



## Preliminary phytochemical analysis, elemental composition and proximate analysis of selected spices

Pavithra B, Anuradha R\*

PG and Research, Department of Biochemistry, Sengamala Thayaar Educational Trust Women's College, (Affiliated to Bharathidasan University), Tiruchirappalli, Tamil Nadu, India

### Abstract

The study is to estimate the phytochemical, proximate, quantitative, and mineral analyses were investigated in *Elettaria cardamomum*, *Trigonella foenum graecum*, *Cuminum cyminum*, *Trachyspermum ammi*, and *Piper nigrum*. The spices were qualitatively analysed for the presence of Phyto-compounds which indicated the presence of various phytoconstituents like terpenoids, alkaloids, phenols, steroids, tannins, flavonoids, saponins, Cardiac glycosides, phlobatannins, sugar, and amino acids. Proximate analysis indicated the presence of moisture content, ash, crude fibre, lipids, protein, and carbohydrates in which *Elettaria cardamomum* has high moisture and *Trachyspermum ammi* has high ash and protein content. *Piper nigrum* has a higher fibre and carbohydrate content. Quantitative analysis indicated the presence of alkaloids phenolic, flavonoids, steroids, tannins, and saponins. The mineral content of spices indicated the presence of calcium, magnesium iron, manganese, and copper. Macro minerals calcium and magnesium (14.3 and 23.8) were higher in fenugreek. The iron content of cardamom is higher than other spices (6.5). The presence of high secondary metabolites and mineral content in the seeds is a good indication that if the spices are subjected to further research novel compounds may also be identified.

**Keywords:** spices, proximate analysis, phytochemical analysis, mineral analysis

### Introduction

Herbs and spices are plant-derived seasoning used for culinary purposes. The terms 'herbs' and 'spices' are often used interchangeably, but they have specific definitions in botany. Herbs store flavour component in their leaves, whereas spices store theirs in seeds, bark, and root. A spice may be the bud (clove), bark (cinnamon), root (ginger), aromatic seed (cumin), and flower stigma (saffron) of a plant. In addition to making food taste good, culinary spices have been used as food preservatives and for their health-enhancing properties for centuries (Kaefer and Milner, 2011) [14]. Moreover, for people of the world, spices stimulate appetite and create visual appeals to food (Opara and Chohan, 2014) [26]. The use of spices in culinary predates recorded history and is said to have been an integral part of local dishes in South Asia and the Middle East as far back as 2000 BCE (Tapsell *et al.*, 2006) [32]. Spices are often gathered from plants when they have stopped flowering (Singletary, 2016) [31]. Spices are functional foods, these are foods that can be demonstrated to have a beneficial effect on certain target functions in the body beyond basic nutritional requirements (Lobo *et al.*, 2010) [19]. Spices occur in a variety of flavours, colours, and aromas contributing a wide range of nutrients to foods (Mann, 2011) [22]. They enhance and complement flavour in foods with no detrimental effect on the organoleptic quality of the food (Kaefer and Milner, 2011) [14]. Herbs and spices elaborate secondary metabolites that form part of the plant's chemical defence. They make food taste good but may not be delicious themselves, and many of them possess marked pharmacological and medicinal properties (Newman and Cragg, 2012) [24]. Their constituents function as defence chemicals to repel insects, snails, and other animals, and to

