DOI: 10.7860/JCDR/2025/79445.21553

Original Article

Nutrition Section

Determination of Nutrient Profiles of Clove, Ajwain and Fennel Seeds: An In-vitro Study

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ABSTRACT

Introduction: Spices have long been a central part of world cuisines, particularly in India, where they are not only prized for their flavoring potential but also for their medicinal properties. Indian spices and condiments, used since ancient times to enhance food flavor, are rich in macro and micronutrients, as well as significant secondary metabolites. Their nutritional and chemical attributes yield considerable medicinal effects, including antibacterial, antioxidant, and anticancer activities, thereby enhancing their economic and therapeutic importance.

Aim: The research aims to investigate the nutritional composition, phytochemical profiles, and antioxidant potential of three widely used spices: ajwain (*Trachyspermum ammi*), clove (*Syzygium aromaticum*), and fennel (*Foeniculum vulgare*).

Materials and Methods: This research employs an in-vitro study to assess the nutritional, phytochemical, and antioxidant properties of ajwain seeds, cloves, and fennel seeds. Ajwain seeds, cloves, and fennel seeds were procured from a local market in Thiruvananthapuram, Kerala. They were dried and powdered into fine powders. Nutrient content such as moisture, ash, crude fiber, fat, protein, iron, calcium, carbohydrates, and vitamin C was determined using standard Association of Official Analytical Chemists (AOAC) procedures, including the anthrone method, solvent extraction, Kjeldahl method, titration, and ascorbic acid assay. Phytochemical assays for alkaloids, phenols, terpenoids, and flavonoids were conducted using standard solvent extraction methods with water, methanol, ethanol, chloroform, and acetone. The antioxidant activity was measured using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay, and absorbance was recorded at 517 nm to determine antioxidant potential.

Results: Clove was found to be abundant in carbohydrates (37.06 g/100 g), crude fiber (17.41 g), protein (9.9 g), calcium (146 mg), iron (9.6 mg), and vitamin C (5.7 mg), while fennel seeds also exhibited a nutrient-dense profile, significantly contributing to a balanced diet. Antioxidant assessments demonstrated significant dose-dependent activity in all three spices, with ajwain seeds achieving up to 33.13% inhibition at the highest concentration, clove reaching 31.09% inhibition at 1.0%, and fennel seeds showing strong antioxidative potential, particularly at higher doses, with 38.32% inhibition at 1.0%.

Conclusion: This research underscores that ajwain seeds, cloves, and fennel are excellent sources of valuable nutrients and bioactive phytochemicals, displaying significant, dose-related antioxidant activities. Such properties mark them as diversified dietary and therapeutically beneficial agents, possessing broad utility in nutrition, medicine, and industry.

Keywords: Antioxidant activity, Functional foods, Health promoting, Medicinal applications, Nutritional composition, Phytochemicals

INTRODUCTION

The World Health Organisation (WHO) estimated that approximately 60-80% of the global population directly depends on herbal preparations for their healthcare needs [1]. Spices have been an integral part of human diets and traditional medicine for centuries, valued for their distinctive flavor and health benefits [2]. Ajwain (*Trachyspermum ammi*) belongs to the Apiaceae family [3]. *T. ammi* has been extensively used in traditional medicine systems such as Ayurveda, Unani, and Siddha [4]. Ajwain seeds exhibit significant pharmacological properties, including antibacterial, antioxidant, and antifungal activities [5]. They are rich in bioactive compounds such as thymol and carvacrol, which contribute to their diverse pharmacological effects, including antioxidant, anti-inflammatory, antimicrobial, antihypertensive, and antidiabetic properties [6]. Its phytochemical composition, therapeutic applications, and pharmacological potential have attracted growing scientific interest [7]. Recent studies also suggest its potential in managing neurodegenerative diseases, cancer, and metabolic disorders [8].

Innovative drug delivery approaches, such as nanoencapsulation and lipid based formulations, are being explored to enhance the stability and bioavailability of Ajwain's active compounds, paving the way for broader applications in modern therapeutics. However, further research is needed to explore its pharmacokinetics, bioavailability, and clinical efficacy [9]. Ajwain seeds contain carbohydrates, fats, proteins, ash, and crude fiber, along with notable antioxidant and phenolic content [10]. Essential oil extraction using n-hexane and petroleum ether yielded thymol as the dominant compound (70-75%) [11].

