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Antioxidant Analysis and Phytochemical Detection of the Some Selected Spices (Ajwain, Coriander, Cumin, Fennel, Fenugreek).

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ABSTRACT

Plants have been used to treat or prevent illness since before recorded history. Naturally occurring plant components are rich sources of antioxidants. The present study focuses on assessing the levels of various enzymatic and non- enzymatic antioxidants in the selected spice samples (Ajwain, Coriander, Cumin, Fennel and Fenugreek). Detection of Phytochemicals such as Carbohydrate, Flavonoids, Glycosides, Proteins, Phenols, Saponins, Tannin, Thiols, Alkaloids, Resins and Steroids were done for evaluating the efficacy of the selected samples to act as a potential antioxidant. The results revealed that Ajwain, Fennel and Cumin possess predominant quantities of enzymatic antioxidant like Catalase. Peroxidase was found abundantly in Ajwain and Coriander, Super Oxide Dismutase activity was maximum in Fennel and Fenugreek. The levels of non-enzymatic antioxidants such as vitamin C, Total phenols, Carotenoids, Reducing sugar in the spices were in the order of Ajwain > Fenugreek > Cumin > Fennel > Coriander. The phytochemicals were found abundant in Ajwain, Fenugreek and cumin. From this study it could be inferred that the antioxidant potential of Ajwain is higher than fenugreek followed by cumin, fennel and coriander respectively.

Keywords: Phytochemicals, Cumin (Cuminum cyminum), Fennel (Foeniculumvulgare miller), Fenugreek (Trigonella foenumgracum), Ajwain (Carumcoptium), and Coriander (Coriandrum sativum)

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INTRODUCTION

The belief in health benefits of foods dates back to approximately 2500 years ago when Hippocrates proclaimed **"Let food be thy medicineand medicine be thy food"** [9]. Antioxidants are dietary substances including nutrients such as beta carotene, Vitamin C, E and selenium that can prevent damage to our body cells or repair oxidative damage caused by free radicals [1]. This is the major reasons for why the people have started opting for antioxidant foods [27]. The high intake of antioxidants may help to maintain an adequate antioxidants status and therefore, the normal physiological function of a living system [13]. Since then the search for antioxidants naturally occurring in plants as alternatives to synthetic antioxidants is of great interest to researchers [7]. Natural antioxidants of plant origin are generally classified as Vitamins, Phenolic compounds including Flavanoids, Phenolic acids and Volatile compounds in herbs and spices [30].

MATERIALS AND METHODS

The spices namely Cumin (Cuminum cyminum), Fennel (Foeniculumvulgare miller), Fenugreek (Trigonella foenumgracum), Ajwain (Carumcoptium), and Coriander (Coriandrum sativum) were purchased from the local market of Coimbatore.

Preparation of Ethanolic extracts:

Samples of spices were pulverized and extracted twice in ethanol (1:10 w/v) at room temperature for 48 hours and filtered. The filtrates were concentrated to dryness under reduced conditions at room temperature. Dried extracts were then suspended in dimethyl sulfoxide (DMSO) for further use.

Determination of enzymatic antioxidants

Estimation of Catalase activity

The activity was done based on the procedure Sadasivamet al[24], About 10g of the sample was grounded and homogenated with phosphate buffer and it is used as enzyme extract**. Test:** To 2ml of enzyme, add 2ml of H_2O_2 (buffer substrate) and note down the time. After 2 min arrest the reaction by adding 2ml of H_2SO_4. The H_2O_2remaning in the tube is assayed by adding 0.5ml of KI solution, 2 drops of ammonium sulphate. Incubate the tube for 3 minutes at room temperature. Add 1-2 drops of starch indicator, titrate the iodine liberated using thiosulphate. **Control:** The initial peroxide concentration is obtained by adding the acid before the enzyme and peroxide solution in a separate test tube and heated.The difference between the titrevalue gives the amount of peroxide used in terms of ml of thiosulphate.

