academicJournals

Vol. 7(26), pp. 1907-1915, 10 July, 2013
DOI: 10.5897/JMPR2013.4453
ISSN 1996-0875 ©2013 Academic Journals
http://www.academicjournals.org/JMPR

Journal of Medicinal Plants Research

Full Length Research Paper

Antioxidant capacity of crude water and ethyl acetate extracts of some Indian spices and their antimicrobial activity against *Vibrio vulnificus* and *Micrococcus luteus*

Oyas Ahmed Asimi*, N. P. Sahu and A. K. Pal

Fish Nutrition, Biochemistry and Physiology Division, Central Institute of Fisheries Education, Mumbai, India.

Accepted 1 July, 2013

The water and ethyl acetate extracts of six Indian spices were screened to identify their antioxidant activity and antimicrobial properties against *Vibrio vulnificus* and *Micrococcus luteus*. The 2,2'-Diphenyl-1-picrylhydrazyl (DPPH), ferric reducing antioxidant power (FRAP) and total phenolic compounds (TPC) assay were used for determining antioxidant activity. The ethyl acetate spice extracts exhibited higher antioxidant activity as compared to water extracts, it can be sorted in the descending order of: clove > cardamom > thyme > black cardamom > black pepper > fenugreek seeds by DPPH assay. By FRAP assay: clove > thyme > cardamom > black cardamom > fenugreek seeds > black pepper and by TPC assay: clove > thyme > cardamom > fenugreek seeds > black cardamom > black pepper. The antimicrobial activity was screened by disk diffusion assay. Only clove and cardamom displayed antimicrobial activity at increasing concentrations in both water and ethyl acetate extracts. Clove displayed the highest 12 mm inhibition zone against *V. vulnificus* and 14 mm inhibition zone against *M. luteus* in both water and ethyl acetate extracted spices at highest concentration. Thus, clove and cardamom can be supplemented for both nutritional purposes and preservation of foods.

Key words: Spices, water extraction, solvent extraction, DPPH, FRAP, TPC, antimicrobial activity.

INTRODUCTION

Antioxidants are compounds or systems that delay autoxidation by inhibiting formation of free radicals or by interrupting propagation of the free radical by one (or more) of several mechanisms: (1) scavenging species that initiate peroxidation, (2) chelating metal ions such that they are unable to generate reactive species or decompose lipid peroxides, (3) quenching $^*O_2^-$ preventing formation of peroxides, (4) breaking the autoxidative chain reaction, and/or (5) reducing localized O_2 concentrations. Highly reactive free radicals and oxygen species are present in biological systems from a wide variety of sources (Nawar, 1996). Antioxidant compounds like phenolic acids, polyphenols and flavonoids scavenge free radicals such as peroxide, hydroperoxide or lipid peroxyl and thus inhibit the oxidative mechanisms that lead to degenerative diseases (Jayasinghe et al., 2003). Usually synthetic antioxidants such as butylhydroxyanisole (BHA) or butylhydroxytoluene (BHT) are used to decelerate these processes (Martinez-Tome et al., 2001). Synthetic phenolic antioxidants (butylated hydroxyanisole [BHA], butylated hydroxytoluene [BHT], and propyl gallate) effectively inhibit oxidation; chelating agents, such as ethylene diamine tetra acetic acid (EDTA), can bind metals reducing their contribution to the process. Some vitamins (ascorbic acid [AA] and α-tocopherol), many herbs and spices (rosemary, thyme, oregano, sage, basil,

*Corresponding author. E-mail-asimioa@gmail.com. Tel: 09702324424.

pepper, clove, cinnamon, and nutmeg), and plant extracts (tea and grapeseed) contain antioxidant components as well (Hinneburg et al., 2006). Additionally, it is still unclear whether prolonged consumption can lead to health risks (Martinez-Tome et al., 2001). Natural antioxidants are considered safe and have consumer acceptant as compared to synthetic antioxidants for example, herbs and spices.

