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Evaluation of *in-vitro* antidiabetic and antioxidant activity of selected Indian spices

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ABSTRACT

Spices act as a flavouring agent in food. They perform a variety of functions, like to provide aroma, texture and colour to food and sometimes as preservatives and they are being used throughout the world for thousands of years, especially in India, China and many other Southern countries. Many spices contain high levels of phenolics and have demonstrated the high antioxidant capacity and potent antidiabetic activity. The study was aimed to investigate the antidiabetic and antioxidant activities of cinnamon, cumin and turmeric. Antidiabetic activity was determined by percentage inhibition of glucose transport across yeast cells. Antioxidant activities were assessed in terms of total phenolics content, total antioxidant capacity and free radical scavenging activity. The findings of *in vitro* studies showed significant antidiabetic and antioxidant activity of each spice as compared to synthetic antioxidant. Turmeric was found to be the more potent antioxidant and antidiabetic agent as compared to cinnamon and cumin.



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INTRODUCTION

Diabetes mellitus is a group of disorders that alter carbohydrate, fat and protein metabolism (Manikandan R *et al.*, 2013). The number of diabetes mellitus cases has been rising worldwide in current years. In 2000, the World Health Organization (WHO) estimated a total of 171 million of people with diabetes mellitus from the global population and this report is projected to increase to 366 million by 2030 (Nadeem M *et al.*, 2012). Extended severe complications of diabetes often result in a high mortality rate, so the treatment of diabetes

depends on some resources including medicines, diets, physical training and so on worldwide. Therefore, there is a need to search a new class of phytochemicals to overcome diabetic complications.

Spices are derived from plant parts like bud, fruit, seed, bark, rhizome, etc. used for flavour, as well as herbal medicine. The term 'spice' originated from the Latin word 'species' meaning of a specific kind (Nadeem M *et al.*, 2012). Many spices have been identified to comprise medicinal properties and acquire many beneficial health effects such as antidiabetic, antioxidant, anti-inflammatory, antimicrobial, renohepato protective, hypolipidemic, antimutagenic, anticarcinogenic potentials, etc. (Wild S *et al.*, 2004, Srinivasan K 2005, Pizzale L *et al.* 2002, Lampe JW 2003, ^aPhatak RS *et al.*, 2017, ^bPhatak RS *et al.*, 2017).

Generally, spices consist of different phytochemicals and active principles such as flavonoids, essential oils, volatile oils, phenolics and polyphenolics (Pizzale L *et al.* 2002). Recently several studies have reported that synthetic antioxidants have shown their carcinogenic and toxic properties if

they are used as a food additive (Phatak RS *et al.*, 2015). Natural antioxidants are assumed to be safe for the consumption. Nowadays medical practitioners prefer to use naturally available antioxidants rather than synthetic antioxidants as additives in the food (Bin Shan *et al.*, 2005). Presently, there is a growing interest to use herbal medicines due to the side effects associated with oral hypoglycemic agents used for the treatment of diabetes mellitus (Barlow SM, 1990). Therefore there is a need to use traditional herbal medicines in the treatment of diabetes mellitus. During recent years, herbal medicines have started to put on importance as a source of hypoglycemic agents. Herbal drugs are prescribed due to their good effectiveness, fewer side effects in clinical experience and relatively they are low cost (Bhalodi M *et al.*, 2008).

Thus, the study was designed to ascertain the *in vitro* antidiabetic activity of ethanolic extracts of cinnamon, cumin and turmeric by studying their effects on the inhibition of glucose transport across yeast cells and also to study free radical scavenging activity and antioxidant capacity by using different *in vitro* models.

MATERIAL AND METHODS

All chemicals were of analytical grade, obtained from Loba Chemie, Pvt. Ltd. India. All spices were procured from local market of Karad (Western Maharashtra) India.

Spice authentication and extraction

Three types of spices were used in the present study viz. cinnamon, cumin and turmeric. They were identified and authenticated from Yashwantrao Chavan College of Science, Karad. The scientific name, common name and sources of the spices used in the present study are depicted in table 1.

