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Comparative Evaluation of Different Brands of Marketed Spices of India with Special Reference to Physico-Chemical Analysis, Total Polyphenolics Content and in Vitro Antioxidant Activities

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ABSTRACT

The present study is carried out for comparative evaluation of different marketed brands of six widely used spices, namely, turmeric, coriander, cumin, mustard, cardamom and chilli powders with respect to physico-chemical analysis, total polyphenolics content (TPC) and in vitro antioxidant assay using 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2′-azino bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) and Ferric Reducing antioxidant Power (FRAP) assays. All the samples found to contain appreciable TPC and in vitro antioxidant activities. Cumin, Chilli and Turmeric powders were found to be most potent with respect to both TPC and in vitro antioxidant activity.

Keywords: Spices, Physico-chemical analysis, Total Polyphenolics Content, Antioxidant.

INTRODUCTION

Herbs and spices have been used in antiquity in adding flavour, color, aroma and pungency to food [(1)]. Spices have been indispensable in the culinary art. Spices have been used for not only seasoning of food but also for medicinal uses, particularly in Ayurvedic/Siddha/Unani systems of medicine as well as in pharmaceuticals due to their hypocholesterolemic, anti-carcinogenic, anti-diabetic, anti-microbial, anti-inflammatory activities [(2,3,4,5,6)]. Spices are also widely used in nutraceuticals, perfumes, cosmetics and other cosmetic products and so on.

Spices and herbs have been found to have many beneficial effects on human health being a part of everyday diet. Spices, in addition to fruits and vegetables, could provide us with additional sources of natural antioxidants, largely due to the presence of an important group of secondary metabolite bioactive, polyphenolics compounds [(7)]. At a low concentration, those compounds acts as antioxidants by interfering with oxidation processes and neutralises the free radicals by donating hydrogen atoms [(8)]. They are known to reduce the risk of cardiovascular diseases, diabetes, and cancer and so on [(9,10,11)]. Polyphenolics are known to exert anti-allergic, anti-microbial, anti-inflammatory and anti-viral properties which arise in part through the antioxidant characteristics of this bioactive. Although, other bioactives, namely, flavonoids, terpenoids etc. play vital role in imparting the antioxidant properties of spices, the role of polyphenolics in contributing towards the antioxidant activity still remains of paramount importance. Therefore, it is essential to quantify the polyphenolics content to evaluate the antioxidant activity. The in vitro antioxidant activity of spices were carried out by three popular assay systems, namely, 2,2′-azino- bis (3-ethylbenzothiazoline- 6- sulfonic acid) (ABTS), 2,2-diphenyl- 1- picrylhydrazyl (DPPH), and ferric reducing ability of

plasma (FRAP) assays were used in this work to study the systematic antioxidant activities of these oils. In general, the assays for the evaluation of antioxidant properties follow two prevalent mechanisms, the electron transfer (ET) and the hydrogen atom transfer (HAT) reaction mechanism. The former includes the DPPH assay, ABTS assay and FRAP assay.

India is not only the largest producer but also the largest consumer and exporter of spices in the World with approximately 5.74 million tonnes of production. India holds monopoly in export of spices to the entire World[12]. Numerous brands of culinary spices are available in the markets of India. This present study is directed towards the comparative evaluation of different brands of spices with respect to physico-chemical properties, total polyphenolics content and in vitro antioxidant activities.

MATERIALS AND METHODS

Samples, Chemicals and reagents:

Samples of 3 different brands of spices were procured from local market of Kolkata, West Bengal, India. All the chemicals and reagents are of AR grade, 2,2'-Azino-bis (3-ethylbenzo-thiazoline-6-sulfonic acid) diammonium salt (ABTS), 2,2-Diphenyl-1-picrylhydrazyl (DPPH) and 2,4,6-Tri(2-pyridyl)-s-triazine (TPTZ) and Capsaicin standards were obtained from Sigma-Aldrich Chemicals.

