ORIGINAL ARTICLE

PHYTOCHEMICAL CHARACTERISATION AND IN VITRO ANTIOXIDANT POTENTIAL OF CAROM (*TRACHYSPERMUM AMMI* L.), A TRADITIONAL MEDICINAL PLANT

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ABSTRACT

Trachyspermum ammi L., commonly known as ajwain or carom, is an important medicinal herb in the Apiaceae family, traditionally employed as a stimulant, carminative, and treatment for ailments ranging from asthma and bronchitis to diarrhea and abdominal pain. This study sought to evaluate the diversity of key health-promoting compounds across seventeen different accessions of this plant, moving beyond agronomic traits to a biochemical focus. After cultivation, methanolic extracts were prepared from the fresh leaves of each accession to analyze their phytochemical profiles. The research specifically quantified the total phenolic and flavonoid content, two major classes of bioactive compounds renowned for their health benefits and evaluated the corresponding in vitro antioxidant potential using a DPPH free radical scavenging assay. The results demonstrated significant variation in the levels of these phytochemicals among the different accessions, with many exhibiting substantial concentrations that correlated strongly with potent, dose-dependent antioxidant activity. These findings are crucial as they provide a scientific basis for the traditional use of ajwain, directly linking its therapeutic effects to its high antioxidant content. The observed biochemical diversity underscores the plant's potential as a rich source of natural antioxidants for nutraceutical or functional food applications. Ultimately, this research highlights the importance of selective cultivation to optimise the medicinal quality of ajwain and deserves further investigation into the specific active constituents responsible for its efficacy.

Keywords *Trachyspermum ammi,* Phenolic Content, Flavonoid Content, Anti-Oxidant Potential

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INTRODUCTION

Trachyspermum ammi L., commonly known as ajwain or carom, is an annual aromatic herb indigenous to Egypt and a member of the Apiaceae family. While it is cultivated as a significant spice crop across Iran, Iraq, Afghanistan, Pakistan, and India, its value extends far beyond the culinary realm into the domain of therapeutic and nutraceutical applications. This botanical species, characterised by a somatic chromosomal number of 2n = 18 and a cross-pollination mechanism facilitated by insects, produces small, Schizocarpic fruits that are among the most potent and fragrant in the spice world, often dominating a dish's flavour profile with their sharp, bitter-herbal notes reminiscent of oregano and anise [1,2].

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The profound health significance of ajwain is fundamentally rooted in its complex and diverse phytochemical constitution. Nutritional analysis reveals a substantial composition of macronutrients, including proteins (17.1%), fats (21.1%), carbohydrates (24.6%), and dietary fibre (11.9%), alongside essential micronutrients such as calcium, phosphorus, iron, cobalt, copper, iodine, manganese, thiamine, riboflavin, and nicotinic acid [3,4]. However, its primary pharmacological activity is attributed to its rich array of secondary metabolites. Phytochemical screening has confirmed the presence of potent bioactive compounds, including tannins, glycosides, saponins, and flavones. The most therapeutically renowned component is the volatile essential oil, often referred to as ajwain oil, which is a complex mixture dominated by the monoterpene phenol thymol, which can constitute up to 50% of the oil's volume. The whole oil itself possesses demonstrated anti-aggregatory, antimicrobial, and fungicidal activities [5].

These properties provide a scientific basis for the plant's extensive history in traditional medicine systems, where it has been employed as a carminative, laxative, stomachic, and anthelmintic agent for treating a wide spectrum of ailments. These include, but are not limited to, abdominal tumours, pains, piles, bronchial problems, cough, pleurisy, atonic dyspepsia, and flatulence.

Thymol is the cornerstone of ajwain's medicinal properties, acting as a powerful germicide, antifungal, and anti-spasmodic agent. This compound is so effective that it is commercially utilised in formulations for toothpaste, mouthwashes, and perfumery due to its antimicrobial and aromatic qualities [6]. This free radical scavenging capacity is further linked to its high phenolic and flavonoid content. The plant's morphology, including the structure of its fruit with a distinct epicarp, mesocarp containing vittae (oil ducts), and endosperm filled with oil globules, is directly correlated to the production and storage of these valuable compounds [7].

