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EFFECT OF THERMAL PROCESSING ON SYNERGISTIC ANTIOXIDANT AND ANTIMICROBIAL ACTIVITIES OF TURMERIC (CURCUMA LONGA) AND RED CHILI PEPPER (CAPSICUM ANNUUM)

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The present study reports possible in vitro synergistic antioxidant and antimicrobial effect of turmeric (*Curcuma longa*) and red chili pepper (*Capsicum annuum*) undergoing processes that resemble cooking. Radical scavenging assays like ABTS and DPPH radical decolorization assay, Hydroxyl (OH^o) radical scavenging assay, FRAP assay, as well as determination of total polyphenols and ascorbic acid content were done. Antimicrobial activities were tested against food borne microorganisms like *Escherichia coli*, *Bacillus subtilis*, *Staphylococcus aureus* and *Bacillus cereus*. Among the three heat treatment methods, pressure cooking method was better in ABTS, DPPH and FRAP assays as the antioxidant capacity was retained most. However, microwave irradiation increased the total phenolic contents and OH^o radical scavenging activities, probably due to dissociation of the conjugated polyphenols to liberate the antioxidative components. This was substantiated by the fact that ascorbic acid contents were decreased after heat treatments, although the ferric reducing activities increased. On the other hand, turmeric after heat treatment at 80 °C showed comparatively more antibacterial activity against *Escherichia coli* and *Bacillus subtilis* than the chili powder preparation, whereas chili was more effective against *Staphylococcus aureus* and *Bacillus cereus*. The combinations of the two showed more potent activities against all the bacterial isolates at all the concentration used in the study. The study revealed that thermal treatments resembling cooking methods would increase the antioxidative and antimicrobial properties of the title spices, which corroborate their use in cuisines alone or in tandem.

Keywords: Turmeric, Chili pepper, Antioxidant, Antimicrobial, Synergism

INTRODUCTION

Dietary spices are such nutrients that are being identified vital to maintain human health by their antioxidative, chemopreventive, antimutagenic, anti-inflammatory and immuno-modulatory effects on cells and a wide array of beneficial effects on human health via action on gastrointestinal, cardiovascular, respiratory, metabolic, reproductive, neural and other systems (Shib *et al*., 2014). Spices and flavoring agents contain volatile essential oils and hydrocarbons which stimulate glandular secretion and may have a weak action on the nervous system (Kochhar, 2008). Since humans, unlike other mammals, cannot survive on raw meat and plants, application of aroma and colors in the form of spices to the foods enhances the acceptability of the cuisine by enhancing the color and flavour of the foods (Pawar *et al*., 2011). Spices and their extracts are long known to be used in ancient Mesopotamia, Egypt, India, China and old Greece, where they were appreciated for their

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specific aroma and various medicinal properties (Greathead, 2003). In modern India, spices are still regarded as important part of the cuisine and cooked in a variety of methods based on tradition and taste preferences.

Among the spices commonly used in Indian cuisines, turmeric (*Curcuma longa*) and red chili pepper (*Capsicum annuum*) are two very important spices and essential flavoring agents. Apart from their culinary uses, they possess excellent pharmacological activities. Turmeric is known to be one of the oldest spices (family – Zingiberaceae) used in India and is an integral part of Ayurvedic medicine. Rhizomes of Turmeric are known to possess antihyperlipidemic, antidiabetic, anti-inflammatory, hepatoprotective, antiasthmatic and anticancer activities (Saha *et al.*, 2015). On the other hand, red chili pepper (family – Solanaceae) showed antihyperlipidemic, antidiabetic, anti-inflammatory, anticancer and anti-arrhythmic properties, principally due to the presence of its' active component – capsaicin (Pawar *et al*., 2011). Both the spices have been used as food, spice and household medicine for a long period of time to abolish common problems such as high cholesterol, high blood pressure, pain to joint or skin problems.

A tendency among modern consumers is the use of antioxidant obtained from natural sources, increased the interest in research. The general public, scientists, medical practitioners, nutritional and health experts are interested to know the antioxidant capacity of the fruits, vegetable or spice we consume. It has been observed that it is very difficult to separate each constituent of the food and then test the antioxidant potential individually as it is not only time consuming but also a costly affair. Therefore scientists are interested in looking a convenient method to test the antioxidant capacity of compounds in food mixture. As early as in 1979, it was observed that natural tocopherol in tandem with partial hydrolyzates of gelatin and organic acids like citric and ascorbic acids could be effective against lipid oxidation of oily foods (Kawashima *et al*., 1979). Another study showed that mixtures of methanolic extracts of clove and turmeric showed better antioxidant activities than a mixture of their active components viz. curcumin and euginol, which suggested probable synergistic activities between the numerous components of the spices (Pandey *et al*., 2014). A lot of studies also came up in the recent years, indicating probable synergistic effects between different herbs and spices (Peytar-Maillard *et al.*, 2003; Roberts and Gordon, 2003; Altunkaya *et al.*, 2009; Bag and Chattopadhyay, 2015; and Chawla *et al.*, 2015). Newer observations also indicated plausible synergism between natural and synthetic antioxidants (Hamdo *et al*., 2014).