Table 1: Sources of the spices used

Scientific name	Family	Common name	Part used
<i>Cinnamomum zeylanicum</i>	Lauraceae	Dalchini	Bark
<i>Cuminum cyminum</i>	Apiaceae	Jeera	Seeds
<i>Curcuma longa</i>	Zingiberaceae	Halad, haldi	Tuber

Spice extraction

Each spice was thoroughly cleaned, dried, finely powdered using electric mixer grinder, placed in airtight containers and was used for *in vitro* experiments. The dried powder was weighed and extracted with 95% ethanol. It was kept in maceration for 15 days at 4° Celsius to maximise the ex-

traction. After 15 days each spice extract was filtered through Whatmann filter paper and transferred to a suitable container and kept for analysis.

Antioxidant assay

Different concentrations of extracts for each spice were prepared. Total phenolics content, total antioxidant capacity, and free radical scavenging activities of each spice extract were estimated in the Folin-Ciocalteu Reagent (FCR) assay, Phosphomolybdenum (PMA) assay, hydroxyl free radical scavenging assay and superoxide free radical scavenging assay respectively.

Folin-Ciocalteu Reagent (FCR) assay

FCR assay was performed by the method of Andressa *et al.* (Andressa B *et al.*, 2013). Ethanolic extract of each spice in different concentrations ranging from 100 µl to 500 µl was added to each test tube. Distilled water was added to make up the volume of 1 ml. About 500 µl of Folin-Ciocalteu reagent solution was added to each tube. About 500 µl of 100 mg/ml sodium carbonate was added after 5 minutes. These tubes were kept at room temperature for 2 hours. Gallic acid was used as a standard. Absorbance was measured at 765 nm. All assays were conducted in triplicate and mean was calculated.

Phosphomolybdenum (PM) assay

PM assay estimated total antioxidant capacity by the method of Prieto *et al.* (Prieto P *et al.*, 1999).

Preparation of molybdate reagent solution:

One ml each of 0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate was added in 20 ml of distilled water and volume was made to 50 ml by adding distilled water.

Different concentrations of ethanolic extracts of spices ranging from 100 µl to 500 µl were added to each test tube containing 3 ml of distilled water and 3 ml of molybdate reagent solution. The tubes were kept incubated at 95° Celsius for 90 minutes. After incubation, all tubes were kept at room temperature for 30 minutes, and the absorbance of the reaction mixture was measured at 695 nm. Mean values from three independent samples were calculated for each extract. Ascorbic acid was used as a standard.

Hydroxyl radical (OH·) scavenging activity

The scavenging ability of spice extract on hydroxyl radicals was determined by the method of Smirnoff and Cumbes (Smirnoff N *et al.*, 1989).

Preparation of Smirnoff reagent:

About 0.041 gm of ferrous sulphate and 0.32 gm of sodium salicylate were mixed in 100 ml of distilled

water. About 4 μ l of hydrogen peroxide was added to it, vortexed for uniform mixing and labelled as Smirnoff reagent. Ethanolic extract of each spice in different concentrations ranging from 100 μ l to 500 μ l was added to each test tube containing 4 ml of distilled water and 1 ml of Smirnoff reagent. All tubes were incubated at 37°Celsius for about 60 minutes. The absorbance of the reaction mixture was read at 562 nm. Ascorbic acid was used as a standard. The percentage scavenging ability on hydroxyl radical of each spice was calculated by using following formula: Scavenged OH⁻ (%) = [(Ac-Ae)/Ac X 100] Where, Ac= absorbance of control and Ae= absorbance of extract.

Superoxide anion radical scavenging assay

Superoxide anion radical scavenging activity of each spice was determined by Nishikimi *et al.* (Nishikimi M *et al.*, 1972). Ethanolic extract of each spice in different concentrations ranging from 100 μ l to 500 μ l was added to each test tube containing 3 ml of Tris-HCl buffer, 0.5 ml of Nitroblue Tetrazolium (NBT) chloride, 0.5 ml of Reduced Nicotinamide Adenine Dinucleotide (NADH) and 0.5 ml of Tris-HCl buffer. The reaction was started by adding 0.5 ml Phenazinemethosulphate (PMS) solution to the mixture, incubated at 25° Celsius for 5 minutes and then the absorbance was measured at 560 nm against a blank sample. Ascorbic acid used as a standard. The percentage inhibition of samples was calculated as

Scavenged superoxide (%) = [(Ac-Ae)/Ac X 100]
Where, Ac= absorbance of control and Ae= absorbance of extract.