Physico-Chemical Analysis:

Moisture content, total ash, ash insoluble in dilute HCl, volatile oil content, non-volatile ether extract, curcuminoid content, crude fiber, added coloring matter and lead chromate were carried out as per method described in Indian Pharmacopoeia and AOAC International [13].

Total Polyphenolics as gallic acid [14]:

Total polyphenolics content was estimated by Folin Ciocalteu's method. 1 ml of aliquots of standard gallic acid (10, 20, 40, 60, 80, 100 µg/ml) was positioned into the test tubes and 5 ml of distilled water and 0.5 ml of Folin Ciocalteu's reagent was mixed and shaken. After 5 minutes, 1.5 ml of 20 % sodium carbonate was added and volume made up to 10 ml with distilled water. It was allowed to incubate for 2 hours at room temperature. Intense blue color was developed. After incubation, absorbance was measured at 750 nm spectrophotometer using UV/Visible spectrophotometer. The blank was performed using reagent blank with solvent. Gallic acid was used as standard. The calibration curve was plotted using standard gallic acid. The data for total polyphenolics as gallic acid contents of spices were expressed as g/100g.

Pungency test in terms of capsaicin[13].:

AOAC official method 995.03 was followed. Briefly samples were extracted with acetone and ethanol and subjected to HPLC analyses after filtration through syringe filter.

HPLC Method:

Shimadzu assembly (LC 2030), equipped with an UV detector, quaternary pump, injector, ZORBAX Eclipse-AAA [RP C18 150 x 4.6 mm; 5 µm] column was used, a mixture of acetonitrile: 1% aqueous acetic acid (40:60) was used as the mobile phase with a flow rate of 1.5 ml/min. the column temperature were kept at 25°C. 10 µl sample was injected and detection was done at 280 nm.

Antioxidant Assays:

2,2-Diphenyl-1-picrylhydrazyl radical (DPPH) scavenging capacity assay[15, 16, 17]

DPPH is one of a few stable and commercially available organic nitrogen radicals and has a UV-vis absorption maximum at λ 517 nm. Upon reduction, the solution color fades. The DPPH assay is typically run by the following procedure. DPPH solution (3 mg in 25 ml ethanol) was mixed with sample solution (0.1 mL). The absorbance of the mixture during the reaction progress was monitored at λ 517

nm for 20 min or until the absorbance was stable. Upon reduction, the color of the solution fades. The concentration that causes a decrease in the initial DPPH concentration by 50% is defined as IC_{50}.

ABTS cation radical decolorisation assay (15, 16, 17)

$ABTS^{-}$, the oxidant, was generated by persulfate oxidation of 2, 2'-azinobis 3-ethylbenzothiazoline-6-sulfonic acid ($ABTS^{2-}$). This solution was diluted with phosphate buffer (pH 7.4) until the absorbance reached 0.7 to 0.8 at λ 734 nm. One ml of the resulting solution was mixed with the sample. The absorbance was read at 30 °C, 4 min after mixing at 30 °C. The difference of the absorbance reading was plotted against the antioxidant concentrations to give a straight line. The concentration that caused a decrease in the initial ABTS concentration by 50% is defined as IC_{50}.

Ferric ion reducing antioxidant power (FRAP) assay (15, 16, 17)

The oxidant in the FRAP assay was prepared by mixing TPTZ, acetate buffer and $FeCl_3.H_2O$. The conglomerate is referred to as "FRAP reagent". The final solution had Fe(III) of 1.67 mM and TPTZ of 0.83 mM concentration, To measure FRAP value, 300 µl of freshly prepared FRAP reagent was warmed to 37 °C and a reagent blank reading was taken at λ 593 nm, then sample and 30 µl of water were added. Absorbance readings were taken λ 593 nm. The concentration that caused an increase in the initial FRAP concentration by 50% is defined as IC_{50}.

RESULTS

Physico-Chemical Analysis:

The samples were characterized for different physico-chemical quality parameters. The results are incorporated in Table 1-6. It is found that all the brands of different spices are acceptable as per physical appearances but somehow differ in chemical properties.