In India, vegetables and spices are treated and cooked in a variety of methods based on tradition and taste preferences. However, various treatment and cooking processes could affect the levels of nutritional and antioxidant factors of the food ingredient (Chakrobarty and Bhattacharyya, 2014). The present study was designed in such a way that thermal extraction of the spices with water, individually or in combination, resembled closely with common cooking procedures used in India. This would certainly indicate probable changes in the pharmacognostic activities of the spices upon thermal treatment. To our knowledge, it is one of the very few studies that dealt with human consumable water extractives of foodstuffs, extracted by methods that resemble cooking, for their radical scavenging and antimicrobial activities – probably the first with turmeric and red chili pepper. One objective was definitely to get some idea about the apposite cooking method, which would retain the most effectiveness of the subject spices for human consumption. The present study reports the achievement of the aim through some common *in vitro* antioxidant and antimicrobial assays.

MATERIALS AND METHODS

Chemicals

2,2'-Azinobis (3-ethylbenzothiazoline-6-sulfonic acid), ABTS, were obtained from Sigma, USA. 2,2'-Diphenyl-1-picryl hydrazyl (DPPH) were obtained from Himedia, India. Analytical grades of 2-Deoxy-D-ribose, thiobarbituric acid (TBA), ascorbic acid, gallic acid, Folin-Ciocalteau's solution, sodium hydroxide and sodium carbonate were obtained from Merck, India. Muller Hinton Agar was purchased from HiMedia. All other reagents and chemicals used were of analytical grade procured from local sources. Deionized distilled water was used in the entire study.

Preparation of Samples

Turmeric and chili samples were procured from local markets of Barasat, Kolkata. The samples were checked for dirt or any visible damages prior to the study. Such samples were discarded. Fresh rhizome of turmeric and fresh red chili fruits were sun dried and made powder using a commercial electrical grinder. 5 gms each of the samples were suspended in 50 ml double distilled water, separately. The samples were extracted by the following procedures:

1. Heating at 80 °C for 10 mins (coded as – HT),
2. Treating in a microwave oven at high power for 5 mins (coded as – MW),
3. Putting in a pressure cooker for 5 mins (coded as – PC).

To estimate the optimum antioxidant capacity, the samples were warmed separately at 60 °C for 10 mins, after suspended in 60% methanol in water. After extraction, the samples were centrifuged at 8000 rpm for 5 mins. The clear supernatants were used for *in vitro* antioxidant and antimicrobial assays. To adjudicate synergism between the two samples, turmeric and chili were taken in ratios 1:1, 2:1 and 1:2 (w/w), separately in 50 ml double distilled water and the treatment protocols were followed as above.

ABTS Radical Decolorization Assay

The ABTS assay was performed using a previously described procedure (Chakrobarty and Bhattacharyya, 2014). $ABTS^{-\bullet}$, the oxidant, was generated by persulfate oxidation of 2,22-azinobis (3-ethylbenzothiazoline)-6-sulfonic acid. This solution was diluted with phosphate buffer (pH 7.4) until the absorbance reached 0.7 to 0.8 at 734 nm in a Systronics spectrophotometer (model – 2202). The oxidant solution was mixed with the sample solutions in such a way that total volume of the solution reached 1 ml. The absorbance was read at room temperature, 4 minutes after mixing. The concentration that causes a decrease in the absorbance of initial oxidants by 50% is defined as IC_{50} of the samples. Gallic acid was used as positive control and comparing with its' IC_{50} and the results were expressed as Gallic acid equivalents (µM/gm fresh leaves).

DPPH Radical Decolorization Assay

The DPPH assay was performed using a previously described procedure (Chakrobarty and Bhattacharyya, 2014). 1 ml DPPH solution (3 mg in 25 ml ethanol) was mixed with 0.5 ml sample solution and the decrease in absorbance of the mixture after 20 minutes of incubation in the dark was monitored at 517 nm in a Systronics spectrophotometer (model – 2202). The concentration that causes a decrease in the absorbance of initial oxidants by 50% is defined as IC_{50} of the samples. Gallic acid was used as positive control and comparing with its' IC_{50} and the results were expressed as Gallic acid equivalents (µM/gm fresh leaves).

Estimation of Total Phenolics Content

Total phenolic compound contents were determined by the Folin-Ciocalteau method (Sarkar *et al.*, 2014). The samples (0.5 ml) were mixed with Folin-Ciocalteau reagent (5 ml, of 1:10 diluted sample with distilled water) for 5 min and aqueous sodium carbonate (4 ml, 1 M) was then added. The absorbance of the reaction mixture was then measured at 765 nm in a UV-Vis spectrophotometer (model – Systronics 2202). Gallic acid was used as standard. The results were expressed in terms of µg gallic acid equivalent/gm fresh leaves.

