RESEARCH ARTICLE

A Comparative study of the antioxidative properties of the different seed spices available in India

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ABSTRACT

The study demonstrates phenolic content and the total antioxidant capacity of common seed spice extracts and establishes the relationship between antioxidant activity and phenolic compounds. Due to its profuse availability in our state, it has turned into a topic of research interest to work with. Many researchers, worldwide claimed that the phenolic compounds in spices were responsible for their antioxidant activity and we established the same through this study. The *in vitro* antioxidant activity confirms the beneficial aspects of dietary seed spices against oxidative stress related disorders within the human body. Four common seed spices of India, fenugreek, cumin, black cumin and coriander, which are consumed along with different meals more or less regularly in the diet of the people of India, were tested for their antioxidative property. 1,1-diphenyl-2-picryl hydrazyl radical (DPPH), hydroxyl radical ($OH^{\cdot}$), 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) radical (ABTS) scavenging activity as well as reducing activity and total polyphenol content were measured to analyze for its antioxidant efficacy. Preliminary phytochemical analysis exhibited the presence of phenolic compounds, saponins, flavonoids, tannins and phytosterols as major phytochemical groups. The extract exhibited significant ($p<0.05$) and concentration dependent antioxidant and free radical scavenging activities of DPPH, $OH^{\cdot}$, ABTS as well as reducing activities when compared to the standard compound. The extract was also reported to possess a high amount of total phenolic content. These findings justify the biological and traditional use of these four seed spices as confirmed by its promising antioxidant efficacy. It was also observed that the anti-oxidative property of the particular spice depends on its polyphenol content and not on the aromatic oil content.

Keywords: Spices, Polyphenols, ROS, Oxidative stress, Antioxidants.

Introduction

Spices or herbs are classified as the exotic crops which may have pungency or flavour of aromatic chemicals [1]. Commonly, spices or herbs could be put into food as full herbs, ground spices or herbs, as well as in concentrated amounts for increasing the flavour. Herbs and spices have been extensively used as food additives not only to add flavours but also play the role of natural antioxidants. They are also considered to be essential in diet and medical therapies for delaying aging and biological tissue deterioration [2]. The search for naturally occurring antioxidants as alternatives to synthetic antioxidants is of great interest both in industry as well as in scientific research. The seed spices can be good choice for that. The antioxidant property of the plant material is due to the presence of many active phytochemicals including vitamins, flavonoids, terpenoids, carotenoids, cumarins, lignin, saponins, plant sterol and etc [3-5]. Numerous spices or herbs happen to be proven to possess medicinal attributes and have quite a few beneficial therapeutic effects along wellness, such as antioxidant action, digestive stimulants, [6] anti-inflammatory, [7,8] germicide, [9] hypolipidemic, [10] antimutagenic, [11] anticarcinogenic prospective, [12] and so on. It has also been shown that the increased consumption of fruits and vegetables has generally been found protective against a variety of diseases [13-15]. Like most herbs, the composition of spices varies with the geographic distribution, time of harvest and agronomic practices [16].

The aromatic seeds of angiospermic plants are cumin and coriander which belongs to *Apiaceae* family [17]. The plants are cultivated in different areas of India and different varieties of them release the aromatic volatile oils. Fenugreek (*Trigonella foenum-graecum* L.) is an annual herb from Papilionaceae Leguminosae family, and is extensively cultivated in Southern

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Europe, Northern Africa, Central Asia, North America, and some parts of Australia [18]. It is generally cultivated as a forage crop because it contains high amounts of protein, vitamins, essential amino acids, and offers good digestibility to cattle [19]. It has been reported that fenugreek seed when supplemented to a dairy cattle diet significantly increased the amount of polyunsaturated fatty acids in the milk, and decreased the level of milk cholesterol [20]. Black cumin plant is botanically known as *Nigella sativa* L, member of Ranunculaceae family, and it produces an economically important seed spice with various beneficial effects. Sprinkles of black cumin seeds are frequently used in Indian cousin and many others. The seeds are also used as antioxidant and in diabetic complications and cancer treatment. These four seed spices were chosen for the study for their significant importance in providing flavour and phyto-chemicals in food items. The current study was designed to characterize indigenous variety of different spice seeds and thus nutritional profile of essential oils which could possibly be used for their potential applications against lifestyle related disorders.