Clove (*Syzygium aromaticum*) is a highly valued spice from the Myrtaceae family and is rich in eugenol [12]. This primary volatile compound is responsible for its aroma and therapeutic properties [13]. Clove buds and essential oil exhibit potent antibacterial, antifungal, antioxidant, anticancer, anti-inflammatory, and antidiabetic activities, making them valuable for food preservation and health applications [14]. Cloves alleviate toothaches, support respiratory health, aid digestion, and help regulate blood sugar levels [15]. Eugenol also serves as a key component in dental treatments, analgesics, and antimicrobial agents [16]. Clove essential oil, rich in bioactive terpenoids and flavonoids, continues to be widely used in traditional medicine [17,18].

Fennel (*Foeniculum vulgare*) is a member of the Apiaceae family and is widely used in traditional medicine for treating digestive issues, diabetes, bronchitis, chronic cough, kidney stones, and bladder diseases due to its diuretic effects [19,20]. It also aids in eye health, relieving conditions such as cataracts and conjunctivitis [21]. Highly aromatic and flavorful, fennel has long been valued in culinary

traditions and was historically served to royalty with fruit, bread, and fish dishes [22].

Phytochemical studies have identified bioactive compounds such as phenolic acids, flavonoids, stilbenes, terpenes, glycosides, hydroxycinnamic acid derivatives, aglycones, iridoid derivatives, lignans, and alkaloids, which contribute to its medicinal properties [23]. Fennel seeds contain fiber, antioxidants, and bioactive substances that contribute to their therapeutic benefits. The essential oil derived from fennel seeds contains anethole, which has antimicrobial characteristics [24,25]. Fennel seeds possess hypolipidemic and hypoglycemic properties, making them useful for obesity management. Additionally, essential oils and fatty acids can help with weight management [26,27].

Despite the extensive use of these spices in both traditional and modern medicine, thorough and comparative evaluations of their nutritional compositions are still limited [28]. Understanding their nutritional content and bioactive compounds is essential for evaluating their dietary importance, therapeutic value, and possible uses in food fortification, nutraceuticals, and pharmaceuticals. This study aims to analyse and evaluate the nutrient compositions of clove, ajwain, and fennel seeds to determine their nutritional importance. This research will provide significant insights into their prospective applications in health and nutrition, facilitating their incorporation into functional food formulations and therapeutic interventions.

MATERIALS AND METHODS

This in-vitro study was conducted at Saveetha Medical College, Chennai, Tamil Nadu, India, from September to December 2024. The research manuscript has been reviewed by the Institutional Review Board of Saveetha Medical College and Hospitals. Institutional Review Board 554/04/2025/Faculty/SRB/SMCH confirmed that this research does not involve any experiments on any human or animal subjects.

Sample Preparation

Ajwain (*Trachyspermum ammi* - local small seed variety), clove (*Syzygium aromaticum* - Zanzibar variety), and fennel (*Foeniculum vulgare* - sweet fennel variety) were purchased from a market in Thiruvananthapuram, Kerala. The ajwain seeds, clove, and fennel seeds were tested for quality and underwent a drying process to eliminate moisture before being ground into a fine powder, thereby ensuring consistency within the sample.

Nutrient Analysis

The assessment of nutrient composition involves the examination of mineral and vitamin levels through standardised analytical techniques [29]. A proximate analysis is conducted to determine the fundamental nutritional constituents of the ajwain seeds, clove, and fennel. The AOAC technique was used to analyse ash moisture, crude fiber, and iron [30]. Carbohydrates were measured using the anthrone technique, fat content was quantified using solvent extraction techniques [31], protein content was analysed using the Kjeldahl method [32], vitamin C was determined using the ascorbic acid test, and calcium was determined through titration against Potassium Permanganate (KMnO4) [33].