Hydroxyl Radical Scavenging Assay

Hydroxyl radical scavenging potentials of the samples were estimated with a previously described procedure with minor modifications (Malik *et al.*, 2011). Briefly, 10 mM each of $FeSO_4.7H_2O$, EDTA, 2-deoxy-D-ribose and H_2O_2 solutions were prepared in water. 0.2 ml each of above four and 0.2 ml sample and/or standard solution was mixed in a test tube and incubated at 37°C for 90 mins. H_2O_2 solution was added last. After the incubation, 1 ml of 2.8% (w/v) aqueous TCA solution and 1 ml of 1% (w/v) aqueous TBA solution were added to the reaction mixture and kept at boiling water bath for 20 mins. Development of the pink chromophore was measured at 532 nm in a UV-Vis spectrophotometer (model – Systronics 2202). Gallic acid is used as standard. Results were expressed as Gallic Acid Equivalents (GAE).

Ferric Reducing Antioxidant Power: FRAP

Ferric reducing potentials of the samples were estimated with a previously established procedure with minor modifications (Rabeta and Nur Faraniza, 2013). Briefly, a maximum of 100 µl of extract solution or standard was mixed with 1.9 mL of FRAP reagent and incubated at 37 °C for 30 mins. FRAP reagent was prepared by mixing 50 mL of 0.1 M acetate buffer (pH 3.6), 5 mL of 10 mM TPTZ solution and 5 mL of 20 mM $FeCl_3$ solution. After the stipulated time period, absorbance was measured at 593 nm in a UV-Vis spectrophotometer (model – Systronics 2202). Gallic acid is used as standard. Results are expressed as Gallic Acid Equivalents (GAE).

Estimation of Ascorbic Acid Content

Ascorbic acid contents of the samples were estimated with a previously described procedure with minor modifications (Kevers *et al.*, 2007). Briefly, a maximum of 100 µl sample (or standard) was mixed with 400 µl 5% metaphosphoric acid solution. Then another 500 µl of 10% metaphosphoric acid solution was added followed by 300 µl of citrate buffer (pH 4.15) and 300 µl of 2,6-DCP-IP solution. Absorbance was read at 520 nm in a UV-Vis spectrophotometer (model – Systronics 2202) within 1 min.

Antibacterial Activity Assay

Antibacterial activities of the samples were evaluated for their antibacterial activity against four common bacteria, viz. *Escherichia coli, Bacillus subtilis, Staphylococcus aureus* and *Bacillus cereus*. The antibacterial activity was measured by agar well diffusion method (Ahmad and Beg, 2001). Each bacterial isolates was previously grown on sterile Muller Hinton Agar (HiMedia M173) plate at 35 °C for 24 hours. Single colony of each of the isolates was grown in Muller Hinton broth (HiMedia M391) for 3 hours at 35 °C. Different dilutions of these two spices were prepared in sterile water. From these different dilutions, 50 µl solution was poured into the wells of the respective culture plates. After incubation for 24 hrs at 35 °C, the plates were observed. The zone of inhibition was measured and expressed in millimeters. Antibacterial activity was recorded if the zone of inhibition was greater than 6 mm. The antibacterial activity results were expressed in term of the diameter of zone of inhibition and <9 mm zone was considered as inactive; 9-12 mm as partially active; while13-18 mm as active and >18mm as very active (Junior and Zanil, 2000).

Statistical Analysis

Values were expressed as mean ± standard deviation of three replicates of each experiment. The analyses were done using the software – SPSS Statistics 17.0 (IBM Corporation).

RESULTS AND DISCUSSION

ABTS Radical Decolorization Assay

The study was performed mainly to compare the change in the antioxidant profile of the spices, individually or in combination, after undergoing processes resembling common cooking methods. To evaluate this, a 60% aqueous methanolic extract was prepared as a comparator having optimum antioxidant capacity for each of the individual or combined samples. The general observation in this assay system indicated that after heat treatments resembling common cooking methods, the antioxidant potentials decreased, probably due to decomposition of the bioactives (Table 1). Among the three heat treatment methods, pressure cooking method was better as the antioxidant capacity was retained most. It has been observed that the decrease in the antioxidative capacity of chili and turmeric were 17% and 21%, respectively, from the optimum value of the comparator (i.e., GAE of 60% aqueous methanolic extract). However, if the two spices were mixed in 1:1 ratio, the decrease comes down to only 12%. This might be due to mutual protection of the bioactives from the two different spices, indicating possible synergism between the two. Mixing the spices into two other ratios surely showed antioxidative qualities, although the effects were not marked.