Estimation of Peroxidase activity

The assay was carried out by the method of Reddy et al., 1995[23]. About 0.5g of the sample was ground in 0.1ml phosphate buffer and used as the source of enzyme. The reaction mixture consisted of 3ml of buffered pyrogallol [0.05 M pyrogallol in 0.1 M phosphate buffer (pH 7.0)] and 0.5ml of 1% H_2O_2. To this added 0.1 ml enzyme extract and O. D. change was measured at 430 nm for every 30 seconds for 3 minutes.

Estimation of superoxide dismutase (SOD) activity

The assay of superoxide dismutase was done according to the procedure Marklund and Marklund, 1988 [18]. About 1g of the sample was homogenated with ethanol and used as an sample extract. **For control:** To 2.9 ml of tris buffer and 0.1ml of pyrogallol solution, mix. The reading noted at 420nm and exactly after 1 minute 30 seconds and 3 minute 30 seconds. The absorbance for to be recorded and the concentration of pyrogallol to be adjusted, so that the rate of change of absorbance/minute to be 0.020-0.023nm approximately. **For sample:** To 2.8ml of tris buffer, add 0.1ml of sample to be added and mixed and started the reaction by adding 0.1ml of pyrogallol solution (as per control). Read at 420nm exactly after 1 minute 30 seconds and 3 minute 30 seconds and recorded the absorbance per minute.

Determination of non- enzymatic antioxidants

Determination of Ascorbic acid

The estimation of ascorbic acid was carried out by the procedure given by Berwalet al., 2004 [3]. To5-10g of sample added 50 ml metaphosphoric acetic acid solution, grinded in the mortar. Adjust pH TO 1.2. Filter rapidly through Watman No.1 and diluted to 100ml with metaphosphoric acetic acid solution. For sample, take 2 ml of sample aliquot in a conical flask. For blank, take 2 ml of metaphosphoric acetic acid solution in a conical flask.For standard, take 2 ml of ascorbic acid standard in a 50 ml conical flask. Add 5 ml of metaphosphoric acetic acid solution to each flask. Titrate rapidly with indophenol solution until light but distinct pink colour persists of more than 5 seconds. Take three readings for each.

Estimation of Carotenoids

The carotenoids estimation was carried out based on the procedure given by Zakariaet al., 1979 [29]. Weighed 5.0g of the sample, Saponified for about 30mins in a shaking water bath at 30·c after extracting the sample in 12% alcoholic KOH. Transferred the saponified extract into a separating funnel (packed with glass wool and $CaCo_3$). Added 10-15ml of petroleum ether & mixed gently. Taken up the carotenoid pigments in petroleum ether layer. Transferred the lower aqueous phase to another separating funnel and collected the upper petroleum ether containing the carotenoids pigments to an amber-coloured bottle. Repeated the extraction of the aqueous phase similarly with phase to the petroleum ether extract added a small quantity of anhydrous sodium sulphate to remove turbidity. Noted the final volume of the petroleum ether extracts and absorbance of the extract was read at 450nm using petroleum ether as a blank.

Estimation of Total phenols

The total phenols were estimated using the procedure given by Malick and Singh, 1980 [17]. Weighed exactly 0.5 1.0g of the sample and grind it with a pestle and mortar in 10-time volume of 80% ethanol. Centrifuged the homogenate at 10,000rpm for 20 min. Save the supernatant. Evaporate the supernatant to dryness. Dissolve the residue in a known volume of distilled water (5ml).Pipetted out different aliquots (0.2 to 2ml) into test tubes. Made up the volume in each tube to 3ml with distilled water. Added 0.5ml of folin-ciocalteau reagent. After 3min, added 2ml of 20% Na_2CO_3 solution to each tube. Mixed thoroughly. Placed the tubes in boiling water for exactly one minutes, cooled and measured the absorbance at 650nm against a reagent blank. Prepared a standard curve using different concentrations of catechol.

