VOLUME-7, ISSUE-12, DECEMBER-2018 • PRINT ISSN No 2277 - 8160

Original Research Paper

Pharmacology

PHENOL QUANTIFICATION AND ANTIOXIDANT ACTIVITY OF SELECTED SPICES

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ABSTRACT Plants have been employed for medicinal purposes long before ancient era. Medicinal plants are considered as rich resources of secondary metabolites. They are also suggested for cookery and therapeutic values. Spices such as *Camellia sinensis*, *Cinnamomum zeylanicum*, *Syzygium aromaticum*, *Brassica nigra*, *Piper longum* and *Trigonella foenum- graecum* was selected for the present study. Total phenolic quantification was done by Folin reagent method and antioxidant activity by DPPH assay. Hot water extract of selected spices contain phenols and they also possess DPPH radical scavenging activity.

KEYWORDS : Camellia sinensis, Cinnamomum zeylanicum, Syzygium aromaticum, Brassica nigra, Piper longum, Trigonella foenum- graecum

INTRODUCTION

Plants, including herbs and spices, have many phytochemicals which are a potential source of natural antioxidant, e.g., phenolic diterpenes, flavonoids, alkaloids, tannins, and phenolic acids. Awareness of medicinal plants usage is a result of the many years of struggles against illnesses due to which man learned to pursue drugs in barks, seeds, fruit bodies, and other parts of the plants (1). Spices are natural plant products, which are used as flavoring agents in culinary and also in folk medicines. The high proportion of various antioxidants is the reason for health benefits imparted by the herbs and spices. These phytochemicals play an important role in the antioxidant activity, hormonal action, stimulation of enzymes, interference with DNA replication, antibacterial effect etc (2). Herbal medicines prepared from various plant parts like leaves, stem, roots, etc. contain secondary metabolites. They are used for treating a number of diseases.

Spices like *Camellia sinensis*, *Cinnamomum zeylanicum*, *Syzygium aromaticum*, *Brassica nigra*, *Piper longum*, *Trigonella foenum-graecum* are used in this study. The objectives of this study were to comparative evaluation of the phenolic content of spices commonly consumed in everyday life. The results of this study will provide useful information for human health and contribute to the potential commercial application of spices as economic natural antioxidants.

MATERIALS AND METHODS

Preparation of crude plant extract

Camellia sinensis (tender leaves), *Cinnamomum zeylanicum*(bark), *Syzygium aromaticum*(dried flower buds), *Brassica nigra* (seeds), *Piper longum*(catkin), *Trigonella foenum- graecum* (seeds) was purchased from the local market. About 10 g of dried, ground plant materials were soaked separately in water (100 ml) for one week, stirred and heated to boiling point and filtered. The extracts were dissolved in distilled water to make a concentration of 1 mg/ml.

Antioxidant activity by DPPH free radical scavenging activity

The antioxidant activity of the plant extracts was assessed on the basis of the radical scavenging effect of the stable 2,2-diphenyl-1-picrylhydrazyl (DPPH)-free radical activity by modified method (3). 0.002% of DPPH was prepared in methanol and 1 ml of this solution was mixed with 1 ml of plant extract. These solution mixtures were kept in dark for 20 min and optical density was measured at 517 nm using Labtronics NT 920 Spectrophotometer. Methanol (1 ml) with DPPH solution (0.002%, 1 ml) was used as control and methanol alone was used as a blank. The optical density was recorded and percentage inhibition was calculated using the formula = (A-B)/A*100, where, A = optical density of the control, B = optical density of the plant extract. The effective concentration of sample required to scavenge DPPH radical by 50% (IC50 value) was obtained by linear regression analysis of dose-response curve plotting between % inhibition and concentrations (4).

Estimation of total phenolic content

The concentration of phenolics in seed extract was determined using the spectrophotometric method (5). The reaction mixture was prepared by taking 5 µl, 2.5 µl, 1µl of hot water extract and made up the volume to 3ml of distilled water, added 0.5 ml of Folin-Ciocalteu's reagent and 2 ml 20% Na_2CO_3. Blank was concomitantly prepared, containing 3 ml water, 0.5ml Folin-Ciocalteu's reagent and 2ml of 20% of Na_2CO_3. The samples were thereafter incubated in a dark for 30min and the absorbance was determined using spectrophotometer at 650 nm. The samples were prepared in triplicate for each analysis and the mean value of absorbance was obtained. The same procedure was repeated for the standard solution of catechol and the calibration line was constructed. Based on the measured absorbance, the concentration of phenolics was read (µg/µl) from the calibration line; then the content of phenolics in extracts was expressed in terms of catechol equivalent (µg of CE/µg of extract).