Spices are a group of esoteric food adjuncts that have been in use for thousands of years to enhance the sensory quality of foods, the quantity and variety consumed in tropical countries is particularly extensive. These spice ingredients impart characteristic flavor, aroma, or piquancy and color to foods. It is a common experience that their distinct aroma stimulates the appetite. Not only are spices used as flavorings and seasonings, but many are also used in perfumery, cosmetics, and toiletries. In addition, several spices have long been recognized to possess medicinal properties such as tonic, carminative, stomachic antispasmodic, and antihelminthic (Nadkarni and Nadkarni, 1976). Although these observations are largely empirical, these undoubtedly efficacious attributes have earned them pharmacological applications in the indigenous system of medicine in India, and other countries.

Many of the spices used today have been valued for their antimicrobial effects and medicinal powers in addition to their flavor and fragrance qualities. Most of the foodborne bacterial pathogens examined were sensitive to extracts from plants such as cinnamon, clove, garlic, mustard, onion and oregano. The antimicrobial compounds in spices and herbs are mostly in the essential oil fraction. Shan et al. (2007) found that of 46 spice extracts evaluated, more than 50% exhibited antibacterial activity against foodborne pathogens (*Staphylococcus aureus* and *Echerichia coli*). The Gram-positive bacteria were more sensitive to the antimicrobial compounds in spices than Gram-negative bacteria. The extent of sensitivity varied with the strain and environmental conditions imposed. Certain spices can have a direct effect on the rate of fermentation by stimulating acid production in starter cultures such as grains, seeds, or nutrient liquids that have been well colonized by the microorganisms used for the fermentation. Phenols, alcohols, aldehydes, ketones, ethers and hydrocarbons have been recognized as major antimicrobial components in spices (Tajkarimi et al., 2001).

Like herbs, spices can have significant antioxidative effects (Suhaj, 2006). Wojdyło et al. (2007) measured total equivalent antioxidant capacities and phenolic contents (Folin–Ciocalteu) of 32 spices. Major phenolic acids identified in these spices include the caffeic, p-coumaric, ferulic, and neochlorogenic acids. Predominant flavonoids are quercetin, luteolin, apigenin, kaempferol, and isorhamnetin.

The aim of the present work was to examine antioxidant activity of spices extracted by two different methods, water and ethyl acetate and also to check their antimicrobial activity against two bacterial strains *Vibrio vulnificus* and *Micrococcus luteus*. *V. vulnificus* is an estuarine bacterium present in temperate waters throughout the world. It is a significant human pathogen, producing potentially fatal infections following ingestion, typically of raw oysters, or through contamination of a wound (Klontz et al., 1988). *M. luteus* and closely related genera occur worldwide and are ubiquitous. They are found on the skin of humans and other animals and in soil, marine and fresh water, plants, fomites, dust, and air (Bannerman and Peacock, 2007). One study (of 115 people) reports that up to 96% of people living in 18 states of USA carried micrococci, with the majority being *M. luteus* (Kocur et al., 2006).

MATERIALS AND METHODS

Spices

Fenugreek (*Trigonella foenumgraecum*), clove (*Syzygium aromaticum)*; thyme (*Folium sennae*), black cardamom (*Amomum subulatum*), black pepper (*Piper nigrum),* cardamom (*Amomum krervanh*) were purchased from local market in Srinagar, Kashmir (J & K State), India.

Spice extraction

The extract preparation was done according to the method previously described (Virdi et al., 2003) with some modification. The spices were ground into powder in a laboratory grinder and sieved into fine powder to be used for extraction. The spice materials were extracted by two methods: water extraction and solvent extraction. In water extraction method, 10 g of finely powdered spice was dissolved in 100 ml distilled water and heated on a hot plate at 80°C for at least one hour. After cooling, the mixture was sieved through a muslin cloth and pure extract was collected in a glass jar. The extract was centrifuged at 3,000 rpm (704 × g) for 15 min and supernatant was again collected in a small beaker. The beaker with extract was then kept in a water bath at 90°C until water evaporates and pure extract remains in a beaker. It was weighed and kept in small tubes in refrigerator at 4°C to be used for checking antioxidant activity and antimicrobial properties. In case of solvent extraction method, ethyl acetate was used as solvent. Again, 10 g of finely powdered spice was weighed and extracted with solvent ethyl acetate in a soxhlet apparatus for at least 24 h at 70°C. The solvent with extract was filtered with Wathman no.1 filter paper and centrifuged for 5 min at 5,000 rpm (1957 × g). In order to obtain pure extract, the extraction solvent was removed by using rotary evaporator (IKA HB10 basic) at 70°C. Then, solvent fee extract was finally stored at 4°C until use. Stock solution of crude ethyl acetate extracts were prepared by diluting the dried extracts with 10% dimethyl sulphoxide (DMSO) solution to obtain a final concentration.

