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ANTIMICROBIAL ACTIVITY AND BIOCHEMICAL PROPERTIES OF VARIOUS SPICE EXTRACTS AGAINST BACTERIAL AND FUNGAL STRAINS

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ABSTRACT

Various cultures of microbes; including gram positive, gram negative and fungi were grown in presence of spice extracts to test their antimicrobial properties. The results revealed that for E. aerogens bacterial strains, 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. Various spice extracts were concerned cumin and chili inhibited the growth of E. aerogens and cumin extract controlled soil bacterial growth. No, any extract inhabited staphylococcus aureus multiplication. The extract of the cinnamon positively controlled growth of Aspergillus niger. Antioxidant activity was found higher in cinnamon (0.57). In methanol extract, cumin, cinnamon, and red chili showed higher phenolic content (0.098, 0.096 and 0.094 respectively). Flavonoid data showed that cumin (0.63) had more flavonoid content in water extract. In methanol extract, Red chili and cinnamon had more flavonoid content (1.98 and 0.959). This biochemical analysis showed that antioxidant activity, phenolic content, and Flavonoid content was higher in cinnamon, cumin, and red chilies. This may be the reason that these spices had also shown strong inhibitory effect on microbial population

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 of bioactive compounds from seeds, peels, leaves and flowers as to promote health due to their antioxidant antimicrobial properties (Geetha *et al*., 2014). Many new chemistry 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 lives 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 strains gram-negative bacterial strains and fungi. They found that *Staphylococcus aureus* was inhibited by Ethyl acetate extract of fruits. In this it was seen that the bacterial actions were more significant whereas no any significant role was seen against fungal. In other study conducted by Pritam *et.al,* (2013), carried out a comparison of the antibacterial activity of two essential oils from cinnamon spices, *Cinnamomum zeylanicum* and *Cinnamomum cassia* and their chemical components in order to prepare economic food spoilage resistant spray. The antibacterial activity of *Staphylococcus aureus* and *Escherichia coli* was tested against food spoilage bacteria. *E. coli* is a waterborne pathogen and food spoiling bacteria. Potential of both essential oils were compared. An antibiotic was used as positive control called Gentamycin. 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 a 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 those 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 determined from these spices and also role of the various compounds of these spices for inhibition of bacterial and fungal growth. Culture includes, gram positive, gram negative and fungi were grown in agar medium. Extract of various spices was 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, 2g peptone, 4g agar and 2g NaCl in 100ml distilled water of 250 ml flask. The media along with Petri-plates were autoclaved for 15 minutes at 121^0C and 15 psi. The sterilized nutrient agar medium could cool at 50^0C and was poured into sterilized petri-plates under aseptic conditions. When media was solidified, small holes were made with the help of sterilized pipette tips. The holes were filled with different spice extracts and labeled accordingly. Then sterilized swab was dipped into the suspension of serial diluted bacterial/fungal cultures and was gently spread onto the entire area of the plate. The petri-plates were covered with parafilm and incubated at 37 ^{0}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 powder 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 through a Whatman NO: 1 filter paper. The supernatant was transferred into 100 ml volumetric 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 flavonoid were some of the compound 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 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 dinitro salicylic 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 colors intensity was observed against reagent blank at 540nm 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 were 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 750nm 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 were 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. 0.2ml 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 capped and incubated in the boiling water bath at 95 C for 90 minutes and the samples were cooled to room temperature and the absorbance was measured at 695nm 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 1ml of 10-fold diluted follin-ciocalteu (10-fold means took 1 ml of follin-ciocal teu and 9ml of water), and 0.8 ml $NaCO_3$ (Sodium carbonate) 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 quer-cetin standard curve. 0.1 ml test sample was added to 0.3ml of 5% sodium nitrate, after 5 minutes 0.3 ml of aluminum chloride was added, again after 5 minutes 2ml 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 510nm with spectrophotometer. A standard curve was prepared by using different concentrations of Quercetin.