Persian practitioners traditionally utilized the hydrosol and oil extracted from ajwain seeds to treat neuropathic pain, chronic fevers, and digestive issues such as gripes. These extracts were also included in medicinal formulations for managing skin conditions like pityriasis and various forms of ecchymosis (bruising). Moreover, ajwain was employed to help ease the symptoms associated with opioid withdrawal. In cosmetics, it has been used for its ability to impart a yellowish tone to the skin. Ajwain seeds possess a range of therapeutic properties, functioning as analgesics, anthelmintics, aphrodisiacs, anti-inflammatories, antioxidants, galactagogues, carminatives, laxatives, and stomachic agents. Additionally, the essential oil derived from these seeds exhibits antimicrobial, anti-aggregatory, and fungicidal effects [8].

Carom, or ajwain, holds a significant place in traditional medical systems across the Indian subcontinent. Its use is documented in classical Ayurvedic texts such as the Sushruta Samhita (where it is named *Bhootika*) and the Charaka Samhita (as *Yavanika*). For centuries, Vaidya gurus and Unani hakims have prepared formulations like *Admoda Arka*Êand prescribed the seeds to treat diverse conditions, including digestive issues (acidity, indigestion), headaches, menstrual pain, and the common cold [9-11]. This long-standing ethnomedicinal use underscores the plant's perceived therapeutic value. The medicinal properties and distinct aroma of ajwain are derived from its phytochemical constituents, primarily volatile essential oils. However, the concentration and profile of these bioactive compounds are not static; they are influenced by a multitude of factors, including soil composition, climatic conditions (temperature, humidity), and post-harvest processing techniques such as extraction duration and method. When obtained via steam distillation, the essential oil of *T. ammi* is characterised by a complex mixture of monoterpenes. Detailed analytical studies have revealed that this volatile fraction is composed of nine predominant monoterpenes, comprising seven hydrocarbons and two alcohols, which are largely

responsible for its antioxidant and pharmacological potential [12].

Phytochemical screening of Trachyspermum ammi seeds confirmed the presence of carbohydrates, glycosides, amino acids, saponins, phenolic compounds, and volatile oils, including thymol, terpinene, para-cymene, and á- and ?-pinene. The seeds are also rich in protein, fats, fibre, and minerals such as calcium, chromium, cobalt, copper, iodine, iron, manganese, phosphorus, and zinc. In addition, they provide essential vitamins and bioactive compounds like thiamine, riboflavin, ascorbic acid, nicotinic acid, and carotene [13,14]. Ajwain essential oil exhibited insecticidal effects against Callosobruchus chinensis during the oviposition stage, and also showed inhibitory activity on egg hatching and further development.

Ajwain (*Trachyspermum ammi* L.), a member of the Apiaceae family, is one of the traditional potential herbs used as a spice in daily life and is frequently used for medicinal purposes. Several illnesses that affect both people and animals. Other names for it in literature include Bishop's weed, carom, Ethiopian cumin, ajwan, and ajowan. The tiny fruit that resembles caraway is the most commonly used part of ajwain and is especially well-liked in Indian savoury recipes, savoury pastries, snacks, and as a spice [15]. By significantly reducing the food transit time, Ajowan exerted its digestive-stimulating effects [16]. The broad-spectrum bioactivity of*Trachyspermum ammi*extends to specific pharmacological targets. Notably, its ethanolic extract demonstrates antibacterial efficacy against*Helicobacter pylori*[17], a key pathogen in the pathogenesis of peptic ulcers and gastric cancer. Beyond antimicrobial effects, the plant's components exhibit significant neuromodulatory properties. For instance, thymol, a major active constituent of*T. ammi*, has been shown to inhibit key neuronal enzymes, including acetylcholinesterase, lactic dehydrogenase, succinic dehydrogenase, and cytooxidase, in the snail*Lymnaea acuminata*, explaining its observed molluscicidal toxicity [18]. Furthermore, the therapeutic potential of its extracts is underscored by their action on mammalian systems. Essential oil and various extracts of*Carum copticum*(a synonym for*T. ammi*) demonstrated potent antihistaminic effects on guinea pig tracheal chains. The rightward shift of the histamine dose-response curve suggests a competitive antagonism at histamine H1-receptors, a mechanism similar to that of the standard drug chlorpheniramine [19].

