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Variation of Phenols, Flavonoids and Antioxidant Potential in Various Parts of *Foeniculum vulgare* on Drying

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ABSTRACT

Foeniculum vulgare is one of the important spices known for its significant use as flavoring agent and carminative. It is proven to treat cancer, diarrhea and show antimicrobial, antifungal, hepato protective activities. Moreover fennel plant is well known for its rich source of quercetin but the content varies in the plant in response to the minor changes in environment. The post harvest treatment of the plant also determines the variability of chemical constituents. Thus drying has been selected as a primary post harvest parameter and procedures were to be developed to validate the yield and activity of different parts of fennel. Methanol extract of various parts of fennel have been investigated for the presence of phyto constituents by performing preliminary phyto chemical screening. Results show the presence of polyphenols, flavonols, carbohydrates, proteins and glycosides in all the parts irrespective of the drying. The total phenol content and total flavonoid content of extracts of various parts of fennel were determined using the standard graphs of gallic acid and quercetin. Out of all, extract of Shade dried stems was found to contain high phenol and flavonoid content of 60.25 and 17.36 and their least concentrations of 16.54 and 8.35 were found in extracts of Hot air oven dried roots. The maximum percentage inhibition of DPPH radicals by different extracts of various parts of fennel on drying was determined in DPPH free radial scavenging method. All the shade dried parts showed better activity compared to sun dried ad hot air oven dried parts. Drying had a significant effect on the antioxidant activity of the fennel plant.

Keywords: Postharvest technology, Drying, Fennel, Total phenol content and Total Flavonoid content.

1. INTRODUCTION

India has a very long, safe and continuous usage of many herbal drugs in the officially recognized alternative systems of health *viz*. Ayurveda, Yoga, Unani, Siddha, Homeopathy and Naturopathy. Millions of Indians use herbal drugs regularly, as spices, home-remedies, health foods as well as over-the-counter (OTC) as self-medication or also as drugs prescribed in the non-allopathic systems. India enjoys a wide range of agronomic and climatic conditions, which enable us to grow a number of spices. Spices have played an important role in the history of civilization, exploration and commerce as these had a universal acceptance as condiments and flavours in human diet as well as in treatment of ailments. According to Hirasa and Takema, the term 'spice' can be defined as the dry part of a plant, such as roots, leaves and seeds, which impart to food a certain flavor and pungent stimuli. Most spices owe their flavouring properties to volatile oils and in some cases, to fixed oils and small amount of resin, which are known as oleoresins. Phytochemicals in spices are secondary metabolites, which are originated for the protection from herbivorous insects, vertebrates, fungi, pathogen, and parasites. Most probably, no single compound is responsible for flavours; but a blend of different compounds such as alcohols, phenols, esters, terpenes, organic acids, resins, alkaloids, and sulphur containing compounds in various proportions produce the flavours. Besides these flavouring components every spice produce contains the usual components such as proteins, carbohydrates, fibre, minerals, tannins or polyphenols.

Quality of spices is assessed by its intrinsic as well as extrinsic characters. The former consists of chemical quality, *i.e.* the retention of chemical principles like volatile oil, alkaloids and oleoresins while the latter emphasizes physical quality. This include appearance, texture, shape, presence or absence of unwanted things, colour etc. In addition certain health requirements are also implemented as export quality standard *viz.* pesticide residue, aflatoxin, heavy metals, sulphur dioxide, solvent residues, and microbiological quality. However, physico-chemical quality remains the ultimate attribute, while considering export requirement of spices as these properties delineate its grade in the market. These qualities vary unpredictably. According to Menon the physicochemical characteristics vary widely depending on the variety, agro-climatic conditions existing in the area of production, harvest, and post-harvest

operations [1]. It is well understood that the quality of spices depends to a large extend upon the post-harvest operations. The post-harvest technology of spices comprises of a whole gamut of operations such as pre-treatment, chemical treatment, curing and similar operations, drying, cleaning, sorting, grading and packaging [2]. Considering each and every step under post-harvest technology of spices, drying remains the most important operation. At the time of harvesting, spices like all other agricultural commodities invariably contain high moisture that must be brought down into the desired level at which attack of micro-organisms would be minimum. At the same time retention of quality attributes should also be at the maximum.

During drying, various other processes also takes place, such as cooling effect, shrinkage effect, case hardening, loss of rehydration ability, scorching or heat damage, loss of flavour, and migration of soluble constituents. Different methods of drying are in vogue for various spices.