Phytochemical Analysis

Qualitative phytochemical analysis is a crucial step in identifying specific chemical constituents of plant materials. This analytical approach seeks to clarify the various chemical groups present in the plants, thereby improving the understanding of their potential therapeutic and nutritional benefits [34]. Among the phytochemicals identified are alkaloids, phenols, terpenoids, and flavonoids, each playing a distinct role in the plant's bioactive characteristics. The combination of qualitative and quantitative analyses provides a thorough insight into the chemical makeup and its relevance for nutritional and pharmacological applications [35]. The phytochemical analysis was conducted following standard procedures [36]. Dry or wet materials were ground into tiny particles for extraction, and a particular amount of solvent was added before shaking rapidly for 5 to 7 minutes or allowing it to sit for 24 hours. The extracts from ajwain seeds, clove, and fennel seed powders were prepared using solvents such as water, methanol, ethanol, chloroform, and acetone.

Antioxidant Analysis

Antioxidants are chemicals present in low concentrations that may postpone or prevent oxidative damage to various biomolecular components associated with various illnesses. The antioxidant effects of ajwain seed, clove, and fennel seed extracts were tested via the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging test. Extracts were assessed at five different dosages: 0.2, 0.4, 0.6, 0.8, and 1.0 mg/mL to measure free radical inhibition based on dosage. The DPPH assay serves as a prevalent method for assessing the antioxidant properties of diverse substances, particularly spices. The procedure involves creating a DPPH solution by dissolving it in a solvent, commonly methanol or ethanol, at a concentration of approximately 0.1 mM and extracting antioxidant compounds from spice samples using distilled water at 0.2-1 mg/mL. Subsequently, the DPPH solution was mixed with the spice extract, incubated in darkness, and the absorbance was measured at 517 nm using a spectrophotometer to determine the amount of unreacted DPPH. The antioxidant activity was quantified by calculating the percentage of DPPH scavenging, where a higher percentage signifies enhanced antioxidant efficacy of the spices [37].

STATISTICAL ANALYSIS

The statistical analysis for this study was conducted using Microsoft Excel.

RESULTS

[Table/Fig-1] shows the nutrient composition of ajwain seeds, clove, and fennel seeds. Ajwain seeds, clove, and fennel seeds each offer a unique nutrient profile per 100 g. In terms of ash content, both ajwain and clove contain 0.9 g, while fennel seeds have slightly less at 0.4 g. Moisture levels are highest in ajwain at 6.9 g, followed by clove at 5.8 g and fennel seeds at just 2.0%. Carbohydrate content is similar in ajwain (37.4 g) and clove (37.0 g), whereas fennel seeds have significantly lower carbohydrates at 7.2 g. Ajwain seeds are richest in protein, with 16.0 g compared to 9.9 g in clove and 1.8 g in fennel seeds. Fat content is high in ajwain (18.0 g), moderately low in clove (1.7 g), and minimal in fennel seeds (0.2 g). Clove stands out for its high crude fiber content (17.4 g), followed by ajwain (12.0 g) and fennel seeds (3.0 g). Regarding vitamin C, clove again leads with 5.7 mg, followed by ajwain (3.0 mg) and fennel seeds (2.9 mg). Iron content is highest in ajwain at 12.0 mg, followed by clove (9.6 mg) and fennel seeds (9.0 mg). Lastly, ajwain seeds provide a significant amount of calcium (992 mg), while fennel seeds offer 340 mg, and clove contains 146 mg.

Nutrients	Nutrient composition of ajwain seeds per 100 g	Nutrient composition of clove per 100 g	Nutrient composition of fennel seeds per 100 g
Ash (g)	0.9	0.9	0.4
Moisture (%)	6.9	5.8	2.0
Carbohydrates (g)	37.4	37.0	7.2
Protein (g)	16.0	9.9	1.8
Fat (g)	18.0	1.7	0.2
Crude Fibre (g)	12.0	17.4	3.0
Vitamin C (mg)	3.0	5.7	2.9
Iron (mg)	12.0	9.6	9.0
Calcium (mg)	992	146	340

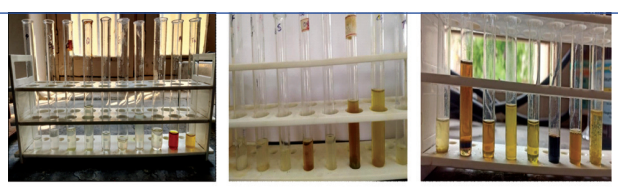
[Table/Fig-1]: Nutrient Analysis of Ajwain, Clove and Fennel Seeds

[Table/Fig-2,3] presents the phytochemical analysis of ajwain seeds using different solvents. Methanol and ethanol effectively extracted alkaloids, while flavonoids were found in chloroform and ethanol. Phenolics were present in chloroform, methanol, and acetone; saponins appeared in aqueous and acetone; and tannins were found in chloroform, methanol, and ethanol. Terpenoids were present in chloroform and ethanol, while quinones were found only in the aqueous extract. The results highlight the solvent-dependent extraction of bioactive compounds from ajwain seeds.