kill Microbes especially parasitic fungi (Adeyemi, 2011) [3]. They sometimes contain allelochemicals, used by certain plants (such as *Thymus vulgaris*) to inhibit the growth of other plant species around them (Linhart *et al.*, 2015) [18]. The importance of spices is underscored by the fact that they are still found in 40% of drugs prescribed till date (Mann, 2011) [22]. Spices and herbs are revered for their potential health attributes. They are reported to have positive effects in the treatment of numerous diseases, especially chronic ones such as cancer, diabetes, and cardiovascular diseases (Kaefer and Milner, 2011) [14]. That nutrition and health are intricately linked is a well-established fact, and the ability of nutrition (in this case, nutrients from spices) to reduce the risk of diseases has engaged the attention of researchers and nutritionist alike in recent decades. Numerous epidemiological, preclinical, and clinical studies providing insights into the mode of action of this relationship has been carried out (Kochhar, 2008; Krishnaswamy, 2008; Iyer *et al.*, 2009) [16, 17, 12]. The rise in demand for cheaper and safer therapeutics due to high cost and apprehension around the side effects of conventional drugs is stimulating interest in the use of phytomedicine for treatment, and management of diseases (Sigh, 2007; Dolui and Segupta, 2012) [30, 9]. This work aims to review previous works done on the nutritional and health benefits of five selected spices—*Elettaria cardamomum*, *Trigonella foenum graecum*, *Cuminum cyminum*, *Trachyspermum ammi* and *Piper nigrum*—commonly used in South Asia culinary with the view to give a summary of what the prospects are for these spices, especially as regards their potential in formulating nutraceuticals used in chronic disease intervention. To address this lacuna, the present study was carried out for

qualitative, quantitative phytochemical analysis, proximate analysis and mineral analysis of seed parts of different spices.

## Materials and Methods

### Plant collection and identification

The plant samples of *Elettaria cardamomum*, *Foeniculum-graecum*, *Cuminum cyminum*, *Trachyspermum ammi* and *Piper nigrum*, were collected around Sundarakottai, during the month of September. These plants were collected from their natural habitat and from identified herbalists.

### Preparation of the samples

The seeds of plant species were cleaned using water until soil and other materials on them were removed. Thereafter, they were then air dried under shade for a week. The plants materials were then ground into fine powder and then wrapped in air-tight containers and placed in the laboratory at room temperature (25°C) prior to further analysis.

### Preliminary qualitative phytochemical analysis

Preliminary qualitative phytochemical analysis was carried out to identify the secondary metabolites present in the various alcoholic and aqueous extracts of leaf and root parts of *H. radicata* (Trease and Evans, 2002; Harborne, 1984) [33, 11].

### Quantitative estimation of chemical constituency

#### Determination of alkaloids

A total of 200 mL of 20% acetic acid was added to 5 g of leaf and root powders taken in a separate 250 mL beaker and covered to stand for 4 h. This mixture containing solution was filtered and the volume was reduced to one quarter using water bath. To this sample, concentrated ammonium hydroxide was added drop-wise until the precipitate was complete. The whole solution was allowed to settle and the precipitate was collected by filtration and weighed (Obadoni and Ochuko, 2001) [25].

#### The percentage of total alkaloid content was calculated as:

Percentage of total alkaloids (%) =  $\frac{\text{Weight of residue}}{100/\text{Weight of sample taken}}$

#### Total phenolics content

The total phenolics content of *H. radicata* was estimated using Folin-Ciocalteu reagent by the method of Sidduraju and Becker (Siddhuraju and Becker, 2003) [28]. About 20 µg of leaf and root extracts were taken separately and it was made up to 1 mL with distilled water. Then 500 µL of diluted Folin-phenol reagent (1:1 ratio with water) and 2.5 mL of sodium carbonate Na<sub>2</sub>CO<sub>3</sub> (20%) were added. The mixture was shaken well and incubated in dark condition for 40 min for the development of colour. After incubation, the absorbance was measured at 725 nm. A calibration curve of gallic acid was constructed and linearity was obtained in the range of 10-50 µg/mL. The total phenolics content in the plant extracts were expressed as mg of gallic acid equivalent (mg GAE/g extract) by using the standard curve.

#### Total flavonoids content

The total flavonoids content was estimated using the procedure described by Jia *et al.* (1999) [13]. A total of 1 mL

of plant extracts were diluted with 200 µL of distilled water separately followed by the addition of 150 µL of sodium nitrite (5%) solution. This mixture was incubated for 5 min and then 150 µL of aluminium chloride (10%) solution was added and allowed to stand for 6 min. Then 2 mL of sodium hydroxide (4%) solution was added and made up to 5 mL with distilled water. The mixture was shaken well and left it for 15 min at room temperature. The absorbance was measured at 510 nm. Appearance of pink colour showed the presence of flavonoids content. The total flavonoids content was expressed as rutin equivalent mg RE/g extract on a dry weight basis using the standard curve.