Materials and methods

Collection of seed samples

Authentic seed samples were directly collected from the field of harvesting from Malda district, West Bengal, India. Seeds were collected, packed in a zip lock packet, brought to laboratory within overnight and stored at -20°C for further analysis.

Preparation of seed extract

The seed samples (10 g) were extracted by stirring with 100 ml of methanol at 25°C for 24 h and filtering through Whatman No. 1 filter paper. The residues were re-extracted with an additional 50 ml of methanol, as described above, for 3 h. The solvent of the combined extract was evaporated under reduced pressure, using a rotary vacuum evaporator at 40°C and the remaining water was removed by lyophilization. The freeze dried extract thus obtained was used directly for measurements of total phenolics, and also for the assessment of antioxidant capacity through various chemical assays.

Determination of DPPH$^{\cdot}$ radical scavenging activity

DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity of seed extract was measured according to the methods described by Braca et al. (2002) with slight modification [21]. The percent of inhibition (PI) was calculated as follows:

$$\%\ \text{of DPPH}^{\bullet}\ \text{radical scavenging activity} = \left(\frac{A0 - A1}{A0}\right) \times 100$$

Where A0 is the absorbance of control and A1 is the absorbance of sample.

Determination of OH$^{\cdot}$ radical scavenging activity

The hydroxyl radical scavenging activity was determined by the method of Halliwell et al. (1992) [22]. The percentage scavenging potential was calculated using the following formula,

$$\%\ \text{of OH}^{\bullet}\ \text{radical scavenging activity} = \left(\frac{A0 - A1}{A0}\right) \times 100$$

Where A0 is the absorbance of the control and A1 is the absorbance of the sample.

Determination of ABTS$^{\bullet+}$ radical scavenging activity

ABTS$^{\bullet+}$ radical cation scavenging activity was evaluated following the method described by Lo˘zien˙et al. (2007) [23]. The percentage scavenging activity of seed extract was calculated using the following formula.

$$\%\ of\ ABTS^{\bullet}\ \text{radical scavenging activity} = \left(\frac{A0 - A1}{A0}\right) \times 100$$

Where A0 is the absorbance of blank and A1 is the absorbance of sample.

Determination of reducing power

The reducing power of different solvent extracts was determined according to the method of Oyaizu (1986) [24]. A higher absorbance of the reaction mixture indicated greater reducing power.

Determination of total polyphenol content

Total phenolic content was estimated by the method of Singleton and Rossi (1965) [25] using Folin Ciocalteau's phenol reagent (FC reagent). A calibration curve was prepared, using a standard solution of gallic

acid (20, 40, 60, 80 and 100 mg/L). Results were expressed as mg gallic acid equivalents (GAE)/100 g of extract.

Statistical analysis

The data were subjected to a one way analysis of variance and the significance of the deference between means was determined by Duncan's multiple range test ($P < 0.05$) using Origin Pro 8.0. Values expressed are mean of triplicate determination ± SEM.

Results and discussion

The biochemical reactions occurring within the living systems are one of the prime driving forces to lead human life normally. If this equilibrium is disturbed in any case, then it may give rise to different pathological conditions. Antioxidants play a major part in maintaining the normal cellular functions and normalcy of human life [26]. Antioxidants are synthesized within the body endogenously. It can also be obtained as a part of a diet or as dietary supplements exogenously [27]. Few dietary compounds that do not neutralize free radicals, but elevate endogenous activity may also be classified as antioxidants [28]. An ideal antioxidant should be readily absorbed and quench free radicals, and chelate redox metals at physiologically relevant levels [29]. Cumin, coriander, fenugreek and black cumin and are some of the seed spices which are widely consumed by human. Spice related research work has been carried out in the different parts of the world widely through the ages. But very little of it has been performed in India, most specifically in our own province West Bengal.