Phytochemicals	Solvent extract	Ajwain seeds	Clove	Fennel seeds
Alkaloids	Aqueous	-	+	+
	Chloroform	-	+	+
	Methanol	+	+	+
	Ethanol	+	+	+
	Acetone	-	-	+
Flavonoids	Aqueous	-	+	+
	Chloroform	+	-	+
	Methanol	-	+	+
	Ethanol	+	-	+
	Acetone	-	+	+
Phenol	Aqueous	-	+	+
	Chloroform	+	+	+
	Methanol	+	-	+
	Ethanol	-	-	+
	Acetone	+	-	+
Saponins	Aqueous	+	-	+
	Chloroform	-	-	+
	Methanol	-	-	+
	Ethanol	-	-	+
	Acetone	+	+	+
Tannin	Aqueous	-	+	+
	Chloroform	+	+	+
	Methanol	+	+	+
	Ethanol	+	+	+
	Acetone	-	-	+
Terpenoids	Aqueous	-	+	+
	Chloroform	+	-	+
	Methanol	-	+	+
	Ethanol	+	+	+
	Acetone	-	+	+
Quinones	Aqueous	+	-	-
	Chloroform	-	+	-
	Methanol	-	-	-
	Ethanol	-	-	-
	Acetone	-	-	-

(+) Presence and (-) Absence

[Table/Fig-2]: Phytochemicals Analysis of Ajwain, Clove and Fennel Seeds

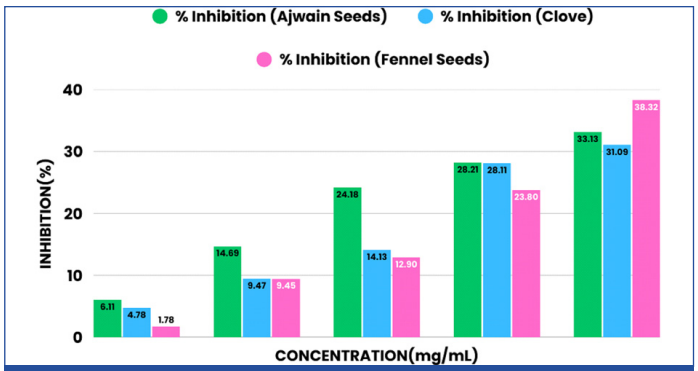


[Table/Fig-3]: Phytochemicals Analysis of Ajwain, Clove and Fennel Seeds

Clove also demonstrated solvent-dependent extraction of bioactive compounds. Alkaloids were present in all solvents except acetone; flavonoids were found in aqueous, methanol, and acetone; while phenolics were only present in aqueous and chloroform. Saponins appeared only in acetone, and tannins were absent in acetone but present in the other solvents. Terpenoids were widely extracted except with chloroform, and quinones appeared only in chloroform. This highlights the influence of solvent choice on extracting specific phytochemicals from clove.

In contrast, fennel seeds showed that all solvents extracted alkaloids, flavonoids, phenols, saponins, tannins, and terpenoids, indicating a rich bioactive profile. These compounds offer antioxidant, anti-inflammatory, and antimicrobial benefits, while quinones were absent in all extracts. Overall, fennel seeds demonstrate strong potential as a functional food with therapeutic value.