#### Estimation of tannins content

Tannins content of *H. radicata* was estimated by the method of Siddhuraju and Manian, (2007) [29]. A total of 500 µL of the extracts were taken in test tube separately and treated with 100 mg of polyvinyl polypyrrolidone and 500 µL of distilled water. This solution was incubated at 4 °C for 4 h. Then the sample was centrifuged at 5 000 r/min for 5 min and 20 µL of the supernatant was taken. This supernatant has only simple phenolics free of tannins (the tannins would have been precipitated along with the polyvinyl polypyrrolidone). The phenolics content of the supernatant was measured at 725 nm and expressed as the content of free phenolics on a dry matter basis. From the above results, the tannins content of the extract was calculated as follows:  
Tannins (mg GAE/g extract) = Total phenolics (mg GAE/g extract) - Free phenolics (mg GAE/g extract)

#### Estimation of total saponins content

Estimation of total saponins content was determined by the method described by Makkar *et al.* (2007) [21] based on vanillin-sulphuric acid colorimetric reaction with some modifications. About 50 µL of plant extract was added with 250 µL of distilled water. To this, about 250 µL of vanillin reagent (800 mg of vanillin in 10 mL of 99.5% ethanol) was added. Then 2.5 mL of 72% sulphuric acid was added and it was mixed well. This solution was kept in a water bath at 60 °C for 10 min. After 10 min, it was cooled in ice cold water and the absorbance was read at 544 nm. The values were expressed as diosgenin equivalents (mg DE/g extract) derived from a standard curve.

#### Proximate Analysis

Determination of moisture content, ash content, crude fibre, crude lipid (Cunniff and AOAC, 1995) [8]. The amount of protein present in the powdered leaves was estimated using Lowry's method (1951). The total carbohydrate content of the powdered sample was estimated using phenol sulphuric acid method of Gornall *et al.*, (1949) [10].

#### Mineral Analysis

For mineral analysis, the samples were digested following the procedure described by Salami and Non, (2002) [27]. Briefly, samples of 1g each plant were digested with 5ml concentrated nitric acid (HN03) and 1ml each of concentrated sulphuric acid (H2SO4) and 60-62% perchloric acid (HClO4) and heated until white fumes of perchloric acid formed. The volume of the digest was reduced by heating but not to dryness. The flask was set aside to cool, after which the content was diluted with distilled deionized water and then filtered into a 50ml volumetric flask. The content was made up to mark with

deionized water and stored until analysed for mineral contents using Atomic Absorption spectrophotometer (AAS).

### Statistical analysis

Statistical analysis was carried out by One-way analysis of variance (ANOVA) test using a statistical package program (SPSS 10.0) and the significance of the difference between means was determined by Duncan's multiple range test at ( $P < 0.05$ ) significant level. Analysis was carried out in triplicate and mean  $\pm$  SD of three parallel measurements.

### Results and Discussion

The present study carried out on the five spices i.e., *Elettaria cardamomum*, *Trigonella foenum graecum*, *Cuminum cyminum*, *Trachyspermum ammi* and *Piper*

*nigrum*, were used in this study and revealed the presence of medicinal active constituents. The phytochemical active compounds of these spices were qualitatively analyzed separately and the results are presented in Table 1 and 2. In these screening process alkaloids, tannins, saponins, flavonoids and terpenoids, glycosides, phenols show different types of results.

Preliminary qualitative phytochemical analysis made for the seed parts of different spices revealed the presence of alkaloids, cardiac glycosides, flavonoids, glycosides, phenols, resins, saponins, steroids, tannins, terpenoids and triterpenoids. These secondary metabolites are reported to have many biological and therapeutic properties (Vishnu *et al.*, 2013; Benedec *et al.*, 2013; Charalampo *et al.*, 2013; Narender *et al.*, 2012) [34, 5, 7, 23] so this species is expected to have many medicinal uses.