Antidiabetic assay by glucose uptake

The assay was performed according to the method of Nair *et al.* (Nair SS *et al.*, 2013) and Cirillo (Cirillo VP 1963). The commercial baker's yeast was procured from the local market. Yeast was subjected to repeat centrifugation (3000 rpm for 5 minutes) until clear supernatant fluids were obtained and about 10% (v/v) suspension was prepared in distilled water. Various concentrations of ethanolic spice extracts ranging from 100 μ l to 500 μ l were added to 1 ml of glucose solution and incubated for 10 minutes at 37°Celsius. The reaction was started by adding 100 μ l of yeast suspension followed by vortexing and further incubation at 37 °Celsius for 60 minutes. After 60 minutes, the tubes were centrifuged (2500 rpm for 5 minutes) and the supernatant was estimated for the amount of glucose. Metformin was used as a standard. The percentage increase in glucose uptake by yeast cells was calculated using the following formula:

$$\text{Increasing in glucose uptake (\%)} = \frac{\text{Abs}_{562\text{nm}} - \text{Abs}_{\text{control}}}{\text{Abs}_{562\text{nm}}} \times 100$$

Where Abs_{control} is the absorbance of the control reaction (containing all reagents except the test sample), and Abs_{sample} is the absorbance of the test sample. All the experiments were carried out in triplicate.

Statistical analysis

A total of five sets of samples from each variable were analysed for antidiabetic and antioxidant parameters. Each sample was further conducted in triplicate, and all values were the mean of three measurements and expressed as mean \pm standard deviation. The results were analysed using one-way ordinary analysis of variance (Ordinary ANOVA).

RESULTS

Ethanolic extracts of cinnamon, cumin and turmeric were analysed by the UV-spectrophotometric method for the quantitative determination of antidiabetic activity and antioxidant capacity.

FCR assay

FCR assay performed the total phenolics content assay. It indicated that total phenolics content was directly proportional to the concentration of all extracts. However, standard gallic acid showed the highest significance except sample number 5 (sample of 500 μ L concentration, in which total phenolics content was higher in turmeric than standard gallic acid) (p<0.0001) for total phenolics content compared to turmeric, cumin and cinnamon. The results of total phenolics content of turmeric, cumin and cinnamon extracts are represented in table 2.

The absorbance of all spice extracts reflects the total phenolics content directly. The order of total phenolics content by FCR assay from different extracts was found as follows: turmeric > cinnamon > cumin.

PM assay

Total Antioxidant Activity (TAC) was used to measure in PM assay and found extremely significant (p<0.0001) in all extracts as well as ascorbic acid. Increase in total antioxidant capacity was directly proportional to the concentration of all extracts and ascorbic acid. Further, TAC in standard ascorbic acid was found to be highly significant (p<0.0001) when compared to turmeric, cumin and cinnamon in all concentrations except sample (300 μ L concentration) in which, TAC was higher in turmeric than ascorbic acid. The order of total antioxidant activity measured by PM assay was found as follows: ascorbic acid > turmeric > cumin

Table 2: Total phenolics content of cinnamon, cumin and turmeric extracts

The concentration of extract (µL)	Cinnamon		Cumin		Turmeric		Gallic Acid	F Value	P Value
	n	Mean ± SD	n	Mean ± SD	n	Mean ± SD	Mean ± SD		
100	5	0.057±0.0008	5	0.014±0.0005	5	0.084±0.0015	0.163 ± 0.01	5746.7	P<0.0001
200	5	0.078±0.0031	5	0.018±0.0004	5	0.113±0.0011	0.314 ± 0.01	3012.3	P<0.0001
300	5	0.152±0.0040	5	0.027±0.0005	5	0.306±0.0104	0.433 ± 0.04	2321.2	P<0.0001
400	5	0.300±0.0120	5	0.039±0.0007	5	0.444±0.0033	0.610 ± 0.03	4051.8	P<0.0001
500	5	0.592±0.0153	5	0.070±0.0005	5	0.834±0.0046	0.792 ± 0.03	8931.5	P<0.0001
F Value	2999.3		7966.1		16119		121475	--	--
P Value	P<0.0001		P<0.0001		P<0.0001		P<0.0001		