Analysis of total phenolic content

The phenolic compounds may contribute directly to the antioxidant action; therefore, it is necessary to investigate total phenolic content [30]. Generally, the mechanisms of phenolic compounds for antioxidant activity are inactivating lipid free radicals and preventing decomposition of hydroperoxide into free radicals[31]. The result revealed that among the four seed spices of the different districts, Black Cumin of Malda district had higher polyphenol content compared to Cumin of Hooghly, Fenugreek of Nadia and lastly Coriander of Malda district **(Table 1).** The data also demonstrated that black cumin seed extract had higher polyphenol content compared to other variety of seed extracts.

Table 1: Total polyphenol content (TPC) of the four different seed extracts expressed as gallic acid equivalent

Spice	TPC Gallic Acid eq. (mg/gm of seed extract)
Fenugreek (Nadia)	0.4
Cumin (Hooghly)	0.5
Black cumin (Malda)	2.1
Coriander (Malda)	0.31

Analysis of DPPH radical scavenging activity

The stable DPPH radical has been used to evaluate antioxidants for their radical quenching capacity and to better understand their antioxidant mechanism(s) each seed extract was evaluated for radical scavenging activity against DPPH free radical [32]. Three different concentrations (100, 200 and 500 µg/ml) of methanolic seed extracts of the four spices were used for the DPPH radical scavenging activity, as shown in **Figure 1**. The results have showed that, all the four spices had high scavenging potential at 500 µg/ml concentration. Cumin showed the highest significant scavenging activity ($p<0.05$) compared to Fenugreek, Coriander and Black Cumin. The result also demonstrated that the scavenging activity of all four different seeds was increased with the increase in concentration.

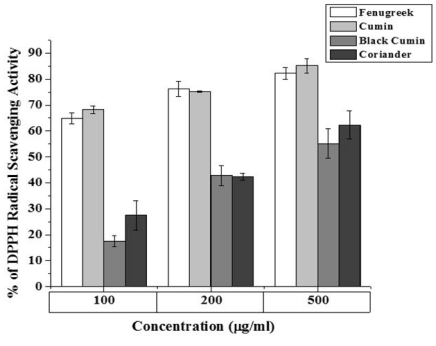

Fig. 1: DPPH free radical scavenging activity of Fenugreek, Cumin, Black Cumin and Coriander seed extract. Values are represents as mean ± SEM of five

parallel measurements. p <0.05 are considered as significant.

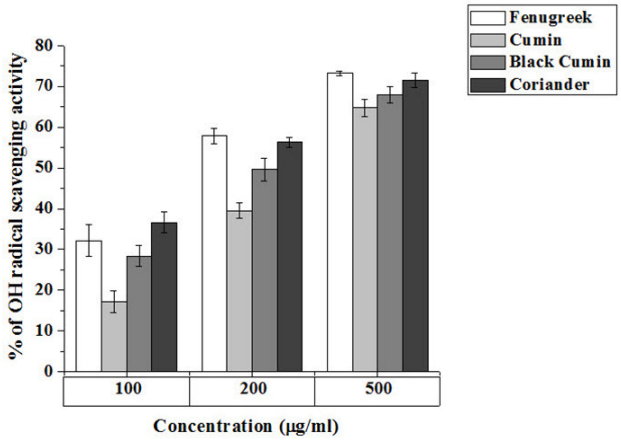

Fig. 2: Hydroxyl radical scavenging activity of the three (100, 200, and 500 µg/ml) different concentrations of the methanolic extracts of Fenugreek, Cumin, Black Cumin and Coriander seed extract. Results are expressed as mean ± SEM of five parallel measurements. P <0.05 was considered as significant.