Determination of Reducing sugars

The reducing sugars was determined using the procedure given by Somogyi, 1952 and Krishnaveniet al., 1984 [26]. Weighed 100mg of the sample and extracted the sugars with hot 80% ethanol twice, collected the supernatant and evaporated it by keeping it on a water bath at 80° C. Added 10ml water and dissolved the sugars. Pipetted aliquots of 0.1or 0.2ml to separate test tubes. Pipette 0.2, 0.4, 0.6, 0.8 and 1ml of the working standard solution in to a series of test tubes. Made up the volume in both sample and standard tubes to 2ml with distilled water. Pipette 2ml distilled water in a separate tube to set a blank. Added 1ml of alkaline copper tartrate reagent to each tube. Placed the tubes in boiling water for 10 minutes. Cool the tubes and add 1ml of phosphomolybdic acid reagent to all the tubes. Make up the volume in each tube 10ml with water. Read the absorbance of blue colour at 620nm after 10 minutes.

Detection of Phytochemicals

The detection of the following phytochemicals (Alkaloids, Phenols, Flavanoids, carbohydrates, Proteins, Tannins, Saponin, Thiols, Glycosides, Resins and Steriods) were carried out by the procedure given by Sadasivam et al, 1984 and 1997 [24].

RESULTS AND DISCUSSION

The presence of enzymatic antioxidants in the foods helps to improve the quality of the product. For e.g., the enzymatic antioxidant in fruit may help in ripening of the fruit. The presence of enzymatic

antioxidants in any food product, thus play as an important factor for the maintenance of the quality of product [19].

Catalase is an important enzymatic antioxidant, widely used in many of the food industry [12]. The peroxidase is also considered as a very important antioxidant. It has a very beneficial role in humans. But the bioavailability of these enzymes in the foods is not completely utilized. These enzymes are nowadays utilized in many of the food industries [8]. SOD is an enzyme that inactivates excess free radicals, preventing them from damaging cell membranes. However when taken orally this enzyme is digested and made useless [21]. But research is been carried out in many of the countries to find out the effective means of consuming as an oral supplement [5,11].

As Mentioned in Table 1, Fennel, ajwain and cumin possess high catalase activity Peroxidase activity was found higher in ajwain and coriander. Cumin and fenugreek possess high SOD activity. The Total enzymatic antioxidant was found to be higher in the selected spice samples such as **Ajwain > Fennel > Fenugreek.**

Table 1: Enzymatic Antioxidants Of Selected Spices

S. No	Spices	Catalase Activity (µmol/ min/g)	Peroxidase Activity (units/ litre/ min)	SOD Activity (units / ml of assay mixture)
1.	Ajwain	2.25	250	1.615
2.	Coriander	1.00	225	1.07
3.	Cumin	2.25	127	3.00
4.	Fennel	2.75	83.6	2.4
5.	Fenugreek	1.00	127.5	2.9

NOTE: 1 Unit of peroxidase activity = 1 mole pyrogallol oxidized/ min1 ml of assay mixture = Tris HCL + pyrogallol + sample extract

Non enzymatic antioxidant thus possesses an effective role in maintaining health status [6]. These non-enzymatic antioxidant are not destroyed completely [28]. These antioxidants can be obtained only through diet and mainly they are used for health oriented benefits.

Ascorbic acid is an antioxidant and water soluble vitamin, which is essential to the construction of connective tissue. The human body stores insignificant amounts of ascorbic acid, therefore requires daily replenishment [15]. Ascorbic acid has an effective role in iron absorption [2], in curing common cold [27], plays a critical role in wound repair and healing, has an effective role in prevention of carcinogen [16] etc. Carotenoids are thought to have a variety of different actions that are related to the decreased risk of some degenerative diseases [10]. Consumption of Carotenoid rich food may enhance the health status of the human. The high antioxidant activity of any food sample can be correlated to the high phonelic content among that food sample [28]. The reducing sugar content differs based on the storage period. Reducing sugar is considered as an important component in the preservation of the sample, they also posses good antioxidant potential [19].

