of these spices which might have some role in antimicrobial activities.

MATERIALS AND METHODS:

The extracts of star ainse, cinnamon, dundicut, Red pepper, black cardamom, and cumin were tested for antimicrobial activity. Biochemical analysis was done from these spices and also role of these spices for inhibition of bacterial and fungal growth was analyzed. Cultures of, gram positive and gram negative bacteria and fungi were grown in agar medium. Extract of various spices were applied to test their microbial inhibition activity. The antimicrobial activity of spice extracts was determined. For determination of antimicrobial activity agar plate method was used. The medium was prepared by dissolving 6g glucose, 2 g peptone, 4 g agar and 2 g NaCl in 100ml of distilled water. The sterilized nutrient agar medium was cooled at 50˚C and was poured into sterilized petriplates under aseptic conditions. When media was solidified, small holes were made. The holes were filled with different spice extracts and labeled accordingly. The suspension of serial diluted bacterial/fungal culture was gently spread onto the entire area of the plate and incubated at 37˚C. The antimicrobial activity was observed after 24 hours and antifungal activity after 72 hours of incubation. Antimicrobial activity was measured in terms of inhibition zone formed around the hole, where spice extract was present. The inhibition zone was compared with the standard. For standard antimicrobial activity, the bacterial/fungal species were grown in presence of antibiotics; Amikacin, Metronidazole, Ceftriaxone, Gentamicin, Penicillin G, Clindamycin, Erythromycin, Tetracycline, and Ampicillin were used.

Preparation of aqueous and methanol extracts of spices for biochemical analysis:

Dried spices were collected and ground in a homogenizer to make a fine powder. 50 gram of spice sample was crushed with glass powder, by adding distilled water or 90% methanol. The mixture was than centrifuged at 7000 rpm for 15 minutes and the pellet was discarded. The supernatant was filtered. The supernatant was transferred into a new flask and this procedure was repeated twice. The final volume was made up to 25 ml with distilled water or 90% methanol. Total sugar, reducing sugar, total protein,

antioxidant activity, phenolic content, and flavonoids were determined during this study.

Determination of total sugar:
Total sugar content from 10% water and methanol extract of black cumin, red chili, black cardamom, cinnamon and star anise was determined by the phenol-sulfuric acid method by (Chatterjee and Montgomery, 1962). A standard curve was used for calculation of total sugar concentration from test samples. 0.5ml of test solution was added in 2.5 ml concentrated sulfuric acid and 0.05 ml of 80% phenol solution. After thoroughly mixing it, it was kept at room temperature for 15 minutes. The blank was prepared by substituting distilled water for test solution. The absorbance was monitored against the blank at 485nm using spectrophotometer. The standard was prepared by using different concentrations of glucose.

Determination of reducing sugar:
The reducing sugar from 10% water and methanol extract of all spices under study was determined by Dinitrosalicylic acid (DNS) method of Miller (1959). The result was calculated from glucose standard curve. 2.0 ml of test solution was added to 2.0 ml of dinitrosalicylic acid in a test tube. The mixture was thoroughly mixed and heated in boiling water bath for 5 minutes. After 5 minutes the tubes were cooled under tap water and color intensity was observed against reagent blank at 540 nm using a spectrophotometer. The concentration of reducing sugar content was calculated from a standard curve prepared with different concentrations of glucose.

Determination of total protein:
Total protein content of 10% water and methanol extract of all spices was determined by (Lowry *et al*., 1951) method. The concentration of test solution was calculated by albumin standard curve. 0.5 ml test solution was added to 2.5 ml alkaline copper reagent. The reaction mixture was mixed thoroughly and allowed to stand at room temperature for 10 minutes. 0.25 ml diluted Folin Cicalteus reagent (1:1 v/v with water) was added and incubated at room temperature for 30 minutes. After 30 minutes the absorbance was read against blank at 750 nm spectrophotometer. Standard protein curve was made by using different concentrations of bovine albumin.