Determination of antioxidant activity 2,2'-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging method

The antioxidant activity of extracts was measured in terms of hydrogen donating or radical scavenging ability, using the stable radical DPPH (Brand-Williams et al., 1995). The ethyl acetate stock solution (20 µl) of the extracts (concentration of stock solutions were 25, 20, 15, 5, 2 and 1 mg/ml) as mentioned above was put into an Eppendroff tubes and 2 ml of 6×10^{-5} mol/L ethyl acetate

solution of DPPH was added. Absorbance measurements at 517 nm commenced immediately using a spectrophotometer and the decrease in absorbance after 1 h was determined for all samples. Ethyl acetate was used to zero the spectrophotometer. Percent inhibition of the DPPH radical by the samples was calculated according to the formula of Yen and Duh (1994).

$$\% \text{ inhibition} = (A_{C(o)} - A_{A(t)} / AC_{(o)}) \times 100$$

Where $A_{C(o)}$ is the absorbance of the control at t = 0 min and $A_{A(t)}$ is the absorbance of the antioxidant at = 1 h.

Determination of ferric reducing antioxidant power (FRAP assay)

The FRAP assay was done according to the method of Benzie and Strain (1996) with some modifications. The stock solutions included 300 mM acetate buffer (3.1 g $C_2H_3NaO_2.3H_2O$ and 16 ml $C_2H_4O_2$), pH 3.6, 10 mM TPTZ (2,4,6-tripyridyl-s-triazine) solution in 40 mM HCL, and 20 mM $FeCl_3.6H_2O$ solution. The fresh working solution was prepared by mixing 25 ml acetate buffer, 2.5 ml TPTZ solution, and 2.5 ml $FeCl_3.6H_2O$ solution followed by warming at 37°C before use. Spice extracts (150 µl) were allowed to react with 2850 µl of the FRAP solution for 30 min in the dark condition. Readings of the colored product (ferrous tripyridyltriazine complex) were then taken at 593 nm. The standard curve was linear between 25 and 800 µM Trolox. Results were expressed in umol (FeII)/g DW.

Determination of total phenolic contents

The total phenolic contents (TPC) were estimated according to the Folin-Ciocalteu method (Singleton et al., 1999). To 50 µl sample were added 250 µl of undiluted Folin-Ciocalteu reagent (diluted 10 times with water). After 1 min, 750 µl of 20% (w/v) aqueous Na_2CO_3 were added, and the volume was made up to 5.0 ml with distilled water. The controls contained all the reaction reagents except the extract. After 2 h of incubation at 25°C, the absorbance was measured at 760 nm and compared to a gallic acid calibration curve. Total phenols were determined as gallic acid equivalents (mg/100 g extract) and the values are presented as means of triplicate analysis. Each assay was carried out in triplicate.

Microbial strain and growth media

A loopful of 24 h surface growth on a nutrient agar (NA) and tryptic soy agar (TSA) slope of each bacterial strain was transferred individually to 5 ml of brain heart infusion (BHI) broth (pH 7.6). After incubation at 37°C for 24 h, bacterial cells were collected by centrifugation at 3,000 rpm for 15 min, washed twice and re-suspended in PBS. Turbidity was adjusted to match that of a 0.5 McFarland standard (10^6 CFU/ml). Then, a 1:10 dilution of the cell suspension was performed to give an inoculum concentration of 10^7 CFU/ml.