DPPH Radical Decolorization Assay

After heat treatments resembling common cooking methods, the antioxidant potentials decreased, probably due to decomposition of the bioactives. Among the three heat treatment methods, pressure cooking method was better as the antioxidant capacity was retained most Table 2). It has been observed that the decrease in the antioxidative capacity of chili and turmeric were 0.21% and 0.08%, respectively, from the optimum value. However, if the two spices were mixed in 1:1 ratio, the decrease comes down to only 0.05%. This might be due to mutual protection of the bioactives from the two different spices, indicating possible synergism between the two. Mixing the spices into two other ratios surely showed antioxidative qualities, although the effects were not marked.

Table 1: ABTS Radical Decolorization Potential of Turmeric, Chili and Combinations Thereof After Processing with Different Thermal Conditions, Results are Expressed as GAE (µg Gallic acid/gm)

Sample	60% Methanol Extract (Comparator)	Treatment Regimen		
		HT	PC	MW
Turmeric	450.85 ± 18.58	315.69 ± 13.86	355.61 ± 15.42	287.72 ± 12.65
Chili	489.61 ± 21.63	370.87 ± 16.51	406.08 ± 17.39	319.28 ± 13.17
Mixture (1:1)	501.49 ± 21.52	413.90 ± 17.09	442.19 ± 18.63	343.96 ± 14.37
Mixture (1:2)	328.20 ± 14.30	212.60 ± 13.80	250.65 ± 10.33	203.63 ± 9.07
Mixture (2:1)	300.87 ± 12.54	150.79 ± 10.58	196.13 ± 12.67	121.47 ± 5.25

Table 2: DPPH Radical Decolorization Potential of Turmeric, Chili and Combinations Thereof After Processing with Different Thermal Conditions, Results are Expressed as GAE (µg Gallic acid/gm)

Sample	60% Methanol Extract (Comparator)	Treatment Regimen		
		HT	PC	MW
Turmeric	63.89 ± 2.61	46.01 ± 1.97	58.57 ± 2.4	50.03 ± 2.18
Chili	54.36 ± 2.27	31.85 ± 1.37	42.44 ± 1.75	40.39 ± 1.96
Mixture (1:1)	67.81 ± 2.81	55.91 ± 2.29	64.74 ± 2.65	61.69 ± 2.61
Mixture (1:2)	52.73 ± 2.45	28.31 ± 1.84	38.02 ± 1.54	37.13 ± 1.67
Mixture (2:1)	58.57 ± 2.41	30.92 ± 1.26	47.80 ± 2.01	41.05 ± 1.7

Table 3: Total Phenolic Contents of Turmeric, Chili and Combinations Thereof After Processing with Different Thermal Conditions, Results are Expressed as GAE (µg Gallic acid/gm)

Sample	60% Methanol Extract (Comparator)	Treatment Regimen		
		HT	PC	MW
Turmeric	481.05 ± 19.98	189.34 ± 8.05	167.24 ± 7.22	227.51 ± 9.46
Chili	604.74 ± 30.52	276.54 ± 7.89	222.63 ± 9.23	294.98 ± 12.44
Mixture (1:1)	395.51 ± 16.37	181.25 ± 7.38	205.47 ± 8.96	371.54 ± 15.9
Mixture (1:2)	462.92 ± 20.54	209.77 ± 8.67	194.11 ± 8.33	259.12 ± 11.5
Mixture (2:1)	283.05 ± 11.75	141.17 ± 8.37	125.84 ± 5.22	255.45 ±10.77

Table 4: Hydroxyl Radical Scavenging Potential of Turmeric, Chili and Combinations Thereof After Processing with Different Thermal Conditions, Results are Expressed as GAE (µg Gallic acid/gm)

Sample	60% Methanol Extract (Comparator)	Treatment Regimen		
		HT	PC	MW
Turmeric	1148.27 ± 48.43	1031.25 ± 43.43	791.99 ±32.67	1027.04 ±46.39
Chili	743.16 ± 31.64	394.95 ± 18.85	521.07 ± 21.99	660.95 ± 28.76
Mixture (1:1)	901.05 ± 37.16	694.50 ± 28.53	472.07 ± 19.47	760.97 ± 31.03
Mixture (1:2)	831.21 ± 35.98	386.26 ± 16.01	594.95 ± 26.46	491.85 ± 19.69
Mixture (2:1)	336.49 ± 16.27	245.39 ± 13.61	259.40 ± 9.59	275.34 ± 11.43

Estimation of Total Phenolics Content

After heat treatments resembling common cooking methods, the antioxidant potentials decreased, probably due to decomposition of the bioactives (Table 3). Microwave cooking method is better than the other methods, probably due to lesser exposure of the bioactives to heat. It has been observed that the decrease in the polyphenol contents of chili and turmeric were 0.51% and 0.53%, respectively, from the optimum value. However, if the two spices were mixed in 1:2 ratio, the decrease comes down to only 0.06%. This

might be due to mutual protection of the bioactives from the two different spices, indicating possible synergism between the two. Mixing the spices into two other ratios surely showed antioxidative qualities, although the effects were not marked.