Determination of antioxidant activity:
The antioxidant activity of 10% water and methanol extract of spices was determined by the method of Voces *et a*l., (1999) and Prieto *et al.*, (1999) with slight modification, the result was calculated from Alpha Tocopherol standard curve. 0.2 ml of test sample was combined with 2 ml of reagent (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The tubes were covered and incubated in the boiling water bath at 95˚C for 90 minutes and the samples were cooled to room temperature. The absorbance was measured at 695 nm against the blank with the help of spectrophotometer instrument. A standard curve was made using different concentrations of Alpha-tocopherol.

Determination of phenolic content: Total phenolic content of 10% water and methanol extract of black cumin, red chili, black cardamom, cinnamon, and star anise was determined by using spectrophotometer by Follin-ciocalteu method (Yasoubi *et al*., 2007). The result was calculated from Gallic acid standard curve. 0.2 ml test sample was added in 1 ml of diluted follin-ciocalteu (1:9 v/v with water), and 0.8 ml $NaCO_3$ was added. After thoroughly mixing, it was left at room temperature for 30 minutes. The blank was prepared by substituting distilled water for test solution. The absorbance was monitored against the blank at 765 nm by a spectrophotometer. The standard was made by using different concentrations of Gallic acid.

Determination of flavonoid content:
Total flavonoid content of 10% water and methanol extract of black cumin, red chili, black cardamom, cinnamon and star anise was determined by aluminum chloride colorimetric method Kim *et al*., (2003). The result was calculated from Quercetin standard curve. 0.1 ml test sample was added to 0.3 ml of 5% sodium nitrate. After 5 minutes 0.3 ml of aluminum chloride was added, again after 5 minutes 2 ml of 1 M sodium hydroxide was added. The final volume was made up to 10 ml with distilled water. The blank was prepared by substituting distilled water for test solution. The absorbance was monitored against blank at 510 nm with spectrophotometer. A standard curve was prepared by using different concentrations of Quercetin.

RESULTS AND DISCUSSION:

Antibacterial activity of different spices
Results of standard antibiotics against bacterial species are given in table-1 depicting that ceftriaxone showed greater inhibition activity against *E. aerogens* bacterial specie followed by Amikacin and Metronidazole. For soil bacteria, Gentamycin antibiotic was most effective followed by penicillin G. Ceftriaxone was found ineffective for soil bacteria. *Staphylococcus aureus* was positively strongly inhibited by Gentamycin, Tetracycline, and

Erythromycin. Geetha *et al*., (2014) had tested peel extracts obtained from various vegetables against bacteria and waterborne pathogens. They found Ampicillin with highest antibacterial effects against all tested microorganisms. The vegetable peel extracts were inhibiting many bacteria tested.

Table -1: Standard inhibition zone of antibiotics against bacterial species

S.no	Bacterial species	Standard inhibition zones of antibiotics		
1	*E.aerogens*	Amikacin	Metronidazole	Ceftriaxone
		++ve	+ve	+++ve
2	Soil bacteria	Gentamycin	Penicillin G	Clindamycin
		+++ve	++ve	-ve
3	*Staphylococcus aureus*	Gentamycin	Erythromycin	Tetracycline
		+++ve	+ve	++ve

Size of inhibition zone: +ve= Small (~ 0.5 cm), ++ve= Medium (~ 1 cm), +++ve Large zone (~1.5 cm) and –ve= No zone.

Antibacterial activities of various extracts of different spices shown in in Table-2. For bacterial specie; *E. aerogens*, Red chili, and cumin extract were found highly effective. Whereas for soil bacteria cumin was effective followed by red chili. However; black cardamom, star anise and cinnamon were found ineffective for soil bacteria. None of the spice extracts were found affective for *Staphylococcus aureus*. Chanda; et al, (2010) tested extract of various fruit peels against microorganisms. *Mangofera indica* peel extract was found highly effective and possess strong antibacterial activity. Clove spice possesses outstanding antibacterial effect followed by Ginger and pepper (Eruteya and Odunfa; 2009). Gram positive bacteria were found more susceptible towards spice extract than gram negative bacteria.

Table-2: Antibacterial activity of different spice extracts

S.no	Bacterial species	Antibacterial activity of spice extracts				
		Black Cardamom	Star Anise	Cinnamon	Cumin	Red Chili
1	*E.aerogens*	+ve	+ve	+ve	+++ve	+++ve
2	Soil bacteria	-ve	-ve	-ve	++ve	+ve
3	*Staphylococcus aureus*	-ve	-ve	-ve	-ve	-ve

Size of inhibition zone: +ve= Small (~ 0.5 cm), ++ve= Medium (~ 1 cm), +++ve Large zone (~1.5 cm) and –ve= No zone.