**Table 1:** Preliminary qualitative phytochemical analysis of some spices

S. No.	Constituents	Cardamom	Fenugreek	Cumin	Ajwain	Pepper
1	Terpenoids	+	+	+	+	+
2.	Alkaloids	+	+	+	+	+
3	Phenols	+	+	+	+	+
4	Steroids	+	+	+	-	+
5	Tannins	+	+	+	+	+
6	Flavonoids	+	+	+	+	+
7	Saponins	+	+	-	+	+
8	Cardiac glycosides	+	+	+	-	+
9	Phlobatannins	-	+	+	+	+
10	Sugar	+	+	+	+	+
11	Amino acids	+	+	+	+	+

(+) Indicate the presence of phytochemicals and (-) Indicate the absence of phytochemicals

**Table 2:** Quantitative Phytochemical composition of spices

S. No.	Parameters	Cardamom (mg/100g)	Fenugreek (mg/100g)	Cumin (mg/100g)	Ajwain (mg/100g)	Pepper (mg/100g)
1.	Alkaloids	31.7 $\pm$ 0.03	29.4 $\pm$ 0.12	26.7 $\pm$ 0.3	32.87 $\pm$ 0.03	33.2 $\pm$ 0.07
2.	Total Phenolic	15.4 $\pm$ 0.1	18.6 $\pm$ 0.22	17.6 $\pm$ 0.04	18.2 $\pm$ 0.11	16.8 $\pm$ 0.8
3.	Total Flavonoids	32.3 $\pm$ 0.12	27.4 $\pm$ 0.4	25.8 $\pm$ 0.08	24.4 $\pm$ 0.03	26.4 $\pm$ 0.54
4.	Steroids	1.64 $\pm$ 0.12	0.84 $\pm$ 0.03	1.84 $\pm$ 0.08	1.56 $\pm$ 0.07	1.24 $\pm$ 0.08
5.	Total Tannins	1.94 $\pm$ 0.6	2.84 $\pm$ 0.12	2.26 $\pm$ 0.8	2.45 $\pm$ 0.1	3.1 $\pm$ 0.18
6.	Total Saponins	4.3 $\pm$ 0.3	5.2 $\pm$ 0.65	4.94 $\pm$ 0.12	3.26 $\pm$ 0.06	2.45 $\pm$ 0.7

The proximate compositions determined in the spices are summarized in Table 3. It shows that the all the spices has a high moisture content and particularly ajwain has (11.6%) and carbohydrate of pepper was about (65.4%) and fenugreek has moderate amount of CHO (42.3%) and the protein content of ajwain was about (16.4%) and ash

(11.5%), and low concentration of fat (0.89 %) in cardamom and fiber (10.5 %) in cumin. Proximate analysis is a scientific inquiry done to partition the approximate amount of both nutrients and non-nutrients (hazardous substances) within an organic material into categories based on common chemical properties.

**Table 3:** Proximate composition of Spices

S. No.	Parameters	Cardamom (%)	Fenugreek (%)	Cumin (%)	Ajwain (%)	Pepper (%)
1.	Moisture content	8.6	7.49	7.8	11.6	5.98
2.	Total Ash	5.4	3.38	4.4	11.5	4.55
3.	Crude Fiber	5.8	5.9	10.5	4.3	6.9
4.	Crude lipid	0.89	1.2	0.9	4.8	2.4
5.	Protein	9.29	4.4	6.7	16.4	12.9
6.	Carbohydrates	43.2	42.3	44.24	47.54	65.4

Mineral content of spices were given in the Table 4 which indicated the high content of minerals. Macro minerals calcium and magnesium (14.3 & 23.8) were higher in fenugreek. The iron content of cardamom is higher than other spices (6.5). Calcium is necessary for the bone formation and it plays a role in blood cell synthesis and functions. For the normal functioning of central nervous

system and haemoglobin synthesis iron has an important role (Adebayo *et al.*, 2015) [2]. The functions of enzymes in body are mainly in association with certain minerals like Fe, Mn, Cu, Zn and Se and they participate in many activities like cell metabolism, anti-oxidant effect, preventing cardiovascular diseases, diabetes and regulating immune responses (Centra and Dayton, 1982) [6]. Along with phytochemicals,

the trace elements also associated in the therapeutic action of medicinal plants. Mg is required to prevent muscular cramps and rigidity. Calcium and phosphorus required for bone formation and in blood coagulation (Abii and Onuha,

2014) [1]. Cu has a role in preventing anemia and maintaining normal healthy CNS (Akinyele and Osibanjo, 1982) [4].