Values were expressed as mean ± SD

Table 3: the total antioxidant capacity of cinnamon, cumin and turmeric extracts by PM assay

Conc. of extract (µL)	Cinnamon		Cumin		Turmeric		Ascorbic Acid		F Value	P Value
	n	Mean±SD	n	Mean±SD	n	Mean±SD	n	Mean±SD		
100	5	0.029±0.0007	5	0.111±0.0007	5	0.160±0.0008	5	0.146±0.0015	15933	P<0.0001
200	5	0.095±0.0015	5	0.391±0.0004	5	0.261±0.0006	5	0.336±0.0012	70642	P<0.0001
300	5	0.108±0.0005	5	0.528±0.0008	5	0.640±0.0003	5	0.532±0.0026	132064	P<0.0001
400	5	0.117±0.0006	5	0.680±0.0011	5	0.780±0.0008	5	0.866±0.0022	297368	P<0.0001
500	5	0.251±0.0010	5	0.749±0.0009	5	0.807±0.0010	5	0.984±0.0007	522412	P<0.0001
F Value	34127		451832		704838		180217		--	--
P Value	P<0.0001		P<0.0001		P<0.0001		P<0.0001			

Values were expressed as mean ± SD

> cinnamon. The results of phosphomolybdenum assay of all variables are represented in table 3.

The absorbance of ascorbic acid standard and all the spice extracts reflects directly the reducing power which is nothing but the total antioxidant capacity.

Hydroxyl Radical Scavenging Activity (HRSA):

It was found extremely significance ($p<0.0001$) in all variable extracts with respect to their concentrations. In hydroxyl radical scavenging activity, ascorbic acid showed extremely significance ($p<0.0001$) compared with cinnamon cumin and turmeric. The results of hydroxyl radical scavenging activity of all variable extracts are represented in table 4.

The order of hydroxyl radical scavenging activity (% inhibition) from different variables was found as follows: ascorbic acid > cinnamon > cumin > turmeric.

Superoxide radical scavenging activity (SRSA):

Though superoxide anion is a weak oxidant, it gives rise to the formation of toxic hydroxyl radicals as well as singlet oxygen, both of which involve in the oxidative stress. In the PMS/NADH-NBT system, the superoxide anion derived from dissolved oxygen from PMS/NADH coupling reaction reduces NBT. The decrease in the absorbance indicates the consumption of superoxide anion in the reaction mixture (Nishikimi M *et al.*, 1972).

The extremely significant difference ($p<0.0001$) was found among all variables. In superoxide radical scavenging activity, ascorbic acid revealed extremely significance ($p<0.0001$) compared to turmeric, cinnamon and cumin. The results of superoxide radical scavenging activity of different variable extracts are represented in table 5.

Table 4: Hydroxyl radical scavenging activity of all variable extracts (% inhibition \pm SD)

The concentration of extract (μ L)	Cinnamon		Cumin		Turmeric		Ascorbic Acid	F Value	P Value
	n	Mean \pm SD	n	Mean \pm SD	n	Mean \pm SD	Mean \pm SD		
100	5	77.75 \pm 0.018	5	72.11 \pm 0.051	5	62.18 \pm 0.010	75.43 \pm 0.079	310742	P<0.0001
200	5	78.03 \pm 0.088	5	72.47 \pm 0.030	5	63.45 \pm 0.136	78.14 \pm 0.110	29759	P<0.0001
300	5	78.99 \pm 0.144	5	73.70 \pm 0.083	5	65.30 \pm 0.086	82.67 \pm 0.091	20445	P<0.0001
400	5	79.55 \pm 0.134	5	75.06 \pm 0.012	5	68.19 \pm 0.077	85.38 \pm 0.148	20489	P<0.0001
500	5	80.08 \pm 0.196	5	75.76 \pm 0.153	5	69.15 \pm 0.074	86.47 \pm 0.130	6769.7	P<0.0001
F Value	286.76		1866.2		5959.4		320.1	--	--
P Value	P<0.0001		P<0.0001		P<0.0001		P<0.0001		