Antifungal activity of different spices

The results of spice extract against fungal activity (Table-3) showed that only cinnamon was found effective for inhabiting *Aspergillus niger* activity. Whereas no other spice extracts were found effective. For *Aspergillus flavous* none of the extract showed any response to control the fungal growth.

Table-3: Antifungal activity of different spice extracts

S.no	Fungal species	Antifungal activity of spices extracts				
		Black Cardamom	Star Anise	Cinnamon	Cumin	Red Chili
1	*Aspergillus niger*	-ve	-ve	++ve	-ve	-ve
2	*Aspergillus flavous*	-ve	-ve	-ve	-ve	-ve

Size of inhibition zone: +ve= Small (~ 0.5 cm), ++ve= Medium (~ 1 cm), +++ve Large zone (~1.5 cm) and –ve= No zone.

Biochemical properties of different spices

Spices possess various chemicals and compounds. These naturally occurring compounds in the spices such as terpenes, phenols and glycosides might have inhibitory or preservative effect (Deis, 1999). Biochemical analysis of these spices will give some clue about the reason behind their antimicrobial activity. Results of biochemical analysis of spices included in this study are briefly described in table 4 and 5.

Table-4. Total sugar, reducing sugar and total protein of spices in water and methanol extract

Biochemical contents of spices (10% water extract)				**Biochemical contents of spices (10% methanol extract)**		
Spice	Total sugar mg/ml	Reducing sugar mg/ml	Total protein mg/ml	Total sugar mg/ml	Reducing sugar mg/ml	Total protein mg/ml
Black cardamom	13.29	5.6	0.89	9.82	4.40	2.90
Star Anise	7.08	3.2	1.03	11.00	1.52	7.76
Cinnamon	12.19	10.5	1.05	20.00	11.50	10.80
Cumin	22.11	7.8	1.77	6.30	1.70	7.50
Red Chili	9.20	6.9	2.40	10.50	5.92	5.79

Table-5: Antioxidant, phenolic and flavonoid contents of different spices in water and methanol extract

Antioxidant, Phenolic and Flavonoid contents of spices (10%water extract)				**Antioxidant, Phenolic and Flavonoid contents of spices (10% methanol extract)**		
Spice	Antioxidant activity mg/ml	Phenolic content mg/ml	Flavonoid content mg/ml	Antioxidant activity mg/ml	Phenolic content mg/ml	Flavonoid content mg/ml
Black cardamom	0.41	0.06	0.50	0.45	0.06	0.22
Star Anise	0.48	0.04	0.50	0.47	0.056	0.46
Cinnamon	0.57	0.16	0.42	0.56	0.096	0.959
Cumin	0.40	0.07	0.63	0.46	0.098	0.098
Red Chili	0.49	0.09	0.508	0.47	0.094	1.98

Highest total sugar was found in cumin (22.11 mg/ml) followed by black cardamom (13.29 mg/ml) and cinnamon (12.19 mg/ml). Methanol extract showed that maximum total sugar was found in cinnamon followed by star anise and chili (20.0, 11.0 and 10.5 mg/ml respectively). Reducing sugar in water extract (10.5 mg/ml) as well in methanol extract (11.5 mg/ml) of cinnamon was found highest. Methanol extracted more protein than water extract may be due to more solubility of the protein in methanol rather than in water. Cinnamon showed more protein 10.8 mg/ml in methanol extract (Table 4). In water, as well as in methanol extract antioxidant activity was also found higher in cinnamon (0.57 and 0.56 mg/ml respectively). In methanol extract, cumin and cinnamon and red chili showed higher phenolic contents (0.098, 0.096 and 0.094 mg/ml respectively). Cumin (0.63 mg/ml) had more flavonoid content in water extract. In methanol extract, red chili and cinnamon had more flavonoid content (1.98 and 0.959 mg/ml respectively) (Table-5). Many different spices possess various bioactive compound in different concentration. Antioxidants, phenolics, and flavonoids are major compounds found in spices and other plant derivatives. There are reports that clove, cardamom, and cinnamon produces certain compounds such as eugenol, cinnamaldehyde, and eucalyptol in varying quantities have antimicrobial activities (El- Baroty et al., 2010).). Thus it is observed from this study that spices have potential against bacterial and fungal pathogens thus, could be used as food preservative and in medicine