Table 1: Physico-chemical characterization of Turmeric powder of different brands

Parameters	Turmeric Powder		
	Brand 1	Brand 2	Brand 3
Odor and flavor	Characteristics	Characteristics	Characteristics
Mustiness and foreign odor	Absent	Absent	Absent
Visible mold	Absent	Absent	Absent
Living/dead insects	Absent	Absent	Absent
Insect fragments	Absent	Absent	Absent
Rodent contamination	Absent	Absent	Absent
Moisture (%)	4.98	7.54	5.82
Total ash on dry basis (%)	5.14	4.52	6.04
Ash insoluble in dil HCL on dry basis (%)	0.15	0.21	0.19
Curcuminoid content on dry basis (%)	3.12	3.98	3.52
Total starch (%)	57.21	54.96	57.11
Test for Lead Chromate	Negative	Negative	Negative

Table 2: Physico-chemical characterization of Coriander powder of different brands

Parameters	**Coriander Powder**		
	Brand 1	**Brand 2**	**Brand 3**
Odor and flavor	Characteristics	Characteristics	Characteristics
Mustiness and foreign odor	Absent	Absent	Absent
Visible mold	Absent	Absent	Absent
Living/dead insects	Absent	Absent	Absent
Insect fragments	Absent	Absent	Absent
Rodent contamination	Absent	Absent	Absent
Moisture (%)	4.31	4.37	5.26
Total ash on dry basis (%)	5.34	4.87	5.69
Ash insoluble in dilute HCL on dry basis (%)	0.17	0.19	0.24
Volatile oil content on dry basis (%)	0.30	0.26	0.21
Added Coloring Matter	Absent	Absent	Absent

Table 3: Physico-chemical characterization of Cumin powder of different brands

Parameters	Cumin Powder		
	Brand 1	**Brand 2**	**Brand 3**
Odor and flavor	Characteristics	Characteristics	Characteristics
Mustiness and foreign odor	Absent	Absent	Absent
Visible mold	Absent	Absent	Absent
Living/dead insects	Absent	Absent	Absent
Insect fragments	Absent	Absent	Absent
Rodent contamination	Absent	Absent	Absent
Moisture (%)	5.61	4.98	5.22
Total ash on dry basis (%)	3.98	4.65	4.21
Ash insoluble in dil HCL on dry basis (%)	0.21	0.19	0.20
Volatile oil content on dry basis (%)	1.25	1.39	1.11
Non-volatile ether extract on dry basis (%)	15.34	16.88	12.89
Added Coloring Matter	Absent	Absent	Absent

Table 4: Physico-chemical characterization of Mustard powder of different brands

Parameters	Mustard Powder		
	Brand 1	**Brand 2**	**Brand 3**
Odor and flavor	Characteristics	Characteristics	Characteristics
Mustiness and foreign odor	Absent	Absent	Absent
Visible mold	Absent	Absent	Absent
Living/dead insects	Absent	Absent	Absent
Insect fragments	Absent	Absent	Absent
Rodent contamination	Absent	Absent	Absent
Moisture (%)	4.21	5.63	4.98
Total ash on dry basis (%)	4.65	5.11	4.21
Ash insoluble in dil HCL on dry basis (%)	0.14	0.21	0.18
Volatile oil content on dry basis (%)	0.46	0.42	0.39
Non-volatile ether extract on dry basis (%)	33.51	31.78	28.69
Crude Fiber (%)	4.96	5.31	5.89
Test for Argemone oil	Absent	Absent	Absent

Table 5: Physico-chemical characterization of Cardamom powder of different brands

Parameters	Cardamom Whole		
	Brand 1	**Brand 2**	**Brand 3**
Odor and flavor	Characteristics	Characteristics	Characteristics
Mustiness and foreign odor	Absent	Absent	Absent
Visible mold	Absent	Absent	Absent
Living/dead insects	Absent	Absent	Absent
Insect fragments	Absent	Absent	Absent
Rodent contamination	Absent	Absent	Absent
Moisture (%)	6.98	7.15	5.69
Total ash on dry basis (%)	5.14	4.29	5.71
Volatile oil content on dry basis (%)	4.25	3.67	3.94
Extraneous matter (%)	Nil	0.19	0.07
Insect Damaged Matter (%)	Nil	Nil	Nil