Hydroxyl Radical Scavenging Assay

After heat treatments resembling common cooking methods, the antioxidant potentials decreased, probably due to decomposition of the bioactives (Table 4). Microwave cooking method is better than the other cooking method, probably due to breakdown of the conjugated bioactives to produce free bioactives by microwave irradiation. It has been observed that the decrease in the antioxidative capacity of chili and turmeric were 0.51% and 0.53%, respectively, from the optimum value. However, if the two spices were mixed in 1:2 ratio, the decrease comes down to only 0.06%. This might be due to mutual protection of the bioactives from the two different spices, indicating possible synergism between the two. Mixing the spices into two other ratios surely showed antioxidative qualities, although the effects were not marked.

Ferric Reducing Antioxidant Power: FRAP

After heat treatments resembling common cooking methods, the reduction power decreased, probably due to decomposition of the bioactives (Table 5). Pressure cooking method is better than the other method, probably due to breakdown of the conjugated bioactives to free bioactives by heating. It has been observed that the decrease in the reducing power of chili and turmeric were 0.30% and 0.39%, respectively, from the optimum value. However, if the two spices were mixed in 1:2 ratio, the decrease comes down to only 0.23%. This might be due to mutual protection of the bioactives from the two different spices, indicating possible synergism between the two. Mixing the spices into two other ratios surely showed antioxidative qualities, although the effects were not marked.

Table 5: Ferric Reducing Antioxidant Potential of Turmeric, Chili and Combinations Thereof After Processing with Different Thermal Conditions, Results are Expressed as GAE (µg Gallic acid/gm)

Sample	60% Methanol Extract (Comparator)	Treatment Regimen		
		HT	PC	MW
Turmeric	476.15 ± 19.98	268.91 ± 12.15	290.80 ± 12.08	165.14 ± 7.25
Chili	882.21 ± 38.21	522.61 ± 22.01	614.52 ± 27.65	466.93 ± 21.22
Mixture (1:1)	707.49 ± 30.87	320.13 ± 14.14	471.10 ± 19.98	337.90 ± 14.57
Mixture (1:2)	1007.46 ± 42.56	644.80 ± 26.54	730.80 ± 30.01	663.19 ± 29.03
Mixture (2:1)	289.79 ± 12.74	167.54 ± 8.44	222.97 ± 9.8	125.74 ± 7.38

Table 6: Ascorbic Acid Contents of Turmeric, Chili and Combinations Thereof After Processing with Different Thermal Conditions, Results are Expressed as µg Ascrorbic acid/gm

Sample	60% Methanol Extract (Comparator)	Treatment Regimen		
		HT	PC	MW
Turmeric	649.03 ± 26.87	496.77 ± 22.23	472.58 ± 13.45	587.16 ± 18.05
Chili	481.67 ± 20.89	455.13 ± 20.28	321.67± 21.39	428.42 ± 25.86
Mixture (1:1)	466.20 ± 19.95	340.04 ± 15.85	250.59 ± 10.28	411.57 ± 14.05
Mixture (1:2)	319.90 ± 13.51	251.69 ± 10.47	271.68 ± 11.59	309.31 ± 11.24
Mixture (2:1)	340.52 ± 14.46	249.11 ± 11.29	281.92 ± 13.08	318.55 ± 12.12

Estimation of Ascorbic Acid Content

After heat treatments resembling common cooking methods, the ascorbic acid content decreased, probably due to decomposition of the bioactive (Table 6). Microwave cooking method is better than the other cooking method, probably due to breakdown of the bioactive by microwave irradiation was minimum due to minimum exposure to the radiation. It has been observed that the decrease in the ascorbic acid content of chili and turmeric were 0.11% and 0.09%, respectively, from the optimum value. However, if the two spices were mixed in 1:1 ratio, the decrease comes down to only 0.03%. This might be due to mutual protection of the ascorbic acid by the bioactives of two different spices, indicating possible synergism between the two. Mixing the spices into two other ratios surely showed total ascorbic acid content, although the effects were not marked.

Antibacterial Activity Assay

Almost all the preparations showed considerable antimicrobial activity against all the experimental bacterial culture. Crude turmeric powder extracted in water at 80 ^{0}C showed comparatively more antibacterial activity against *E. coli* and *Bacillus subtilis* than the chili powder preparation (Table 7). On the contrary, chili powder was more effective against *Staphylococcus aureus* and *Bacillus cereus (*Table 8). Some researchers have found that capsaicin, the main active compound found in chili for pungency or heat sensation, has an antimicrobial property against *Helicobacter pylori* (Jones *et al*., 1997). However, no antimicrobial effect of dried chili was found in this study. This may be because the capsaicin compound has less water solubility (Lopez-Carrillo *et al*., 2003). The different combinations of mixture of chili and turmeric showed that the mixture of chili and turmeric was more potent against all the bacterial isolates at all the concentration used in the study (Tables 7 and 8). This showed that the mixture of these two spices was more useful for the destruction of the microorganism than their single used. Heating of the different extracts alone and also in different combination of mixture of chili and turmeric showed that the antimicrobial activity of these preparations decreased after heating the preparations at higher temperatures but still their antimicrobial activities were not completely destroyed even after heating in pressure cooker and also in microwave oven