REFERENCES:

1. Arya, V., S. Yadav, S. Kumar and J. Yadav. 2010. Antimicrobial of Cassia occidentalis l. (Leaf) against various human pathogenic microbes. Life Sci. Med. Res. 9: 1-11
2. Chanda, S., Y. Baravalia, M Kaneria and K. Rakholiya, 2010. Fruit and vegetable peels: strong natural source of antimicrobics in Current Research, pp. 444-450. Technology and Education Topics in Applied Microbiology and Microbial Biotechnology A. Méndez-Vilas (Ed.)
3. Chatterjee, A. K and R. Montogomery, 1962. The carbohydrate of ovomucoid. Archives of Biochemistry and Biophysics. 99(3): 426-432.
4. Deis, R.D. 1999, Secret world of spices. Food Product Design 5: 1-7
5. Eeuteya, O.C and S. A.Oduna, 2009. Antimicrobial properties of three spices used in the preparation of suya condiment against organisms isolated from formulated samples and individual ingredients. African Journal of Biotechnology 8(10): 2316-2320

6. ElBaroty G.S. E-BH., R.S.Farag and M.A. Saleh, 2010. Characterization of antioxidant and antimicrobial compound of cinna- mon and ginger essential oils. Afr. J. Biochem. Res. 4: 167-174.
7. Geetha S., S. deepikad, L. Somayak, 2014. Anti-microbial activity of selected vegetable peels against water borne pathogens. International Journal of Advances in Pharmacy, Biology and Chemistry 3(4): 937-940.
8. Hancock E.W. 2005. Mechanisms of action of newer antibiotics for Gram-positive path- ogens. Lancet Infectious Diseases 5: 209-218
9. Kim, D. O., S.W. Jeong and C.Y. Lee, 2003. Anti-oxidant capacity of phenolic phytochemicals from various cultivars of plums. Food Chemistry 81(3): 321-326.
10. Lowry, O.H., N.J. Rosebrough, A.L. Farr and R.J. Randall, 1951. Protein measurement with Folin reagent. J. Boil. Chem. 193(1): 265-275.
11. Miller, G. l., 1959. Use of dinitrosalicylic acid reagents for determination of reducing sugar. Analytical Chemistry 31(3): 426-428.
12. Montgomery, R., 1961. Further studies of phenol sulfuric acid reagent for carbohydrates. Biochemica et Biophysica Acta 48(3): 591-593.
13. Nimjee, P.D., H. Garg, A. Gupta, N. Srivastav, M. Katiyar and C. Rama- lingam, 2013. Comprasion of antimicrobial activity of cinnamomum zeylanicum and of cinnamomum cassia on food spoilage bacteria and water borne bacteria. Der Pharmacia Lettre 5(1): 53-59
14. Thomas, F., S.P. Daoust and M. Raymond, 2012. Can we understand modern human consi- dering pathogens? Evolutionary Applica- tions 5 (4): 368-379.
15. Voces, J., A. Alvaarez, L. Vila, A. Ferrando, C. Cabral de Oliveira and J. Prieto, 1999. Effects of administration of the standa- rdized Panax ginseng extract G115 on hepatic antioxidant function after exhaustive exercise. Comparative Biochemistry and Physiology part c: Pharmacology, Toxicology and Endocrinology 123(2): 175-184.
16. Vijayakumar, A., V. Duraipandiyan, B. Jeyaraj, P. Agastian, M.K. Raj and S. Ignacimuthu, 2012. Phytochemical analysis and invitro antimicrobial activity of Illicium griffithi hook f. and Thomas extracts. Asian Pacific Journal of Tropical Disea-ses 2(3):190-199.
17. Yasoubi, P., M. Barzegar, M. Sahari and M. Aziz, 2007. Total phenolic contents and anti- oxidant activity of pomegranate (Punica granatum L.) peel extracts. Journal of Agricultural Science and Technology 9(1): 35-42.