RESULTS AND DISCUSSION

Antimicrobial activity of different spice of against Bacterial pathogen

Results of standard antibiotics against bacterial species are given in Table 1 depicts that ceftriaxone showed greater inhibition activity against E. aerogens bacterial species followed by Amikacin and Metronidazole. For soil bacteria, Gentamycin antibiotic was most effective followed by penicillin G. ceftriaxone were 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 pathogen. They found Ampicillin with highest antimicrobial inhibition effects against all tested microorganisms. They had reported that inhibitory effect of vegetable peel extracts was spice-specific means dependent on which bacterial strain which vegetable was tested for antimicrobial activity.

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, ++ve= Medium +++ve Large zone and –ve= No zone.

Antibacterial activities of various extracts of different spice are given in table 2. For bacterial species, E. aerogens, Red chili, and cumin extract were found highly effective. A positive response was also found of other spice extract for E. aerogen species 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 (Table 2.). Chanda et al., 2010 tested peel extract of various fruits against microorganisms. *Mangofera indica* peel extract was found highly effective and possess strong antibacterial activity. In other study conducted by Eruteya and Odunfa (2009) reported that Clove spice possesses outstanding anti-bacterial effect followed by Ginger and pepper. Gram-positive bacteria were found more susceptible of spice extract than gram negative.

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, ++ve= Medium, +++ve Large zone and –ve= No zone.

Antifungal activity of different spice against fungus: The standard antibiotic such as Penicillin G, Tetracyclin, and ampicillin was checked against *Aspergillus niger* and *Aspergillus flavous* strain, none of the antibiotics was found affective (Table 3). Where-as the results of spice extract against fungal activities are described in table 4. Data showed that only cinnamon was found effective for inhabiting *Aspergillus niger* activities. Whereas no other spice extracts were found reactive for this strain. For Aspergillus flavous none of the extract showed any response to control fungal growth.

Table- 3: Standard inhibition zone of antibiotics against fungal species

S.no	**Fungal species**	**Standard inhibition zones of antibiotics**		
1	*Aspergillus niger*	Penicillin G	Tetracyclin	Ampicillin
		-ve	-ve	-ve
2	*Aspergillus flavous*	Gentamicin	Penicillin G	Tetracyclin
		-ve	-ve	-ve

Size of inhibition zone: +ve= Small, ++ve= Medium, +++ve Large zone and –ve= No zone.

Table -4: 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, ++ve= Medium, +++ve Large zone 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, glycoside and another compound which might have inhibitory or preservative affect (Deis 1999). Investigation of various spice and their biochemical will give some clue about the reason behind their antimicrobial activity. Results of analysis of biochemical of spices included in this study are briefly described in table 5 and 6.

Table -5: Total sugar, reducing sugar and total protein of spices in water and methanol extract

Total sugar, reducing sugar and total protein of 10% water extract of spices				Total sugar, Reducing Sugar and total protein of 10% methanol extract of spices		
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	4.4	2.9
Star Anise	7.08	3.2	1.03	11.0	1.52	7.76
Cinnamon	12.19	10.5	1.05	20	11.5	10.8
Cumin	22.11	7.8	1.77	6.3	1.7	7.5
Red Chili	9.20	6.9	2.4	10.5	5.92	5.79
Blank	2.73	0.48	1.86	1.3	0.69	0.27

Table- 6: Antioxidant, Phenolic and flavonoid contents of different spices in water and methanol extract

Antioxidant, Phenolic and Flavonoid contents of 10% water extract of spices				Antioxidant, Phenolic and Flavonoid contents of 10% methanol extract of spices		
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	0.5	0.45	0.057	0.22
Star Anise	0.48	0.04	0.5	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
Blank	0.38	0.17	0.098	0.39	0.039	0.098

Total sugar mg/ml in water extract ranged from 2.73mg/ml in blank to 22.11mg/ml in cumin. 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 highest total sugar was found in cinnamon followed by star anise and chili (20, 11.0 and 10.5mg/ ml respectively). Reducing sugar in water extract as well in methanol extract cinnamon was found possessing highest reducing sugar of 10.5mg/ml in water extract and 11.5 in methanol extract. 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-5). In water, as well in methanol extract antioxidant activity was found higher in cinnamon (0.57 and 0.56 mg/ml respectively). For phenolic content cinnamon and the blank was higher (0.16 and 0.17mg/ml respectively). In methanol extract, cumin and cinnamon and red chili showed higher phenolic content (0.098, 0.096 and 0.094mg/ml respectively). Flavonoid data showed that cumin (0.63) 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) (Table 6). Many different spices possess various bioactive compound in different concentration. Antioxidant, phenol, and flavonoid 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).