Therefore, given the established critical link between its biochemical constituents and its wide-ranging health benefits, there exists a compelling rationale for the biochemical characterization of different ajwain accessions. The present study was consequently designed to determine the diversity of*Trachyspermum ammi*across 17 distinct accessions based on both morphological and biochemical characters. The ultimate objective is to identify and select superior germplasm with enhanced concentrations of these health-promoting phytochemicals, thereby contributing to its optimised application in functional foods, nutraceuticals, and evidence-based phytomedicines.

METHODOLOGY

Seventeen accessions of*Trachyspermum ammi*L., comprising both local and exotic varieties, were obtained from the Seed Bank of the Plant Genetic Resources Institute (PGRI) at the National Agricultural Research Centre (NARC), Islamabad, Pakistan. The plants were cultivated in the experimental fields of NARC. Seeds were sown on 15 December 2022. Phenological monitoring recorded the onset of initial flowering on 16 February 2023. Agronomic parameters, including plant height and number of branches, were measured from three randomly selected plants per accession at full maturity. The total days to initial flowering and 50% flowering were also recorded for each accession. Fresh leaves were harvested from all 17 accessions at a uniform growth stage. The leaves were rinsed with distilled water to remove surface contaminants and air-dried at room temperature. Briefly, fresh leaves were manually triturated using a pestle and mortar with liquid nitrogen to create a fine powder. A measured quantity of the ground leaf material

from each accession was transferred into individually labelled test tubes. A 70% (v/v) methanol solution was added to each tube in a volume sufficient to fully immerse the plant material (approximately a 1:10 w/v ratio). The tubes were then sealed and placed on an orbital shaker at 120 rpm for 24 hours at room temperature to facilitate exhaustive extraction. Following maceration, the crude extracts were filtered through Whatman No. 1 filter paper. The filtrates were transferred to pre-weighed petri dishes and allowed to dry completely in a fume hood for 48 hours. The resulting dried extracts were carefully scraped from the petri dishes using a sterile steel spatula and stored in labelled 1.5 mL Eppendorf tubes. All prepared extracts were stored at 4°C until further phytochemical and antioxidant analysis.

Determination of Total Polyphenols

The total polyphenolic content (TPC) of the methanolic leaf extracts was determined using the Folin-Ciocalteu (F-C) method with slight modifications. Briefly, 0.05 g of each plant extract was dissolved in 5 mL of methanol. From this solution, a 500 µL aliquot was mixed with 2.5 mL of Folin-Ciocalteu reagent (diluted 1:5 with distilled water) in a test tube. After 5 minutes, 2.5 mL of a sodium bicarbonate (Na_2CO_3) solution was added. The reaction mixture was then vortexed, covered with aluminum foil to protect it from light, and incubated in a water bath at 25°C for 30 minutes. Following incubation, the absorbance of the resulting blue complex was measured at 700 nm using a UV-VIS Spectrophotometer (Lambda 5) against a prepared reagent blank. The total polyphenolic content was quantified by comparison to a gallic acid standard curve and is expressed as milligrams of gallic acid equivalents per 100 grams of dry plant mass (mg GAE/100 g DW) [20].