The present investigation is aimed to formulate appropriate methods for the quality improvement of Fennel plant. However large quantities of fennel produced are under graded every year due to the mishandling and unhygienic methods adopted after the harvest, for processing and storage. The rejection of the plant from the export market is also reported to be due to these unscientific approaches. Moreover fennel plant is well known for its rich source of quercetin but the content varies in the plant in response to the minor changes in environment. The post harvest treatment of the plant also determines the yield of chemical constituents. The environment is such a factor that it cannot be controlled or maintained economically. Therefore the objective of this study was to devise methods of post harvest technology to improve the export quality of fennel. Thus drying has been selected as a primary post harvest parameter and procedures were to be developed to validate the yield and activity of different parts of fennel.

2. MATERIALS AND METHODS

2.1. Collection

The fresh fennel plants were collected from Tirumala hills, Tirupathi, India in the month of December 2011. Fresh fruits were collected from a local herbal drug store, Tirupathi.

2.2. Identification and Authentication

The taxonomical identification and authentication of the plant was done by Dr. P. Jayaraman, Director, National Institute of Herbal Medicine, Plant Anatomy Research Centre, Chennai. The voucher specimen (PARC/2010/721) was preserved in laboratory, Department of Pharmacognosy, Sree Vidyanikethan College of Pharmacy for further reference.

2.3. Post harvest drying

Fresh fennel plants were collected and various parts like leaves, stems, fruits and roots were separated.

These parts were individually dried under following conditions

a. Sun dried at 45^0c
b. Shade dried at room temperature (25-30^0c)
c. Hot air oven at 40-45^0c.

After ensuring complete drying for 2days the plant material was ground and powdered and tested for physical parameters like loss on drying and ash values.

2.4. Extraction

50g of powdered material of each part of the fennel plant was extracted separately using methanol using soxhelt apparatus. The extract was concentrated and traces of the solvent were completely removed under reduced pressure and stored in vacuum desiccators for further use. These extracts were named consecutively as:

a. Sun dried Leaves (SDL)
b. Sun dried Stems (SDS)
c. Sun dried Roots (SDR)
d. Sun dried Fruits (SDF)
e. Shade dried Leaves (SHL)
f. Shade dried Stems (SHS)
g. Shade dried Roots (SHR)
h. Shade dried Fruits (SHF)
i. Hot air oven dried Leaves (HDL)
j. Hot air oven dried Stems (HDS)
k. Hot air oven dried Roots (HDR)
l. Hot air oven dried Fruits (HDF)

2.5. Preliminary Phytochemical analysis

The concentrated extracts were subjected to chemical tests for the identification of the various constituents as per the standard procedures given by Kokate [3].

2.6. Determination of total phenolic content

The total phenolic content was determined using Folin–Ciocalteu reagents with analytical grade gallic acid as the standard [4].

2.6.1. Standard curve of gallic acid

1mg of gallic acid was weighed and dissolved in 100ml of distilled water and successive dilutions were made to make up the concentrations 2,4,6,8 and 10 µg/ml. A volume from above aliquots was taken and mixed with 1.25ml of FC reagent. It was left for 5 mins. Then 2.5ml of 20% sodium carbonate was added and it was let to react for 30 min then the volume was made upto 10ml. Then the absorbance was measured at 765nm. The calibration curve was drawn plotting the absorbance and concentrations.

2.6.2. Sample preparation

0.5g of Methanol extract was weighed and dissolved in 100ml of water. From this 0.1ml was taken into 10ml standard flask and 1.25ml of FC reagent was added and let to react for 5 min. Then 2.5ml of 20% sodium carbonate was added and the volume was made upto 10ml. It was kept for 30 min for complete reaction. Now the absorbance was measured at 765nm. Total phenolic content was calculated from the calibraition curve of gallic acid and the value was expressed in gallic acid equivalents.

2.7. Total flavonoid content

2.7.1. Standard curve of quercetin

1mg of quercetin was weighed and dissolved in 100ml of methanol and successive dilutions were made to make up the concentrations 2,4,6,8 and 10 µg/ml. 5mL of 2 % aluminium trichloride ($AlCl_3$) in methanol was mixed with the same volume of the quercetin solution (0.4 mg/mL). Absorption readings at 415 nm using UV-VIS spectrophotometer were taken after 10 minutes against a blank sample consisting of a 5 mL quercetin solution with 5 mL methanol without $AlCl_3$.

2.7.2. Sample preparation

The total flavonoid content was determined using the Dowd method [16]. 5 mL of 2 % aluminium trichloride ($AlCl_3$) in methanol was mixed with the same volume of the extract solution (0.4 mg/mL). Absorption readings at 415 nm using UV-VIS spectrophotometer and readings were taken after 10 minutes against a blank sample consisting of a 5 mL extract solution with 5 mL methanol without $AlCl_3$. The total flavonoid content was determined using a standard curve of quercetin. Total flavonoid content is expressed as mg of quercetin equivalents (QE) / g of extract.