[Table/Fig-4] presents the antioxidant analysis of ajwain, clove, and fennel seeds. At the lowest concentration of 0.2 mg/mL, ajwain seeds exhibit the highest inhibition at 6.11%, followed by clove at 4.78% and fennel seeds at 1.78%. As the concentration increases to 0.4 mg/mL, ajwain continues to lead with 14.69%, while clove and fennel seeds show nearly identical inhibition at 9.47% and 9.45%, respectively. At 0.6 mg/mL, the inhibition rises to 24.18% for ajwain, 14.13% for clove, and 12.9% for fennel seeds. A notable increase is observed at 0.8 mg/mL, where ajwain and clove exhibit nearly the same inhibition levels at 28.21% and 28.11%, while fennel seeds follow at 23.8%.



[Table/Fig-4]: Antioxidant Activity of Ajwain Seeds, Clove and Fennel Seeds

Interestingly, at the highest concentration of 1.0 mg/mL, fennel seeds show a significant jump in inhibition to 38.32%, surpassing ajwain (33.13%) and clove (31.09%), indicating a potentially stronger antioxidant effect at higher concentrations.

DISCUSSION

The present study highlights the nutritional and phytochemical richness of *S. aromaticum*, *T. ammi*, and *F. vulgare* seeds, affirming their potential role as functional foods. Nutritional profiling revealed that all three seeds are valuable sources of macronutrients such as carbohydrates, proteins, fats, and dietary fiber, as well as micronutrients including calcium, iron, and vitamin C, which are essential for various physiological functions.

Nutrition refers to the ingestion and application of essential nutrients necessary for growth, development, and sustaining a healthy, productive life [38]. Inadequate sleep, imbalanced diets, and detrimental eating habits can adversely affect children's quality of life [39]. Adequate nutrition is crucial for fulfilling energy requirements and is instrumental in preserving overall health [40]. A balanced, nutritious diet serves as an effective means of preventing numerous health conditions [41].

Fennel seeds had lower carbohydrate content (7.2 g) and the lowest protein (1.8 g) and fat (0.2 g) content among the seeds studied. The moisture level was the lowest at 2.0%, and the ash content was 0.4 g, with 3.0 g of crude fiber and 2.9 mg of vitamin C content in this study. These findings contrast with those reported in a study by Ozel OT et al., who found that fennel seeds had a vitamin C content ranging from 87 to 347 mg/kg, significantly higher than the 2.9 mg observed in the present study [42]. A recent study by Kiki MJ, highlighted the antioxidant potential of fennel seeds, largely attributed to their rich phytochemical composition [43].

Fennel seeds have been shown to contain a wide range of bioactive compounds, including alkaloids, flavonoids, phenolics, saponins, tannins, and terpenoids across various solvent extracts, indicating their broad-spectrum solubility and stability. Fennel seed extracts exhibited notable antioxidant activity, with DPPH radical scavenging percentages ranging from 35.68% to 66.76%, depending on the extraction solvent used [44]. These compounds are well known for their antioxidant, anti-inflammatory, and antimicrobial properties, which contribute to fennel's role as a functional food [45].

In the present study, clove exhibited 4.78% inhibition at the lowest concentration (0.2 µg/mL). Clove contains various phytochemicals, especially in its acetone and ethanolic extracts, which have shown significant bioactivity [46]. Eugenol, known as 4-allyl-2-methoxyphenol, is an aromatic compound classified under the phenols category, specifically as a phenylpropanoid [47]. The elevated eugenol concentration is strongly associated with the demonstrated antibacterial and antiproliferative effectiveness of clove extracts, highlighting clove's potential as a functional dietary and medicinal agent. Clove extracts may be important in formulating therapies for resistant fungal strains and adjunctive cancer treatments [48].