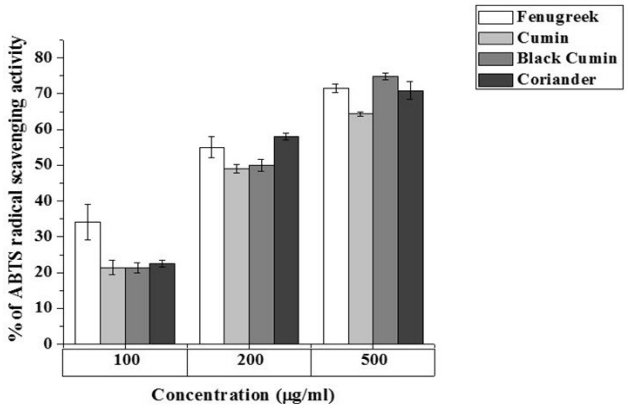

Fig. 3: ABTS free radical scavenging activity of Fenugreek, Cumin, Black Cumin and Coriander seed extract. Results are expressed as mean ± SEM of five parallel measurements. P <0.05 was considered as significant.

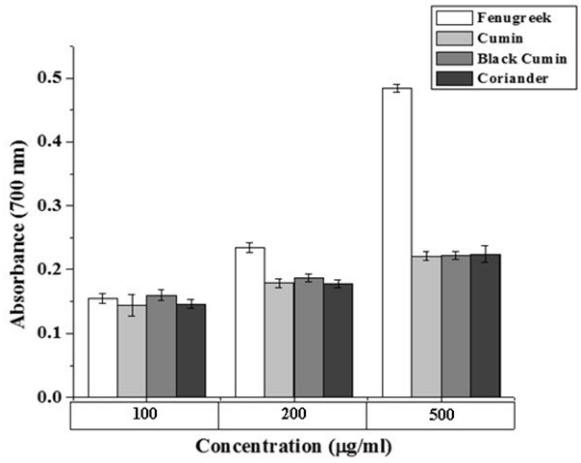

Fig. 4: Reducing activity of Fenugreek, Cumin, Black Cumin and Coriander seed extracts. Results are expressed as mean ± SEM of five parallel measurements. P <0.05 was considered as significant.

Analysis of OH· Scavenging activity

Among the oxygen radicals, hydroxyl radical is the most active and induces severe damage to adjacent biomolecules [33]. As shown in **Figure 2**, all four studied samples exhibited potent or moderate scavenging activity in concentration dependent manner. Among the four seed extract, fenugreek seed extract showed higher OH· scavenging potential compared to other seed extract.

Analysis of ABTS radical scavenging activity

ABTS radical scavenging activities [34] of four seed extracts are presented in **Figure 3.** The results indicated that methanolic seed extract had potent ABTS radical scavenging activity at three concentrations (100, 200 and 500 µg/ml). The result showed that all the four spices had significant higher scavenging activity ($p<0.005$) at 500 µg/ml concentration compared to lower concentration. At 100 µg/ml concentration fenugreek seed extract showed higher scavenging activity than other three seed extract but with the increase in concentration; the activity did not increase significantly. On the other hand black Cumin seed extract showed the highest significant scavenging activity ($p<0.05$) among all the said concentration, in comparison with Fenugreek, Coriander and Cumin seed extract respectively.

Analysis of reducing power

Earlier reports demonstrated that the reducing power of bioactive compounds is associated with antioxidant activity. The reducing power of the extracts increased with an increase in the amount of the extract as shown in **Figure 4**. Among four seed extract, only fenugreek seed extract showed significant ($p<0.05$) reducing activity with increasing in concentration. The relationship between the amount of total phenolic content and reducing power is established by the study.

In this study, four different spices Fenugreek, Black Cumin, Cumin and Coriander of the different districts of West Bengal was a matter of interest. These seed

spices are consumed along with different meals more or less regularly in the diet of the people of West Bengal. Due to its profuse availability in the state, it has turned into a topic of research interest to work with. Many researchers, worldwide claimed that the phenolic compounds in spices were responsible for their antioxidant activity, but few could establish real correlative relationships and provide convincing statistical data to reveal the relationship between the activity and phenolics on the basis of large numbers of spice samples. No significant research has been done with dietary seed spices in such a localized pattern till date in India. Hence the work has ample significance.

Conclusion

The results of the study showed that, though oil seeds are used in very small quantity in food, but they are potential sources of antioxidants. Thus, spices might be regarded as a promising strategy for the protection of cells and different organ systems against oxidative stress. The novel scientific merit of this study is the validation of the age old practice of the use of herbs and spices for several health benefits and for therapeutic measures across the continents.

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