According to Table 2, Ascorbic acid content was found in higher level in the spices namely cumin and fenugreek; carotenoid content was found in higher level in ajwain and fennel, total phenols was found abundantly in fenugreek and ajwain, the reducing sugar content was found higher in cumin and ajwain. The amounts of non- enzymatic antioxidant, found in the spices was in the order of ajwain > fenugreek > cumin > fennel > coriander.

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Table 2: Non Enzymatic Antioxidants Of Selected Spices

S.No	Spice	Ascorbic acid(mg/ 100g)	Carotenoids (mg/100g)	Total Phenols (mg/100g)	Reducing Sugar (%)
1.	Ajwain	1.5	4.0	9000	2.0
2.	Coriander	0.7	1.2	7000	0.5
3.	Cumin	3.0	3.5	2000	2.5
4.	Fennel	0.5	4.5	4000	1.0
5.	Fenugreek	2.5	2	11000	1.5

Phytochemicals are non-nutritive plant chemicals that contain protective, disease-preventing compounds [20]. Flavanoids are important components in the human diet. Flavanoids are essential compound which possess medicinal values [22]. Protein is also considered as an important nutrient which possesses numerous health benefits and also possesses antioxidant property [19]. Phenolic compounds have a very beneficial effect on human body [20]. Tannins are incompatible with alkali, gelatin, heavy metals, iron, lime water, metallic salts, strong agents & zinc sulfate. It is considered as an important photochemical. Thiols are effective phytochemical which is found in cruciferous vegetables and their benefit in human health is, it is considered as a detoxification of cancer causing agents. Different forms of phenolic glycoside compounds are found in nature. Some of these phenolic glycosides have a most potent scavenging activity [4]. The saponins are generally referred as hypocholesterolemicsaponins [20]. This exhibits the truth that fenugreek will be having an effective hypocholesterolemic compound but coriander since they are deficit in saponin they might not have the efficacy to act as hypocholestrolemic compound.

Table 3 indicates that Ajwain and fenugreek possess enormous amount of flavanoids. All the selected spices samples possess enoromous amount of carbohydrate and protein. Phenol was found in higher amount in ajwain, coriander and fennel. Tannin content was higher in cumin and fennel. Except coriander, all the other selected samples possess enormous amount of glycosides, and saponin was found higher in fenugreek. Ajwain possess high amount of resin, steroids and alkaloids. Ajwain and Fenugreek exhibited a similar pattern of phytochemical content. Same trend was observed between cumin and fennel. Based on the Phytochemical content estimated the spices can be arranged in the following order.

- Ajwain and Fenugreek
- Cumin and Fennel
- Coriander
-

The noticeable phytochemical present in coriander was only Phenol.

Table 3: Detection Of Phytochemicals

S.No	Spices	Flavanoids	Carbohydrates	Protein	Phenol	Tannin	Thiols	Glycocides	Resins	Steroids	Alkaloids	Saponin
1.	Ajwain	+ + +	+ + +	+ + +	+ + +	+	-	+ + +	+ +	+	+ + +	+ +
2.	Corriander	+ +	+ + +	+ +	+ + +	+	-	+ +	+	-	-	-
3.	Cumin	+ +	+ + +	+ + +	+ +	+ +	-	+ + +	+	-	+	+
4.	Fennel	+ +	+ + +	+ +	+ + +	+ +	-	+ + +	+	-	+	+
5.	Fenugreek	+ ++	+ + +	+ + +	+ +	+	-	+ + +	++	+	+ +	+ + +

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Figure 1: Spices used in this study

cumin seeds (*cuminum cyminum*)

fennel seeds (*foeniculumvulgare miller*)

fenugreek (*trigonellafoenumgracum*)

ajwain (*carumcoptium)*

coriander seeds (*coriandrum sativum*)

CONCLUSION

It was noted that increased amounts of the phytochemicals were found to be present in the spices like ajwain and fenugreek and cumin.From the study it can be concluded that Ajwain possess an increased amount of both enzymatic and non- enzymatic antioxidants and also the phytochemicals too. Thus the antioxidant potential can be found in the order such as **Ajwain > Fenugreek > Cumin > Fennel > coriander.**

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