Table 7: Antibiogram Pattern of Chili and Turmeric and Their Mixtures for *Escherichia coli* and *Bacillus subtilis*

Sample		Concentration (mg/ml)	Microorganisms	
			Escherichia coli	*Bacillus subtilis*
HT	Chili	1	13.33 ± 0.67	13.33 ± 0.67
		0.1	9 ± 0.49	8.67 ± 0.43
		0.01	-	-
	Turmeric	1	16 ± 0.80	16.51 ± 0.83
		0.1	8.67 ± 0.43	11.33 ± 0.57
		0.01	-	-
	Mixture (1:1)	1	17.33 ± 0.87	18.33 ± 0.92
		0.1	11.67 ± 0.58	12.67 ± 0.63
		0.01	8.67 ± 0.43	9 ± 0.47
	Mixture (1:2)	1	17.67 ± 0.89	18.66 ± 0.93
		0.1	13.33 ± 0.58	12.67± 0.63
		0.01	8.65 ± 0.51	13.33 ± 0.58
	Mixture (2:1)	1	13.12 ± 0.61	13.57± 0.6
		0.1	8.33 ± 0.42	9.68 ± 0.48
		0.01	-	-

Table 7 (Cont.)

PC	Chili	1	8.67 ± 0.43	10.33 ± 0.52
		0.1	-	-
		0.01	-	-
	Turmeric	1	12.67 ± 0.63	10.33 ± 0.52
		0.1	9.67 ± 0.48	8 ± 0.41
		0.01	-	-
	Mixture (1:1)	1	13.33 ± 0.58	11 ± 0.56
		0.1	7.67 ± 0.38	8.33 ± 4.4
		0.01	-	-
	Mixture (1:2)	1	13.67 ± 0.68	11.67 ± 0.58
		0.1	8.33 ± 0.42	9 ± 0.42
		0.01	-	-
	Mixture (2:1)	1	11.33 ± 0.57	9.67± 0.48
		0.1	8 ± 0.39	-
		0.01	-	-
MW	Chili	1	8.67 ± 0.43	9.33 ± 0.47
		0.1	-	-
		0.01	-	-
	Turmeric	1	12.67 ± 0.03	11 ± 0.55
		0.1	9.57 ± 0.50	-
		0.01	-	-
	Mixture (1:1)	1	11.33 ± 0.57	11.33 ± 0.57
		0.1	-	-
		0.01	-	-
	Mixture (1:2)	1	12 ± 0.62	12.67 ± 0.63
		0.1	8 ± 4.4	-
		0.01	-	-
	Mixture (2:1)	1	10.33 ± 0.52	8.67 ± 0.43
		0.1	-	-
		0.01	-	-

and almost all these preparations showed a considerable amount of zone of inhibition against all the used bacterial culture (Tables 7 and 8).

Spices and herbs have been used for thousand of the centuries by many cultures to enhance the flavour and aroma of food. Spices in the past decade confirm that the growth of both Gram-positive and Gram-negative food borne bacteria, yeasts and molds can be inhibited by garlic, onion, cinnamon, clove, thyme, sage and other spices. Although, the primary purpose of spices is to impart flavour and piquancy to food, the medicinal, antimicrobial and

Table 8: Antibiogram Pattern of Chili and Turmeric and Their Mixtures for *Staphylococcus aureus* and *Bacillus cereus*

Sample		Concentration (mg/ml)	Microorganisms	
			Staphylococcus aureus	*Bacillus cereus*
HT	Chili	60	-	-
		80	9 ± 0.50	9 ± 0.50
		100	11 ± 0.55	12 ± 0.60
	Turmeric	60	-	-
		80	-	-
		100	8.67 ± 0.43	10 ± 0.51
	Mixture (1:1)	60	-	-
		80	8.33 ± 0.38	9 ± 0.50
		100	11.67 ± 0.58	12.33 ± 0.61
	Mixture (1:2)	60	-	9 ± 0.50
		80	9 ± 0.50	13.33 ± 0.58
		100	9 ± 0.50	13.67 ± 0.68
		150	12.33 ± 0.61	15 ± 0.75
	Mixture (2:1)	60	-	-
		80	10 ± 0.51	8 ± 0.41
		100	11.33 ± 0.57	10.67 ± 0.53
		150	14.67 ± 0.73	12 ± 0.60
PC	Chili	60	-	10 ± 0.51
		80	8.33 ± 0.43	10 ± 0.51
		100	11.33 ± 0.57	14 ± 0.70
	Turmeric	60	9 ± 0.50	9 ± 0.50
		80	12 ± 0.60	10.33 ± 0.52
		100	13.67 ± 0.68	13.67 ± 0.68
	Mixture (1:1)	60	10 ± 0.50	9.33± 0.57
		80	10 ± 0.50	12 ± 0.60
		100	14.33 ± 0.73	13 ± 0.27
	Mixture (1:2)	60	9 ± 0.50	9.67 ± 0.56
		80	9.33 ± 0.52	11.67 ± 0.58
		100	12.33 ± 0.65	12 ± 0.60
		150	15.33 ± 0.68	13.67 ± 0.68
	Mixture (2:1)	60	-	8 ± 0.40
		80	9.33 ± 0.52	9 ± 0.50
		100	10 ± 0.50	10.67 ± 0.57
		150	13 ± 0.70	13.67 ± 0.69

Table 8 (Cont.)