RFERENCES:

Arya, V., S. Yadav, S. Kumar and J. Yadav, Antimicrobial of Cassia occidentalis l. (Leaf) against various human pathogenic microbes. Life Sci. Med. Res. 9: 1-11 (2010).

Chanda, S., Y. Baravalia, M Kaneria and K. Rakholiya, Fruit and vegetable peels– strong natural source of antimicrobics in Current Research, Technology and Edu-cation Topics in Applied Microbiology and Microbial Biotechnology A. Méndez -Vilas (Ed.) Pp. 444-450 (2010).

Chatterjee, A.K. and R. Montogomery, The carbohydrate of ovomucoid. Archives of Biochemistry and Biophysics. 99(3): 426 - 432 (1962).

Deis, R.D., Secret world of spices. Food Product Design 5: 1-7 (1999).

Eeuteya, O.C. and S.A. Oduna, 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 (2009).

El-Baroty G.S. E-BH., R.S.Farag and M.A. Saleh, Characterization of antioxidant and antimicrobial compound of cinna-mon and ginger essential oils. Afr. J. Biochem. Res. 4: 167-174 (2010).

Geetha S., S. Deepikad, L. Somayak, Antimicrobial activity of selected vegetable peels against water borne pathogens. International Journal of Advances in Pharmacy, Biology and Chemistry 3(4): 937 -940 (2014).

Hancock E.W., Mechanisms of action of newer antibiotics for Gram-positive pathogens. Lancet Infectious Diseases 5: 209-218 (2005).

Kim, D.O., S.W. Jeong and C.Y. Lee, Antioxidant capacity of phenolic phytochemicals from various cultivars of plums. Food Chemistry 81(3): 321-326 (2003).

Lowry, O.H., N.J. Rosebrough, A.L. Farr and R.J. Randall, Protein measurement with Folin reagent. J. Boil. Chem. 193(1): 265-275 (1951).

Miller, G.L., Use of dinitrosalicylic acid reagents for determination of reducing sugar. Analytical Chemistry 31(3): 426-428 (1959).

Montgomery, R., Further studies of phenolsulfuric acid reagent for carbohydrates. Biochemica et Biophysica Acta 48(3): 591-593 (1961).

Nimjee, P.D., H. Garg, A. Gupta, N. Srivastav, M. Katiyar and C. Rama-lingam, Comprasion of antimicrobial activity of cinnamomum zeylanicum and of cinnamomum cassia on food spoilage bacteria and water borne bacteria. Der Pharmacia Letter 5(1): 53-59 (2013)

Thomas, F., S.P. Daoust and M. Raymond, Can we understand modern human considering pathogens? Evolutionary Applications 5 (4): 368-379 (2012).

Voces, J., A. Alvaarez, L. Vila, A. Ferrando, C. Cabral de Oliveira and J. Prieto, Effects of administration of the standardized 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 (1999).

Vijayakumar, A., V. Duraipandiyan, B. Jeyaraj, P. Agastian, M.K. Raj and S. Ignacimuthu, Phytochemical analysis and invitro antimicrobial activity of Illicium griffithi hook f.

and Thomas extracts. Asian Pacific Journal of Tropical Diseases 2(3):190-199 (2012).

Yasoubi, P., M. Barzegar, M. Sahari and M. Aziz, Total phenolic contents and anti-oxidant activity of pomegranate (Punica granatum L.) peel extracts. Journal of Agricultural Science and Technology 9(1): 35-42 (2007).