**Table 4:** Mineral composition of spices

S. No.	Parameters	Cardamom (mg/100g)	Fenugreek (mg/100g)	Cumin (mg/100g)	Ajwain (mg/100g)	Pepper (mg/100g)
1.	Calcium	10.2	14.3	10.4	9.8	8.7
2.	Magnesium	19.2	23.8	22.4	18.4	15.6
3.	Iron	6.5	5.6	5.2	4.9	4.2
4.	Manganese	1.54	1.65	0.8	0.95	1.1
5.	Copper	0.08	0.06	0.09	0.2	0.25

### Conclusion

The spices have been screened for phytochemical constituents seemed to have the potential to act as a source of useful drugs and also to improve the health status of the consumers as a result of the presence of various compounds that are vital for good health.

### Acknowledgment

We would like to express our sincere and heartfelt thanks to our beloved Correspondent Dr. V.Dhivaharan, M.Sc., D E M., Ph.D., Department of Life Sciences, S.T.E.T Women's College, Sundarakkottai, Mannargudi, for his encouragement and for providing adequate facilities to complete the project work successfully.

### References

- Abii TA, Onuha EN. A preliminary investigation in to the phytochemicals, vitamins and mineral constituents of the leaf of two tradomedicinal plants – *Urena lobata* and *Cassia alata* used in Nigeria. *IOSR Journal of Applied Chemistry*,2014;7(2):01-04.
- Adebayo OR, Efunwole OO, Raimi MM, Oyekanmi A M, Onalapo IO. Proximate, Mineral element, anti-bacterial activity and Phytochemical screening of *Bombacum* leaves (*Calotropis procera*). *International Journal of Contemporary Applied Sciences*,2015;2(9):40-51.
- Adeyemi MMH. A review of secondary metabolites from plant materials for post harvesting. *International Journal of Pure and Applied Science and Technology*,2011;6:94-102.
- Akinyele IO, Osibanjo O. Level of some trace elements in Hospital diet. *Food Chemistry*,1982;9:81-84.
- Benedec D, Vlase L, Oniga I, Mot AC, Damian G, Hanganu D. Polyphenolic composition, antioxidant and antibacterial activities for two Romanian subspecies of *Achillea distans* Waldst. et Kit. ex Wild. *Molecules*,2013;18:8725-8739.
- Centra RK, Dayton DH. Trace element regulation of immunity and infection. *Nutrition Research*,1982;2:721-733.
- Charalampos P, Konstantina L, Olga KM, Panagiotis Z, Vassileia JS. Antioxidant capacity of selected plant extracts and their essential oils. *Antioxidants*,2013;2:11-22.
- Cunniff P. Association of Official Analytical Chemists. Official methods of analysis of AOAC international, Washington, DC: Association of Official Analytical Chemists, 1995.
- Dolui AK, Segupta R. Antihyperglycemic effect of different extracts of leaves of *Cajanus cajan* HPLC profile of the active extracts. *Asian Journal of Pharmaceutical and Clinical Research*,2012;5:116-119.
- Gornall AG, Bardawill CJ, David MN. "Determination of serum protein by means biuret reaction." *J Bio Chem*,1949;177(2):751-766.
- Harborne JB. *Phytochemical methods - a guide to modern techniques of plant analysis*. 2nd ed. London: Chapman and Hall, 1984, 4-16.
- Iyer A, Panchal S, Poudyal H, Brown L. Potential health benefits of Indian spices in the symptoms of the metabolic syndrome: a review. *Indian Journal of Biochemistry and Biophysics*,2009;46:467-481.
- Jia Z, Tang M, Wu J. The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. *Food Chem*,1999;64(4):555-559.
- Kaefer CM, Milner JA. Herbs and spices in cancer prevention and treatment. Chapter 17. In: Benzie, I. F. F, Wachtel-Galor, S. (eds.) *Herbal Medicine: Biomolecular and Clinical Aspects*. CRS Press/Taylor and Francis, Boca Raton, FL, 2011.
- Klein BP, Perry AK. Ascorbic acid and vitamin A activity in selected vegetables from different geographical areas of the United States. *J Food Sci*,1982;47:941-945.
- Kochhar KP. Dietary spices in health and diseases (II). *Indian Journal of Physiology and Pharmacology*,2008;52:327-354.
- Krishnaswamy K. Traditional Indian spices and their health significance. *Asia Pacific Journal of Clinical Nutrition*,2008;17(Suppl)1:265-268.
- Linhart YB, Gauthier P, Keefover-Ring K, Thonpson JD. Variable phytotoxic effects of *Thymus vulgaris* (Lamiaceae) terpenes on associated species. *International Journal Plant Science*,2015;176:20-30.
- Lobo V, Patil A, Phatak A, Chandra N. Free radicals, antioxidants and functional foods: impact on human health. *Pharmacognosy Reviews*,2010;4:118-126.
- Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent, *Journal of Biological Chemistry*,1951;193(1):265-75.
- Makkar HP, Siddhuraju P, Becker K. *Methods in molecular biology: plant secondary metabolites*. Totowa: Human Press, 2007, 93-100.
- Mann A. Biopotency role of culinary spices and herbs and their chemical constituents in health and commonly used spices in Nigerian dishes and snacks. *African Journal of Food Science*,2011;5:111-124.
- Narender PD, Ganga R, Sambasiva E, Mallikarjuna T, Praneeth VS. Quantification of phytochemical constituents and in vitro antioxidant activity of *Mesua*