MW	Chili	60	8 ± 0.41	8 ± 0.41
		80	9.33 ± 0.50	12 ± 0.60
		100	12 ± 0.60	12 ± 0.60
	Turmeric	60	10 ± 0.50	9 ± 0.45
		80	10 ± 0.50	9 ± 0.45
		100	14 ± 0.07	13 ±0.73
	Mixture (1:1)	60	9 ± 0.45	8.67 ± 0.43
		80	9.33 ± 0.48	10 ± 0.50
		100	12 ± 0.60	11 ± 0.59
	Mixture (1:2)	60	8 ± 0.40	9 ± 0.50
		80	10 ± 0.50	9.67 ± 0.56
		100	11.67 ± 0.58	11.33 ± 0.57
		150	12.67 ± 0.67	12.33 ± 0.63
	Mixture (2:1)	60	-	-
		80	9 ± 0.50	8 ± 0.41
		100	9.6 ± 0.57	9 ± 0.50
		150	11 ± 0.59	11 ± 0.59

antioxidant properties of spices have also been exploited (Shib *et al.*, 2014). The present study indicated possible *in vitro* synergistic antioxidant and antimicrobial effect of the above two spices undergoing processes that resemble cooking with the help of the methods like ABTS radical cation decolorization assay, DPPH radical decolorization assay, Determination of total polyphenols, Hydroxyl ($OH^{\bullet}$) radical scavenging assay, FRAP assay, Determination of ascorbic acid content and determination of total curcuminoids content. It is assumed that 60% methanol extract showed optimum antioxidant capacity as almost all bioactives are extracted with this solvent mixture. After heat treatments resembling common cooking methods, the antioxidant potentials decreased, probably due to decomposition of the bioactives. In all the above methods, possible synergism of the two spices was observed. The study also helped us to understand the *in vitro* synergistic antimicrobial activity against four bacterial cultures, viz. *E. coli*, *Bacillus cereas*, *Bacillus subtilis* and *Staphylococcus aureus*.

CONCLUSION

The major conclusion arising out of this research was that the antioxidant capacities of turmeric and red chili pepper were somehow deteriorated from optimum capacities by thermal processing methods that resemble cooking. Among three different heat treatment methods, radical scavenging activities like ABTS and DPPH was maximum after pressure cooking. This was substantiated by the fact that ferric reducing potential was also optimum after pressure cooking treatment regimen. However, total phenolic content and ascorbic acid contents of the samples were most after microwave treatment. This might be due to the fact that bound phenolics or ascorbic acids were released by this type of treatment, thereby improving antioxidant potential. Turmeric, after heat treatment at 80°C, showed comparatively more antibacterial activity against *Escherichia coli* and *Bacillus subtilis* than the chili powder preparation, whereas chili was more effective against *Staphylococcus aureus* and *Bacillus cereus*. The combinations of the two showed more potent activities against all the bacterial isolates at all the concentration used in the study. A possible synergism was also observed with the mixture of the two spices, probably due to mutual protection of the bioactives from the two different spices. The study thus indicated that mixing of the spices would be beneficial upon heat treatment that resembles cooking, which lends credence to their culinary uses.