Screening of spice extracts using disk diffusion technique

The disk diffusion test was performed using the standard procedure as described by Bauer et al. (1966). The bacteria, *V. vulnificus* was incubated in nutrient broth (NB) and *M. luteus* was incubated in tryptone soy broth (TSB) at 37 ± °C for 24 h. The inoculums suspension of bacterial stains, *V. vulnificus* (MTCC 1145) and *M. luteus* (MTCC 106) was swabbed on the entire surface of Mueller-Hinton agar (MHA, pH 7.3 ± 0.1). Sterile 6 mm filter paper discs (Schleicher & Schuell) were aseptically placed on MHA surfaces by pressing slightly, and solvent extracts of spices were immediately added to discs in volume of 15 µl, respectively. The plates were left at ambient temperature for 15 min to allow excess re-diffusion of extracts prior to incubation at 37°C for 24 h. Diameters of inhibition zones were measured. Each experiment was done in duplicate. All of the extracts individually were injected into sterile paper discs having a diameter of 6 mm in the amount of 15 µl. Discs injected by micropipette with pure ethyl acetate and distilled water served as negative control whereas disc containing 10 µg amoxicillin was placed in the plate as a positive control.

Statistical analysis

Univariate analysis of variance (ANOVA) was applied to the data to determine differences ($p < 0.05$). To discover where there were significant differences between the levels of the main factor, least significant difference was used. The statistical analyses were made using statistical package for social sciences (SPSS)-17(Microsoft TechNet).

RESULTS

The radical scavenging capacity of both water and ethyl acetate spice extracts was tested using the 'stable' free radical, DPPH, FRAP and TPC. Tables 1 and 2 shows the effective concentrations of each extract required to scavenge DPPH radical and the scavenging values as inhibition (%). It was observed that the extracts analyzed exhibited varying degrees of scavenging capacities. Cardamom extracted with water exhibited the highest ($p < 0.05$) radical scavenging effect which was higher than the other extracts. Clove extracted with ethyl acetate exhibited the highest ($p < 0.05$) radical scavenging effect. The lowest activity was shown by black pepper extracted with water and fenugreek seeds extracted with ethyl acetate. A concentration dependent ferric reducing capacity was found for all the spice extracts (Tables 3 and 4). Clove extract again exhibited the strongest ($p < 0.05$) radical scavenging effect which was highest in both water and ethyl acetate extracts followed by cardamom, thyme, black cardamom, fenugreek seeds and black pepper. Total phenolic contents of the clove was highest ($p < 0.05$) among all extracts in both water and ethyl acetate extracts. Other extracts were in the order of thyme, cardamom, fenugreek, black cardamom and black pepper (Tables 5 and 6).

The results of the disk diffusion test indicated that spice extracts clove, cardamom, thyme, black cardamom, fenugreek seeds and black pepper exhibited different degrees of growth inhibition at different concentrations depending on the bacterial stains (Tables 7 and 8). Only clove and cardamom displayed the zone of inhibition at all concentrations. Clove displayed the highest 12 mm inhibition zone against *V. vulnificus* and 14 mm inhibition zone against *M. luteus* in both water and ethyl acetate extracted spices at highest concentration. Cardamom displayed 11 mm inhibition zone against *V. vulnificus* and 12 mm zone against *M. luteus* while it displayed 13 mm zone against *M. luteus* in ethyl acetate extraction. Other

Table 1. Antioxidant activity of water extracts of spices using the corresponding concentrations (A = 25, B = 20, C = 15, D = 5, E = 2 and F = 1 mg/ml) measured by DPPH (% inhibition) method.

S/No.	Spices	Concentration (mg/ml)						Overall mean
		1	2	5	15	20	25	
1	Fenugreek seeds	1.659±0.27	5.490±0.29	16.601±0.29	39.857±0.22	42.894±1.10	79.974±.99	31.079±27.67
2	Clove	25.947±0.22	50.861±0.69	76.415±0.22	75.676±1.08	96.553±0.33	97.415±0.58	64.327±20.32
3	Thyme	18.901±0.16	32.525±0.67	48.734±1.63	51.642±0.33	76.858±0.47	80.206±0.14	57.628±30.71
4	Black cardamom	3.082±1.47	11.560±0.14	2.793±0.22	15.125±0.08	42.581±0.22	57.080±0.28	22.037±21.16
5	Black pepper	0.820±0.46	17.317±0.22	29.570±0.46	51.423±0.60	59.623±0.28	83.695±0.22	40.408±28.43
6	Cardamom	30.776±0.30	60.766±0.14	62.831±0.29	65.929±0.14	78.761±0.14	89.429±0.88	64.749±18.69
Overall mean		15.298±10.57	31.537±16.76	45.316±22.06	57.267±18.32	70.134±17.01	82.484±10.44	50.339±27.89