In this study, ajwain seeds were found to contain per 100 g: protein (16.0 g), fat (18.0 g), crude fiber (12.0 g), carbohydrates (37.4g), moisture (6.9%), ash (0.9 g), vitamin C (3.0mg), iron (12.0 mg), and calcium (992 mg). A study by Arshadullah et al., (2024) reported ajwain seeds containing protein (15.4%), fat (18.1%), fiber (11.9%), carbohydrates (38.6%), moisture (8.9%), and mineral matter (7.1%), including calcium, phosphorus, and iron [14]. Ajwain seeds are known for their rich phytochemical composition, predominantly containing bioactive compounds such as thymol and carvacrol. Thymol plays a central role in the antioxidant potential of ajwain [49]. The seeds exhibit significant antioxidant activity due to the presence of these phenolic compounds, which help neutralise free radicals and reduce oxidative stress. This antioxidant capacity underpins ajwain's protective effects against neurodegenerative diseases, cancers, and metabolic disorders [50]. Recent studies have confirmed that higher concentrations of thymol, especially in essential oils extracted at elevated temperatures, correlate with enhanced hydrogen peroxide scavenging activity, emphasising the role of ajwain in modern therapeutic applications targeting oxidative damage [48,49]. Ajwain seeds exhibit high protein (16.0 g), fat (18.0 g), and calcium (992 mg) content. Comparative studies from recent literature corroborate these findings. For instance, a 2023 study reported ajwain seeds containing approximately 15.4% protein, 18.1% fat, and 1034mg calcium per 100 g, aligning closely with the current data [50,51]. In the present study, ajwain exhibited the highest inhibition (6.11%) at the lowest concentration (0.2 mg/mL). A study reported by Ishtiaque S et al., indicated that ajwain extracts exhibited significant DPPH radical scavenging activity, with inhibition percentages increasing from 66.3% at 50 µg/100 µL to 95.7% at 250 µg/100 µL, indicating strong antioxidant potential [52].

The extensive investigation of *S. aromaticum*, *T. ammi*, and *F. vulgare* seeds highlights their substantial nutritional and phytochemical potential, confirming their significance as functional foods with future medicinal uses. The existence of powerful bioactive chemicals enhances their significant antioxidant efficacy, supporting the mitigation of oxidative stress and associated chronic diseases. However, comprehensive investigations into their pharmacokinetics, bioavailability, and clinical efficacy are necessary to fully exploit their therapeutic potential and promote their incorporation into evidence-based medical and dietary practices.

Limitation(s)

While this study highlights the nutritional and phytochemical richness of *S. aromaticum*, *T. ammi*, and *F. vulgare*, several limitations should be acknowledged. The antioxidant and phytochemical analyses were primarily based on in-vitro methods, which may not fully replicate the complex biological interactions in vivo. Moreover, clinical validation and long-term safety studies are lacking, limiting the direct translation of these findings into therapeutic or dietary recommendations. Future research involving animal models and human clinical trials is necessary to substantiate these functional food claims and establish standardised formulations for practical use.

CONCLUSION(S)

In conclusion, the nutrient, phytochemical, and antioxidant profiles of ajwain seeds, clove, and fennel highlight their potential as multifunctional dietary and therapeutic agents. Nutrient analysis per 100g of ajwain, fennel seeds, and clove showed that they are rich sources of essential nutrients, particularly high in protein, crude fiber, fat, and micronutrients like calcium and iron, which are vital for overall health. The antioxidant analysis demonstrated a dose-dependent increase in free radical inhibition with the highest concentration yielding significant inhibition, emphasising their strong antioxidant properties. The phytochemical profile of these plants reveals bioactive compounds such as alkaloids, flavonoids, phenols, tannins, and terpenoids that vary in solubility across different solvents. These findings collectively affirm the status of ajwain seeds, clove, and fennel as functional foods with extensive applications in nutrition, medicine, and industry.

Authors contributions: MRS, AB: Methodology, Validation, Visualisation, Writing - original draft and Writing - review & editing. VSS, MD, MKDJ: Conceptualisation, Data curation, Formal analysis; Investigation.

Acknowledgement

We sincerely thank everyone, including our mentors, colleagues, and institution, for their support and guidance. Their contributions were invaluable to the successful completion of this study.

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PLAGIARISM CHECKING METHODS: [Jain H et al.]

- Plagiarism X-checker: Mar 26, 2025
- Manual Googling: Apr 19, 2025
- iThenticate Software: Jun 05, 2025 (5%)

ETYMOLOGY: Author Origin

EMENDATIONS: 7

AUTHOR DECLARATION:

- Financial or Other Competing Interests: None
- Was Ethics Committee Approval obtained for this study? Yes
- Was informed consent obtained from the subjects involved in the study? NA
- For any images presented appropriate consent has been obtained from the subjects. NA

Date of Submission: **Mar 19, 2025**
Date of Peer Review: **Apr 02, 2025**
Date of Acceptance: **Jun 07, 2025**
Date of Publishing: **Oct 01, 2025**