Table 6: Physico-chemical characterization of Red Chilli powder of different brands

Parameters	Chilli Powder		
	Brand 1	**Brand 2**	**Brand 3**
Odor and flavor	Characteristics	Characteristics	Characteristics
Mustiness and foreign odor	Absent	Absent	Absent
Visible mold	Absent	Absent	Absent
Living/dead insects	Absent	Absent	Absent
Insect fragments	Absent	Absent	Absent
Rodent contamination	Absent	Absent	Absent
Moisture (%)	6.87	4.52	6.07
Total ash on dry basis (%)	3.18	4.22	3.89
Ash insoluble in dil HCL on dry basis (%)	0.27	0.21	0.18
Pungency in terms of Capsaicin (SHU)	4780.31	5634.57	5214.29
Non-volatile ether extract on dry basis (%)	15.72	18.21	17.05
Crude Fiber (%)	24.06	22.39	26.14
Added Coloring Matter	Absent	Absent	Absent

Total Polyphenolics as gallic acid

Total Polyphenolics as gallic acid was estimated in all the samples by Folin Ciocalteu's method. The results are incorporated in Figure 1. It is found that the polyphenolics content of the samples are in order of Cumin > Chilli > Turmeric > Mustard > Coriander > Cardamom.

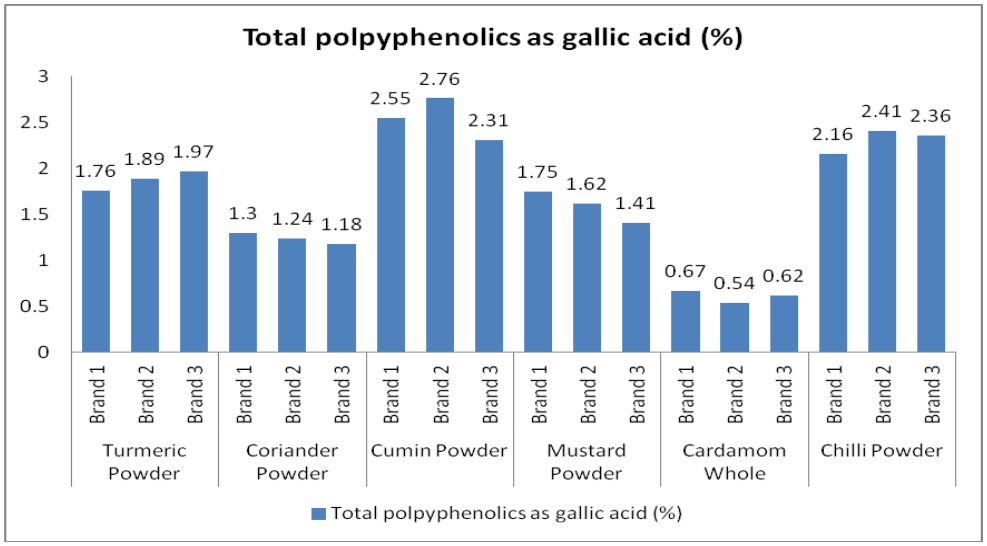

Figure 1: Content of total polyphenolics as gallic acid of Spices

Evaluation of the antioxidant capabilities of Spices

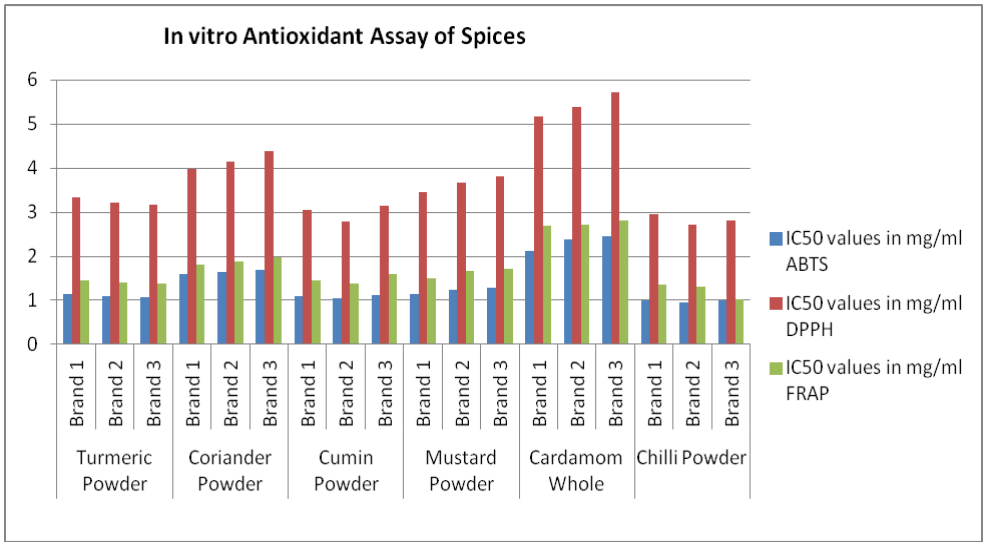

Figure 2: In vitro antioxidant assays of Spices

Table 7: In vitro antioxidant assays of Spices

		IC50 values in mg/ml		
		ABTS	**DPPH**	**FRAP**
Turmeric Powder	Brand 1	1.15	3.34	1.46
	Brand 2	1.09	3.21	1.41
	Brand 3	1.06	3.17	1.38
Coriander Powder	Brand 1	1.59	3.98	1.81
	Brand 2	1.64	4.15	1.89
	Brand 3	1.68	4.38	1.98
Cumin Powder	Brand 1	1.09	3.05	1.45
	Brand 2	1.04	2.78	1.38
	Brand 3	1.12	3.14	1.59
Mustard Powder	Brand 1	1.13	3.46	1.51
	Brand 2	1.24	3.68	1.67
	Brand 3	1.29	3.82	1.72
Cardamom Whole	Brand 1	2.12	5.16	2.69
	Brand 2	2.37	5.39	2.72
	Brand 3	2.46	5.71	2.81
Chilli Powder	Brand 1	1.01	2.96	1.35
	Brand 2	0.95	2.72	1.31
	Brand 3	0.99	2.82	1.028