CD: ($p < 0.05$), Conc: 0.42, Spices: 0.57, Spices*Conc: 1.40.

Table 2. Antioxidant activity of ethyl acetate extracts of spices using the corresponding concentrations (A = 25, B = 20, C = 15, D = 5, E = 2 and F = 1) measured by DPPH (% inhibition) method.

S/No.	Spices	Concentration (mg/ml)						Overall mean
		1	2	5	15	20	25	
1	Fenugreek seeds	1.291±0.40	4.974±0.29	12.919±0.62	21.447±1.16	33.462±0.40	43.540±0.29	19.605±15.51
2	Clove	31.166±3.96	42.737±0.17	69.473±0.69	76.760±0.83	88.773±0.29	88.084±0.22	66.166±22.62
3	Thyme	15.885±0.33	20.840±0.42	35.164±0.24	48.411±0.24	84.598±0.61	85.998±0.24	48.483±28.84
4	Black cardamom	11.127±0.52	15.510±0.71	20.423±0.36	28.564±0.36	45.712±0.30	69.267±0.30	31.767±20.71
5	Black pepper	10.805±0.36	19.585±0.22	38.446±0.84	51.085±0.25	71.924±0.52	86.589±0.68	46.406±27.67
6	Cardamom	18.043±0.30	30.973±0.44	42.182±0.14	62.536±0.14	81.465±0.61	91.838±0.55	54.506±27.29
Overall mean		12.760±8.49	23.850±9.33	41.028±14.61	53.812±15.72	70.281±16.53	80.383±13.74	47.019±27.44

CD: ($p < 0.05$), Conc: 0.39, Spices: 0.53, Spices*Conc: 1.30.

extracts displayed no inhibition zone below 20 mg/ml concentration.

DISCUSSION

A variety of tests expressing antioxidant potency of food components has been suggested. These can be categorized into two groups: assays for radical scavenging ability and assays that test the ability to inhibit lipid oxidation under accelerated conditions (Schwarz et al., 2001). The features of an oxidation are a substrate, an oxidant and an initiator, intermediates and final products. Measurement of any of one of these can be used to assess antioxidant activity (Antolovich et al., 2002).

In this study, the antioxidant activities of six aromatic spice plants belonging to different plant families were determined.

DPPH assay

The radical scavenging capacity of the spice extracts both in water and ethyl acetate solvent was tested using the 'stable' free radical, DPPH. Tables 1 and 2 showed the effective concentrations of each spice extract required to scavenge DPPH radical and the scavenging values as inhibition (%). It can be seen that the extracts analyzed exhibited varying degrees of scavenging

Table 3. Antioxidant activity of water extracts of spices using the corresponding concentrations (A = 25, B = 20, C = 15, D = 5, E = 2 and F = 1) measured by FRAP (umol(FeII)/g DW) method.

S/No.	Spices	Concentration (mg/ml)						Overall mean
		1	2	5	15	20	25	
1	Fenugreek seeds	110.125±0.43	130.125±0.25	131.708±0.38	132.791±0.76	135.125±0.50	146.291±1.25	131.027±11.05
2	Clove	146.291±0.62	172.708±0.62	193.708±0.62	221.791±0.38	238.458±0.38	275.458±16.09	208.069±44.25
3	Thyme	129.208±0.52	145.458±1.94	172.041±7.91	169.791±2.87	195.958±2.74	209.625±13.46	170.347±28.76
4	Black cardamom	115.208±0.38	131.458±0.76	131.041±0.87	134.291±7.37	148.291±8.50	143.708±1.23	134.000±11.53
5	Black pepper	75.958±4.19	90.625±17.33	104.875±1.39	131.708±2.02	133.875±1.08	196.875±0.66	122.319±40.84
6	Cardamom	131.875±0.25	122.125±4.44	140.625±3.92	167.291±7.28	194.458±4.65	212.208±2.12	161.430±34.20
Overall mean		118.503±20.21	129.549±28.89	141.594±30.26	163.238±29.74	182.958±35.11	204.125±40.57	156.661±43.21

CD: ($p<0.05$), Conc: 2.34, Spices: 3.181, Spices*Conc: 7.79.