2.8. *Invitro* DPPH radical scavenging activity

In order to determine the radical scavenging ability, the method reported by Lilian was used[5]. Briefly, 0.3 mM alcohol solution of DPPH (1 mL) was added to samples (2.5 mL) containing different concentrations of extracts like 200, 400, 600, 800 and 1000 mcg/ml. The samples were first kept in a dark place at room temperature and their absorbance was read at 518 nm after 30 min. The antiradical activity (AA) was determined using the following formula:

AA% = 100 x (Abs:sample - Abs:empty sample) x 100)/ Abs:control

Blank samples contained 1 mL ethanol and 2.5 mL from various concentrations of each extract; control sample contained 1 mL of 0.3 mM DPPH and 2.5 mL ethanol. The optical density of the samples, the control and the empty samples were measured in comparison with ethanol. One antioxidant, BHT (Butyl Hydroxyl Toluene) used as positive control (STD).

3. RESULTS AND DISCCUSION

Studies on loss on drying and ash values were performed and the results were tabulated in table 1.

Table -1: Physical parameters of various parts of fennel

Drug sample	Drying type	Loss on drying (%w/w)	Ash values (%w/w)		
			Total ash	Acid insoluble ash	Water soluble ash
leaves	Sun dried	2.10	4.24	1.58	2.44
	Shade dried	7.54	4.26	1.51	2.39
	Hot air oven dried	5.27	4.21	1.54	2.41
Stems	Sun dried	3.36	4.89	1.65	2.77
	Shade dried	9.98	4.9	1.61	2.54
	Hot air oven dried	6.51	4.85	1.6	2.71
Roots	Sun dried	5.36	6.12	2.47	3.14
	Shade dried	12.57	6.09	2.51	3.08
	Hot air oven dried	6.14	6.15	2.54	3.17
Fruits	Sun dried	3.68	4.32	1.72	2.57
	Shade dried	8.87	4.31	1.76	2.64
	Hot air oven dried	6.25	4.36	1.7	2.6

Methanol extract of various parts of fennel have been investigated for the presence of phyto constituents by performing preliminary phyto chemical screening. Results show the

presence of polyphenols, flavonols, carbohydrates, proteins and glycosides in all the parts irrespective of the drying.

The total phenol content and total flavonoid content of extracts of various parts of fennel were determined using the standard graphs of gallic acid with r^2=0.988 and quercetin r^2=0.998 which are given in figures 1 and 2.

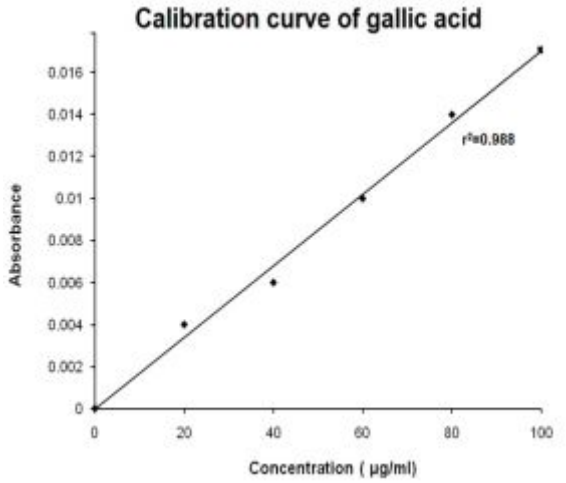

Figure 1: Standard curve of gallic acid

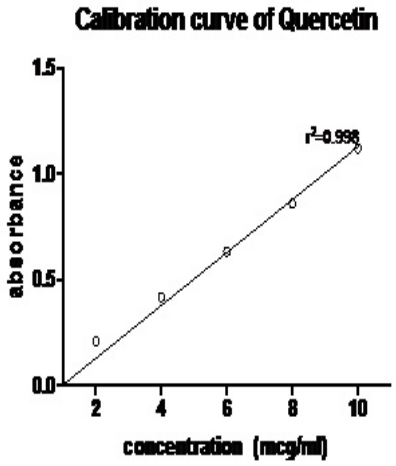

Figure 2: Standard curve of quercetin

The values of total phenol content and total flavonoid content of extracts of various parts of fennel were tabulated in table 2. Out of all, extract of Shade dried stems was found to contain high phenol and flavonoid content of 60.25 and 17.36 and their least concentrations of 16.54 and 8.35 were found in extracts of Hot air oven dried roots.