Values were expressed as mean \pm SD

Table 5: Superoxide radical scavenging activity of cinnamon, cumin and turmeric extracts (% inhibition \pm SD)

The concentration of extract (μ L)	Cinnamon		Cumin		Turmeric		Ascorbic Acid	F Value	P Value
	n	Mean \pm SD	n	Mean \pm SD	n	Mean \pm SD	Mean \pm SD		
100	5	37.09 \pm 0.0123	5	70.55 \pm 0.0867	5	75.08 \pm 0.0084	76.69 \pm 0.070	834502	P<0.0001
200	5	39.17 \pm 0.0084	5	71.20 \pm 0.0396	5	76.47 \pm 0.0438	78.68 \pm 0.111	1717222	P<0.0001
300	5	45.42 \pm 0.0259	5	73.06 \pm 0.0179	5	77.16 \pm 0.0515	79.80 \pm 0.081	1227936	P<0.0001
400	5	47.21 \pm 0.0054	5	73.43 \pm 0.0653	5	78.27 \pm 0.2446	81.25 \pm 0.125	65319	P<0.0001
500	5	51.60 \pm 0.1152	5	75.00 \pm 0.0885	5	79.07 \pm 0.0929	84.40 \pm 0.044	110923	P<0.0001
F Value	62104		3727.7		827.81		890.42		
P Value	P<0.0001		P<0.0001		P<0.0001		P<0.0001		

Values were expressed as mean \pm SD

Superoxide radical scavenging activity (% inhibition) was found in following order: Ascorbic acid > turmeric > cumin > cinnamon.

Antidiabetic activity

Antidiabetic activity was measured by the percentage increase in glucose uptake by yeast cells. A significant increase ($p<0.0001$) showed in percentage glucose uptake by yeast cells in cinnamon and turmeric. However, no significance ($p>0.05$) was found in all concentrations of cumin except sample number 5 (500 μ L concentration).

Similarly results were not significant in the case of Metformin ($p>0.05$). Further, turmeric found an extremely significant increase in glucose uptake ($p<0.0001$) among cinnamon, cumin and Metformin. The results of the antidiabetic activity of turmeric, cumin, cinnamon extracts and standard metformin are depicted in table 6.

DISCUSSION

The antioxidant activity exhibited by cinnamon, turmeric and cumin were determined regarding total phenolic content, PM assay, hydroxyl radical and superoxide radical scavenging activity. *In vitro* antidiabetic activity was determined by using glucose uptake by yeast cells.

In the last few decades, research on spices has been directed to understand their medicinal, antidiabetic, antioxidant, and anticarcinogenic properties. Thus, spices like turmeric, cinnamon and cumin protect the human body against cellular oxidation reactions, bacterial infections and other metabolism-related disorders (Manikandan R *et al.*, 2013). The extracts of many spices have become popular in recent years for their antimicrobial and antioxidant properties and attempt to characterise their bioactive principles have gained momentum for

Table 6: Comparative % increase in glucose uptake by yeast cells due to the effect of cinnamon, cumin, turmeric extracts and standard Metformin (25 mM glucose concentration)