RESULT AND DISCUSSION

DPPH assay is a standard method for measurement of free radical scavenging potential of an antioxidant molecule. 1 µg, 2.5 µg and 5 µg of extracts of various spices were taken for the study. When compared to the control, extracts showed the decrease in absorbance of DPPH free radical (Table 1). These results demonstrated that the hot water extracts of spices have shown effectiveness against the free radical scavenging activity. All the extracts showed DPPH activity in a dose-dependent manner.

TABLE-1 ANTIOXIDANT ACTIVITY OF HOT WATER EXTRACT OF SELECTED SPICES

Sl No	Plant Extract	Concentration (µg)	Optical density (OD)		% inhibition	IC50 (µg)
			Initial	Final		
1.	Control		0.340			
2.	Camellia *sinensis*	1	0.62	0.047	86	0.912
		2.5	0.030	0.024	91	
		5	0.047	0.020	94	
3.	*Cinnamomum zeylanicum*	1	0.063	0.135	61	1.91
		2.5	0.129	0.129	62	
		5	0.135	0.032	91	
4.	*Syzygium aromaticum*	1	0.051	0.051	85	0.928
		2.5	0.032	0.032	91	
		5	0.024	0.024	93	
5.	*Brassica nigra*	1	0.157	0.188	45	2.64
		2.5	0.172	0.133	60	
		5	0.340	0.101	70	

6.	*Piper longum*	1	0.169	0.306	10	3.59
		2.5	0.207	0.178	48	
		5	0.309	0.123	64	
7.	*Trigonella foenum-graecum*	1	0.257	0.253	26	8.02
		2.5	0.253	0.251	26	
		5	0.288	0.234	31	

Considering that the phenolic compounds are potent antioxidants in spices, the total phenolic content of the selected spices was checked. The amount of total phenolic content measured spectrophotometrically by the Folin–Ciocalteu method. It was expressed as microgram (µg) catechin equivalents (CE) / µg of extract. Quantification of phenolic content of spices was found to be in the order *Trigonella foenum- graecum*>*Piper longum* (6)>*Camellia sinensis*>*Syzygium aromaticum*>*Cinnamomum zeylanicum*>*Brassica nigra* (7) (Table 2). Phenolic compounds exhibit a wide range of activities, such as anti-allergenic, anti-atherogenic, anti-inflammatory, anti-microbial, antioxidant, anti-thrombotic, cardioprotective and vasodilatory effects. Phenolic compounds have been associated with the health benefits derived from consuming high levels of fruits and vegetables (8). Many studies have shown a good positive linear correlation between antioxidant capacity and total phenolic content of spices, medicinal herbs, and other dietary plants. Moreover, these results have also suggested that phenolic compounds are responsible for their antioxidant capacity (9).

TABLE - 2 QUANTIFICATION OF TOTAL PHENOLS OF HOT WATER EXTRACT OF SELECTED SPICES

Sl. No	Plant extract (5 µg)	OD	Phenol Quantification	IC50	Phenol Quantification (µg)
1.	*Camellia sinensis*	0.845	168	0.912	153.22
2.	*Cinnamomum zeylanicum*	0.567	112	1.91	42.74
3.	*Syzygium aromaticum*	0.309	62	0.928	57.54
4.	*Brassica nigra*	0.218	42	2.64	22.17
5.	*Piper longum*	0.157	150	3.59	538.5
6.	*Trigonella foenum-graecum*	0.442	84	8.02	134.73

CONCLUSIONS

The main focus of current research relies on natural products obtained from plants. They can be easily available and also used in traditional medicines. This interest primarily arises from the belief that green medicine is safe and dependable. In this study showed that hot water extracts of *Camellia sinensis*, *Cinnamomum zeylanicum*, *Syzygium aromaticum*, *Brassica nigra*, *Piper longum*, *Trigonella foenum- graecum* contain phenols and also possess antioxidant activity. The identification of the active components not only reveals its biological activity but also confirms its use time immemorial.

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