Table -2: Total phenol content and total flavonoid content

Sample	Drying	Total phenol content (mg gallic acid eq's / g extract)	Total flavonoid content (mg quercetin eq's / g extract)
Leaves	Sun (SDL)	55.71	8.55
	Shade (SHL)	54.49	14.57
	Hot air oven (HDL)	21.39	5.52
Roots	Sun (SDR)	19.31	8.99
	Shade (SHR)	36.12	12.74
	Hot air oven (HDR)	16.54	8.35
Stems	Sun (SDS)	32.19	10.29
	Shade (SHS)	60.25	17.36
	Hot air oven (HDS)	29.75	9.76
Fruits	Sun (SDF)	28.66	10.75
	Shade (SHF)	45.87	15.22
	Hot air oven (HDF)	41.58	9.63

Comparing the drying efficiency in stems, Hot air oven dried stems yielded in least content of phenols and flavonoids. This suggests that Shade drying is better than Sun drying better than Hot air oven drying. In contrast, Hot air oven dried fruits showed a better yield compared to sun dried fruits. This may be due to degradation of chemical constituents due to UV radiation from direct sunlight. In most cases, Sun drying and Hot air oven drying gave almost similar results which can be concluded that temperature had its role in maintaining the yield of chemical constitutents in respective parts. Interestingly, fruits showed contrast results proving sun drying results in further decrease in the chemical constituents due to electromagnetic radiation which is reported to destroy the chemical constituents in a plant. Overall it suggests that stems contain more flavonoids comparable to fruits better than roots better than leaves.

DPPH has been used by various researchers as a quick and reliable parameter to assess the invitro antioxidant activity of crude plant extracts. In DPPH test the ability of a compound to act as donar for hydrogen atoms or electrons was measured spectrophotometrically. The maximum percentage inhibition of DPPH radicals by different extracts of various parts of fennel on drying was determined in DPPH free radial scavenging method. The results were tabulated in table 3.

Table -3: Effect of drying on antioxidant activity of various parts of fennel

Sample	Drying	DPPH radical scavenging activity (AA%)				
		200 mcg/ml	400 mcg/ml	600 mcg/ml	800 mcg/ml	1000 mcg/ml
Leaves	Sun (SDL)	71.25	78.86	84.21	86.79	87.48
	Shade (SHL)	75.24	80.68	86.54	90.46	92.49
	Hot air oven (HDL)	35.69	39.42	42.38	45.94	49.78
Roots	Sun (SDR)	32.77	36.24	39.85	42.18	45.85
	Shade (SHR)	65.12	69.58	72.41	75.26	79.82
	Hot air oven (HDR)	25.31	27.84	29.45	30.7	31.27
Stems	Sun (SDS)	42.85	45.76	48.69	50.27	51.87
	Shade (SHS)	80.56	86.2	92.14	94.78	96.38
	Hot air oven (HDS)	40.26	43.12	45.08	46.95	48.22
Fruits	Sun (SDF)	39.27	42.55	45.81	48.66	49.99
	Shade (SHF)	78.2	82.57	88.65	89.81	90.93
	Hot air oven (HDF)	65.84	68.26	70.8	72.09	75.57
	BHT	82.07	86.97	92.78	95.79	96.99

Drying had a significant effect on the antioxidant activity of the fennel plant. From the table it is clear that stems showed the best antioxidant activity of 96.38% at 1000mcg/ml comparable to the standard, BHT of 96.99%. It is even better compared to leaves, fruits and roots. Correlating the phenol and flavonoid content in the extracts it can be stated that these are mainly responsible for the activity. A change in their concentration resulted in the variationin antioxidant activity. Very less activity was showed by Hot air oven dried roots of 31.27%. It can also be stated that all the extracts showed a dose dependant activity against DPPH radicals.

From the results it was understood that drying also plays important role in determining the activity irrespective of the part. All the shade dried parts showed better activity compared to sun dried ad hot air oven dried parts. This can be again correlated with the variation in phenols and flavonoids on drying in the respective parts.

4. CONCLUSION

Fennel is one of the important spices known for its significant use as flavoring agent, carminative. It is proven to treat cancer, diarrhea and pocess antimicrobial, antifungal, hepato protective activities. Moreover fennel plant is well known for its rich source of quercetin but the content varies in the plant in response to the minor changes in environment. The post harvest treatment of the plant also determines the content of chemical constituents. So issues to maintain postharvest technologies aiming in better yield are of concern.

From the results it was understood that Drying had a significant effect on the antioxidant activity of the fennel plant. All the shade dried parts showed better activity compared to sun dried ad hot air oven dried parts. Further steps have to be developed to estimate the effect of drying on individual chemical constituent using accurately reliable methods like HPLC and GC. Investigations on the distribution of chemical constituents in various parts of fennel are also to be performed.

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