The DPPH, ABTS, and FRAP methods were employed to evaluate the radical scavenging capabilities of the spices. As shown in Table 7 and Figure 2, their antioxidant capabilities were varied. This phenomenon might be attributed to the varied amounts of antioxidant active compounds in these spices. The results are depicted in IC50 values in mg/ml.

DISCUSSION

The present study was designed for a thorough evaluation of popular Indian spices sold under different brands. It was found that neither of the samples found to contain added coloring matter and was devoid of mustiness, visible mold and insect

fragments. Although, all the samples found to contain appreciable polyphenolics content, but the maximum polyphenols were observed in Cumin followed by chilli, turmeric, mustard, coriander and cardamom. Similar trends were also observed in all three in vitro antioxidant assays.

CONCLUSION

The present study is carried out for comparative evaluation of different marketed brands of six popular spices, with respect to physico-chemical analysis, total polyphenolics content (TPC) and in vitro antioxidant assays. All the samples were found to contain appreciable TPC and in vitro antioxidant activities. None of the products were found to contain any added color, mold and insect fragments, though they varied appreciably from one brand to another brand in physico chemical properties. This may be attributed to the fact that polyphenolics are largely responsible for imparting antioxidant properties of these spices. Spices not only enhance the flavor, aroma and color of food and beverages, but also protect people from acute and chronic diseases, due to their high antioxidant activity. This study presents abundant data on the antioxidant activities of spices and culinary herbs, as well as information related to their content of total polyphenols. All of this information will hopefully add to an already high level of interest toward spices and culinary herbs. Spices and herbs should certainly be incorporated as integral parts of healthy, nutritious eating, and as functional food ingredients

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