Table 4. Antioxidant activity of ethyl acetate extracts of spices using the corresponding concentrations (A = 25, B = 20, C = 15, D = 5, E = 2 and F = 1) measured by FRAP (umol(FeII)/g DW) method.

S/No.	Spices	Concentration (mg/ml)						Overall mean
		1	2	5	15	20	25	
1	Fenugreek seeds	118.375±.25	129.625±.25	132.875±.25	152.125±.25	156.875±.25	159.291±1.23	141.527±15.81
2	Clove	149.541±.38	179.375±.25	205.708±.62	229.375±.25	249.791±1.42	286.875±.25	216.777±46.41
3	Thyme	144.708±9.19	151.708±2.98	154.125±2.64	178.375±1.63	211.375±18.85	232.791±2.25	178.847±34.61
4	Black cardamom	124.458±2.37	113.708±.62	131.875±.90	156.458±.76	176.125±.50	209.791±6.33	152.069±34.13
5	Black pepper	74.625±2.17	83.208±1.94	104.208±.62	143.041±5.24	153.791±2.89	149.958±.38	118.138±33.18
6	Cardamom	132.458±.38	149.125±.25	158.458±.38	179.708±.38	196.541±.62	230.125±.50	174.402±33.28
Overall mean		125.905±23.26	138.943±31.66	155.231±33.74	180.473±28.92	193.390±44.69	223.541±38.51	169.580±47.44

CD: ($p<0.05$), Conc: 6.23, Spices: 8.44, Spices*Conc : 20.69.

capacities. Clove extract showed the strongest ($p < 0.05$) radical scavenging effect (97.415 ± 0.58) extracted with water and (88.084 ± 0.22) extracted with ethyl acetate at 25 mg/ml which is highest as compared with other extracts. Black pepper showed the lowest scavenging activity ($p < 0.05$). The antioxidant activity of clove extract is mainly due to the high content of eugenol. The same result was previously indicated by the lipid-malonaldehyde assay (Lee and Shibamoto, 2001). Comparing 16 spices, Khatun et al. (2006) found that clove had the highest radical-scavenging activity followed by all spice and cinnamon. The antioxidant activity of glycosidically bound volatile compounds in clove essential oil has been reported to be significantly greater than that of the volatile aglycones (Politeo et al., 2010). The glycosides can undergo enzymatic hydrolysis releasing their aglycones, therefore, could be considered as potential antioxidant precursors.

Heating at 100°C for up to 6 h increases the peroxyradical-scavenging activity of clove (Khatun et al., 2006). Matthäus (2002) reported a significant decrease ($p < 0.01$) in the concentration of DPPH radical due to the scavenging ability of clove oil and standards. BHA, BHT, α-tocopherol and trolox were used as references for radical scavengers. The scavenging effect of clove oil and standards on the DPPH radical decreased in the order of clove oil > BHT > α-

Table 6. Antioxidant activity of ethyl acetate extract of spices using the corresponding concentrations (A=25 mg/ml, B=20mg/ml, C=15mg/ml, D=5mg/ml, E=2mg/ml and F=1mg/ml) measured by TPC (mg/100g) method.