- ferrea leaves. Asian Pac J Trop Biomed,2012.:2(Suppl 2):S539-S542.
24. Newman DJ, Cragg GM. Natural products as sources of new drugs over the 30 years from 1981 to 2010. Journal of Natural Products,2012:75:311-335.
  25. Obadoni BO, Ochuko PO. Phytochemical studies and comparative efficacy of the crude extracts of some homeostatic plants in Edo and Delta States of Nigeria. Glob J Pure Appl Sci,2001:8(2):203-208.
  26. Opara EI, Chohan M. Culinary herbs and spices: their bioactive properties, the contribution of polyphenols and the challenges in deducing their true health benefits. International Journal of Molecular Sciences,2014:15:19183-19202.
  27. Salami S, Non D. Delamination of Trace Elements in water Lily and Lettuce. Journal of Chemical Society of Nigeria,2002:27(1):92-94.
  28. Siddhuraju P, Becker K. Antioxidant properties of various solvent extracts of total phenolic constituents from three different agroclimatic origins of drumstick tree (*Moringa oleifera* Lam.) leaves. J Agric Food Chem,2003:51(8):2144-2155.
  29. Siddhuraju P, Manian S. The antioxidant activity and free radical scavenging capacity of dietary phenolic extracts from horse gram (*Macrotyloma uniflorum* (Lam.) Verdc.) seeds. Food Chem,2007:105(3):950-958.
  30. Sigh S. from exotic spice to modern drug? Cell,2007:130:765-768.
  31. Singletary K. Thyme: history, applications, and overview of potential health benefits. Nutrition Today,2016:51:40-49.
  32. Tapsell LC. Health benefits of herbs and spices: the past, the present, the future. The Medical Journal of Australia,2006:185:S4-S24.
  33. Trease G, Evans SM. Pharmacognosy. 15th ed. London: Bailer Tindal, 2002, 23-67.
  34. Vishnu R, Nisha R, Jamuna S, Paulsamy S. Quantification of total phenolics and flavonoids and evaluation of in vitro antioxidant properties of methanolic leaf extract of *Tarenna asiatica* - an endemic medicinal plant species of Maruthamali hills, Western Ghats, Tami Nadu. J Res Plant Sci,2013:2(2):196-204.