Conc. of extract (μL)	Cinnamon		Cumin		Turmeric		Metformin		F Value	P Value
	n	Mean \pm SD	n	Mean \pm SD	n	Mean \pm SD	n	Mean \pm SD		
100	5	85.07 \pm 0.17	5	51.79 \pm 1.08	5	50.87 \pm 0.50	5	40.00 \pm 0.00	5266.6	P<0.0001
200	5	87.45 \pm 0.18	5	53.45 \pm 2.48	5	91.24 \pm 0.51	5	40.00 \pm 0.00	1978	P<0.0001
300	5	91.58 \pm 0.03	5	66.83 \pm 1.43	5	93.92 \pm 0.72	5	40.00 \pm 0.00	4959.4	P<0.0001
400	5	93.02 \pm 0.37	5	68.48 \pm 0.74	5	96.18 \pm 0.11	5	50.00 \pm 0.00	13524	P<0.0001
500	5	95.84 \pm 0.18	5	81.73 \pm 1.19	5	97.55 \pm 0.42	5	66.53 \pm 0.30	2452.5	P<0.0001
F Value	2029.2		331.75		8040.4		38516		--	--
P Value	P<0.0001		P<0.0001		P<0.0001		P<0.0001			

Values were expressed as mean \pm SD

various pharmaceutical and food processing applications (Nadeem M *et al.*, 2012).

Spices are rich sources of polyphenolic compounds having strong antioxidant capacities and could potentially replace the synthetic antioxidants in food Systems and offer additional health benefits (Nadeem M *et al.*, 2012). Use of spices has been concerned in the prevention of many chronic diseases such as diabetes, cardiovascular diseases, cancer, inflammation etc. (Wild S *et al.*, 2004).

Plant polyphenolics, a varied group of phenolic compounds (flavanols, flavonols, anthocyanins, phenolic acids, etc.) acquire ideal structural chemistry for free radical scavenging activity (Nair SS *et al.*, 2013). Antioxidant properties of polyphenolics arise from their high reactivity as hydrogen or electron donors from the ability of polyphenol derived radical to stabilise and delocalize the unpaired electron (chain braking function) and from their potential to chelate metal ions (termination of Fenton reaction) (Cirillo VP 1963).

The antioxidant properties of flavonoids are due to several different mechanisms, such as scavenging of hydroxyl, superoxide ions and free radicals, chelation of metal ions like iron, copper and inhibition of enzymes responsible for the free radical generation (Rohit Yadav *et al.*, 2012).

Many researchers have found that *in vitro* studies on various medicinal plants showed antioxidative properties either by chelating metal ions, chain braking function, scavenging free radicals or by inhibiting enzymes of free radical generation (Phatak RS *et al.*, 2015, Bin Shan *et al.*, 2005, Rohit Yadav *et al.*, 2012, Patel RM *et al.*, 2011, Chanda S *et al.*, 2009, Salazar R *et al.*, 2008). Similar findings were observed in our study which indicated a

strong correlation between total phenolics and total antioxidant activity found in cinnamon, cumin and turmeric.

Increased glucose concentration in the blood leads to binding of glucose to haemoglobin that may result in the formation of Reactive Oxygen Species (ROS) (Nair SS *et al.*, 2013). The spices such as turmeric, cinnamon and cumin play an important role in the inhibition of reactive oxygen species formation by inhibiting glycation end products which ultimately leads to an increase in glucose uptake by yeast cells. In our study, different antioxidant capacities were observed related to different extracts of the turmeric, cinnamon and cumin. Turmeric was found to have the highest antioxidant and antidiabetic activity compared to cinnamon and cumin.

The *in vitro* assay of the present study indicated that all the three spice extracts possess good antidiabetic activity. In yeast, glucose transport takes place through facilitated diffusion (Cirillo VP 1963). After treatment of the yeast cells with ethanolic extracts of spices, the glucose uptake was found to increase. In our study, we found the highest antidiabetic activity in turmeric than cinnamon, cumin and Metformin.

CONCLUSION

The overall evaluation of this study concludes that the turmeric found to have potent antioxidant and antidiabetic property as compared to cinnamon and cumin. Phenolics, flavonoids, essential oils are the essential components present in spices which showed highest antioxidant and antidiabetic activity. There is also a good correlation between total phenolics content and total antioxidant activities, so this study revealed that spices such as cinna-

mon, cumin and turmeric showed better antioxidant and antidiabetic activity and could be safer than the synthetic antioxidant. Further research is needed to understand detailed mechanisms through which these effects are exerted and to study the biological effects of antioxidant-rich herbs and spices on oxidative stress-related diseases.

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CONFLICT OF INTEREST

The authors affirm that they have no conflict of interest.

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