S/No.	Spices	Concentration (mg/ml)						Overall mean
		1	2	5	15	20	25	
1	Fenugreek seeds	5.916±0.14	8.833±0.38	10.083±0.14	33.333±4.64	55.666±1.25	84.000±2.00	32.972±29.63
2	Clove	93.333±0.76	111.666±2.02	133.666±1.52	153.750±1.01	174.666±1.08	186.083±1.37	142.194±33.90
5	Thyme	10.750±1.75	12.666±0.76	19.750±0.90	39.166±0.38	71.416±3.44	107.166±1.75	43.486±36.26
7	Black cardamom	4.083±0.38	6.500±0.50	11.416±0.14	17.000±1.80	37.000±3.90	44.833±1.60	20.138±15.93
8	Black pepper	1.500±0.25	3.666±0.38	6.583±3.25	27.500±2.13	42.500±2.00	51.000±5.07	22.125±20.21
9	Cardamom	6.166±0.28	10.166±0.62	25.750±0.25	31.583±0.38	50.166±1.66	69.500±3.26	32.222±22.73
Overall mean		12.492±26.08	19.962±30.05	29.515±36.34	45.757±38.66	65.780±40.53	92.143±45.67	44.275±45.68

CD($p<0.05$), Conc: 1.06, Spices: 1.44, Spices*Conc: 3.53.

Table 7. Antimicrobial activity of water extracts of spices against *Vibrio vulnificus* and *Micrococcus leutieus* using the corresponding concentrations (A = 25, B = 20, C = 15, D = 5, E = 2 and F = 1).

Spices	Diameter of inhibition zone (mm)[a]											
	V. vulnificus						*M. luteus*					
	A	***B***	***C***	***D***	***E***	***F***	***A***	***B***	***C***	***D***	***E***	***F***
Fenugreek seeds	8	7	-	-	-	-	8	7	-	-	-	-
Clove	12	10	9	9	8	7	14	12	10	9	9	8
Thyme	9	8	-	-	-	-	10	7	-	-	-	-
Black cardamom	9	8	-	-	-	-	9	7	-	-	-	-
Black pepper	8	7	-	-	-	-	9	7	-	-	-	-
Cardamom	11	10	9	8	8	7	12	11	10	9	8	7
Amoxycillin (positive control)			21						25			
Dist. Water (negative control)			Nil						Nil			

[a]Data are mean of two replications. [b]No inhibition was observed.

tocopherol > BHA > trolox, which were 83.6, 67.8, 64.9, 62.5 and 29.4%, at the concentration of 45 µg/ml, respectively. In the present study, radical scavenging effect were in the order of clove > cardamom > thyme > black cardamom > black pepper > fenugreek seeds at different concentration by DPPH assay.

FRAP assay

Different studies have indicated that the electron donation capacity, reflecting the reducing power of bioactive compounds is associated with antioxidant activity (Siddhuraju et al., 2002; Arabshahi-Delouee and Urooj, 2007). A concentration dependent ferric reducing capacity was found for all the spice extracts. Clove extract at all the concentrations analyzed showed the highest ($p < 0.05$) ferric reducing capacity followed by cardamom, thyme, black cardamom, fenugreek seeds and black pepper. In a study for the measurements of the reductive ability of clove oil,

Table 8. Antimicrobial activity of spices extracted by ethyl acetate against *Vibrio vulnificus* and *Micrococcus luteus* using the corresponding concentrations (A = 25, B = 20, C = 15, D = 5, E = 2 and F = 1).

Spices	Diameter of inhibition zone (mm)[a]											
	V. vulnificus						*M. luteus*					
	A	*B*	*C*	*D*	*E*	*F*	*A*	*B*	*C*	*D*	*E*	*F*
Fenugreek seeds	7	-	-	-	-	-	9	-	-	-	-	-
Clove	12	11	10	9	8	7	14	12	12	10	9	8
Thyme	9	8	7	-	-	-	11	9	7	-	-	-
Black cardamom	9	7	-	-	-	-	8	7	-	-	-	-
Black pepper	8	7	-	-	-	-	8	7	-	-	-	-
Cardamom	11	11	10	9	7	7	13	12	11	10	8	7
Amoxycillin (positive control)			22						25			
Ethyl acetate (negative control)			Nil						Nil			

[a]Data are mean of two replications. [b]No inhibition was observed.

the Fe^{3+}–Fe^{2+} transformation was investigated in the presence of clove oil using the method of Oyaizu (1986). At different concentrations (15 to 45 µg/ml), clove oil demonstrated powerful reducing ability (r^2: 0.9677) and these differences were statistically very significant ($p < 0.01$).