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REFERENCES

- Ahmad I and Beg A Z (2001), "Antimicrobial and Phytochemical Studies on 45 Indian Medicinal Plants Against Multi-Drug Resistant Human Pathogens", *J. Ethnopharmacol*, Vol. 74, pp. 113-123.
- Altunkaya A, Becker E M, Gokmen V and Skibsted L H (2009), "Antioxidant Activity of Lettuce Extract (*Lactuca sativa*) and Synergism with Added Phenolic Antioxidants", *Food Chem*, Vol. 115, pp. 163-168.
- Bag A and Chattopadhyay R R (2015), "Evaluation of Synergistic Antibacterial and Antioxidant Efficacy of Essential Oils of Spices and Herbs in Combination", *PLOS ONE*, Vol. 10, No. 7, pp. 1-17.
- Chakrobarty A and Bhattacharyya S (2014), "Thermal Processing Effects on *in vitro* Antioxidant Activities of Five Common Indian Pulses", *J. App Pharm Sci.*, Vol. 4, pp. 65-70.
- Chawla P, Gaur H, Tripathi M, Tripathi M, Agarwal B and Pandey A (2015), "Synergistic Antioxidant Activity of Lipoic, Ferulic and Ellagic Acid", *Int J. Pharm Sci Res*, Vol. 6, No. 6, pp. 2551-2556.
- Greathead H (2003), "Plants and Plant Extracts for Improving Animal Productivity", *Proc. Nutri Soc*, Vol. 62, pp. 279-290.
- Hamdo H H, Khayata W and Al-Assaf Z (2014), "Synergistic Effect of Combined Some Natural and Synthetic Antioxidants to Increase Oxidative Stability Using DPPH Test", *Int J. Chem Tech Res*, Vol. 6, No. 4, pp. 2539-2545.
- Jones N L, Shabib S and Sherman P M (1997), "Capsaicin as an Inhibitor of the Growth of the Gastric Pathogen *Helicobacter pylori"*, *FEMS Microbiol Lett*, Vol. 146, No. 2, pp. 223-227.
- Junior A and Zanil C (2000), "Biological Screening of Brazilian Medicinal Plants", *Braz J. Sci*, Vol. 95, pp. 367-373.
- Kawashima K, Itoh H and Chibata I (1979), "Synergistic Ternary Antioxidant Compositions Comprising Tocopherol, Partially Hydrolyzate of Gelatin and Organic Acid", *Agric Biol Chem*, Vol. 43, No. 4, pp. 827-831.
- Kevers C, Falkowsky M, Tabart J, Defraigne J O, Dommes J and Pincemail J (2007), "Evolution of Antioxidant Capacity During Storage of Selected Fruits and Vegetables", *J. Agric Food Chem*, Vol. 55, No. 21, pp. 8596-8603.
- Kochhar K P (2008), "Dietary Spices in Health and Diseases: I", *Ind J. Physiol Pharmacol*, Vol. 52, No. 2, pp. 106-122.
- López-Carrillo L, López-Cervantes M, Robles-Díaz G, Ramírez-Espitia A, Mohar-Betancourt A, Meneses-García A, López-Vidal Y and Blair A (2003), "Capsaicin Consumption, *Helicobacter pylori* Positivity and Gastric Cancer in Mexico", *Int J. Cancer*, Vol. 106, No. 2, pp. 277-282.
- Malik A, Kushnoor A, Saini V, Singhal S, Kumar S and Yadav Y C (2011), "*In vitro* Antioxidant Properties of Scopoletin", *J. Chem Pharm Res*, Vol. 3, No. 3, pp. 659-665.
- Pandey A, Gupta R K, Lawrence R, Lawrence K and Srivastava R (2014), "Synergistic Study of Antioxidant Potential of Different Spices and their Bioactive Constituents", *Int J. Pharm Sci Res*, Vol. 5, No. 8, pp. 3267-3272.
- Pawar S S, Bharude N V, Sonone S S, Deshmukh R S, Raut A K, Umarkar A R (2011), "Chillies as Food, Spice and Medicine: A Perspective", *Int J. Pharm Biol Sci*, Vol. 1, No. 3, pp. 311-318.
- Peytar-Maillard M N, Cuvelier M E and Berset C (2003), "Antioxidant Activity of Phenolic Componds in 2, 2- Azobis(2-amidopropane) Dihydrochloride (AAPH)-Induced Oxidation: Synergistic and Antagonistic Effects", *J. Am Chem Soc*, Vol. 80, No. 10, pp. 1007-1012.
- Rabeta M S and Nur Faraniza R (2013), "Total Phenolic Content and Ferric Reducing Antioxidant Power of the Leaves and Fruits of *Garcinia atrovirdis* and *Cynometra cauliflora*", *Int Food Res J.*, Vol. 20, No. 4, pp. 1691-1696.
- Roberts W G and Gordon M H (2033), "Determination of the Total Antioxidant Activity of Fruits and Vegetables by a Liposome Assay", *J. Agric Food Chem*, Vol. 51, pp. 1486-1493.
- Saha P, Shib M, Pal T K and Bhattacharyya S (2015), "Thermal Processing Effects on *in vitro* Antioxidant Potential of Fresh and Packaged Turmeric (*Curcuma longa*), Coriander (*Coriandrum sativum*) and Cumin

(*Cuminum cyminum*)", *Am J. Pharm Health Res*, Vol. 3, No. 4, pp. 102-112.

- Sarkar S, Saha S, Rai C and Bhattacharyya S (2014), "Effect of Storage and Preservatives on Antioxidant Status of Some Refrigerated Fruit Juices", *Int J. Curr Microbiol App Sci*, Vol. 3, pp. 1007-1013.
- Shib M, Saha P, Pal T K and Bhattacharyya S (2014), "Thermal Processing Effects on *in vitro* Antioxidant Potential of Fresh and Packaged Black Pepper (*Piper nigrum*) and Indian Red Chili (*Capsicum annuum*)", *Annals Biol Sci*, Vol. 2, pp. 72-78.


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