The results of the present study on reducing power demonstrated the electron donor properties of clove extract, thereby neutralizing free radicals by forming stable products. The outcome of the reducing reaction is to terminate the radical chain reactions that may otherwise be very damaging. Clove had highest antioxidant capacity as measured in FRAP and MLP assays and the antioxidant potential of clove extracts may be due to its strong hydrogen-donating and metal chelating ability, as well as its effectiveness as a scavenger of hydrogen peroxide, superoxide and free radicals, which is in agreement with results from the FRAP values for clove which is higher than other spice extracts values. The high antioxidant capacity of Myrtaceae spices is well known (Shan et al., 2005; Khatun et al., 2006; Zheng and Wang, 2001).

TPC assay

The total phenolic compound (TPC) content of the clove, cardamom, thyme, black cardamom, black pepper and fenugreek seeds are given in Tables 5 and 6. In the clove extract both in water and ethyl acetate solvent, a high content of total phenols was obtained. Gülçin et al. (2004) also demonstrated that clove extracts have high phenol content. Black pepper and black cardamom extracts were seen to be a less rich source of total phenols. The phenolic compounds content could be used as an important indicator of the antioxidant capacity, which may be used as a preliminary screen for essential oils when intended as natural sources of antioxidants in functional foods (Liu et al., 2008). Many authors (Hernández-Hernández et al., 2009; Gómez-Estaca et al., 2009) have described the potential antioxidant properties of polyphenols. These compounds act as antioxidants by donation of a hydrogen atom, as an acceptor of free radicals, by interrupting chain oxidation reactions or by chelating metals (Gramza et al., 2004).

Antimicrobial activity

The *in vitro* test of spice extracts both in the water and ethyl acetate solvent was done to determine which spice extract has the strongest antimicrobial activity against *V. vulnificus* and *M. luteus.* Most of these spices are reported to have antimicrobial properties (Rusenova and Parvanov, 2009). In the present study, it was found that clove extract both in water and ethyl acetate solvents had the highest inhibitory activity when compared with the other spice extracts. The clove is known to contain biologically active compounds that is, eugenol which were reported to have antibacterial properties (Burt, 2004). The water extract of clove showed antibacterial activity (12 mm inhibitory zone) and ethyl acetate extract showed little higher (14 mm inhibitory zone) against both *V. vulnificus* and *M. luteus* at higher concentration. Burt and Reinders (2003) obtained similar results of clove extract like Liu and Nakano (1996), Agaoglu et al. (2006) and Shan et al. (2007). The water extract of cardamom also showed antibacterial activity (11 and 12 mm inhibitory zone) against both *V. vulnificus* and *M. luteus* and ethyl acetate extract showed (11 and 13 mm inhibitory zone) against both *V. vulnificus* and *M. luteus* at higher concentration. The oil of cardamom is also highly inhibitory to the tested bacterial so they may contain potent antimicrobial compounds. The major constituent of cardamom oil is α-terpinyl acetate (20 to 53%) (Guzman and Siemonsma, 1999). Rest of the spices extracts showed inhibition zone only at higher concentration while as clove and cardamom showed inhibition zone at all

concentrations. Natural antimicrobial compounds in spices were found to possess antimicrobial activity (Kim et al., 1995).

Conclusion

Many previous studies have reported the antibacterial activity, and antioxidant activities of spices and herbs. But it was not easy to compare directly the results of different studies and to establish reasonable relationships between antibacterial activity and antioxidant activity because of the low number of spice samples tested, different determination methods and different bacterial strains used. The results obtained using three different methods to evaluate the antioxidant activity (DPPH, FRAP and TPC) showed that the spice extracts used in the present study can be considered good sources of natural compounds with significant antioxidant activity and antimicrobial activity against tested organisms.

ACKNOWLEDGEMENT

The first author is highly thankful to the Director/Vice-chancellor, Central Institute of Fisheries Education, Mumbai, India for providing facilities for carrying out the work.

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