Determination of Total Flavonoid Content

The total flavonoid content (TFC) was determined using a colorimetric assay based on the aluminum chloride method. All reagents were prepared fresh: a 5% sodium nitrate solution, a 10% aluminum chloride solution, and a 1M sodium hydroxide (NaOH) solution. For the assay, a stock solution was prepared by dissolving 0.001 g of the plant extract in 5 mL of methanol. A 1 mL aliquot of this stock was then transferred to a 10 mL volumetric flask. To this, 4 mL of distilled water was added, followed by 0.3 mL of the 5% sodium nitrate solution. After standing for 5 minutes, 0.3 mL of the 10% aluminium chloride solution was added, and the mixture was allowed to stand for another 5 minutes. Subsequently, 2 mL of the 1M NaOH solution was added, and the total volume was made up to 10 mL with distilled water. The absorbance of the resulting pink solution was measured immediately at 510 nm using a UV-Vis spectrophotometer. The total flavonoid content was calculated from a quercetin standard calibration curve and expressed as milligrams of quercetin equivalents per gram of dry weight (mg QE/g DW) [21].

Determination of Anti-oxidant potential by DPPH Assay

The free radical scavenging activity of the extracts was evaluated using the 2, 2-diphenyl-1-picrylhydrazyl (DPPH) assay. A 0.1 mM DPPH solution was prepared by dissolving 0.012 g of DPPH in ethanol within a volumetric flask, which was then wrapped in aluminum foil to protect it from light and stored at 4°C until use. For the assay, a stock solution of each plant extract was prepared by dissolving 0.01 g of the sample in 25 mL of methanol with stirring until fully dissolved, and the beaker was covered to prevent solvent evaporation. The reaction was initiated by adding 200 µL of the extract stock solution to 3 mL of the freshly prepared DPPH solution in a test tube. The mixture was vortexed and incubated in the dark for 20 minutes at room temperature. After incubation, the absorbance was measured at 517 nm against a methanol blank using a UV-Vis spectrophotometer. The radical scavenging activity, expressed as a percentage of inhibition (I%), was calculated using the formula: $I\% = [(A_{blank} - A_{sample}) / A_{blank}] \times 100$, where A_{blank} is the absorbance of the control (DPPH solution without extract) and A_{sample} is the absorbance of the test reaction [22].

RESULTS

The phenology and morphology of the seventeen carom accessions were recorded during the growth cycle. Plants were sown on 15 December 2022, with initial flowering observed in most accessions on 16 February 2023, and 50% flowering reached by 9 March 2023. Significant morphological variation was evident among the accessions (Fig. 1a). Plant height varied considerably, with accession 20613 being the tallest at 43 cm (Fig. 1b). Furthermore, accession 21514 produced the highest number of branches at 13 per plant (Fig. 1c). Raw data for plant height and branch number for all accessions are provided in Table 1. The average values for these morphological traits, along with the average days to initial and 50% flowering, are summarized in Table 2. The descriptive statistical analysis confirmed significant morphological diversity among the seventeen *Trachyspermum ammi* accessions (Table 3). Plant height averaged 22.92 cm with substantial variation, as indicated by a standard deviation of 7.96 cm and a wide range from 11.10 cm to 43.90 cm. Similarly, the number of branches per plant showed considerable variability, with a mean of 12.62, a standard deviation of 6.02, and values spanning from 5 to 27. The distribution of both traits was positively skewed (Skewness: 1.08 for height, 0.85 for branches), indicating a concentration of accessions with lower values and a tail of higher-performing ones. Furthermore, the kurtosis value for height (1.88) suggests a distribution that is more peaked with longer tails than a normal distribution, while the branches (0.40) show a relatively flatter distribution. The high standard deviations and the nature of their distributions underscore the pronounced phenotypic variation present in the germplasm collection, highlighting its potential for selecting superior accessions for breeding programs.

Table 01: Height and Number of Branches of 17 Accessions of Ajwain

Accessions	Height			No of branches			Total days (initial flowering)	Total days (50% flowering)
	P1	P2	P3	P1	P2	P3		
20565	18.5	16.5	21.5	7	6	5	63	83
20579	20.5	24.5	21	21	9	18	63	83
20591	15.4	30	24.5	7	5	4	63	83
20613	13	16.4	14.5	6	11	9	63	83
20698	21.2	12.2	Nil	13	6	Nil	63	83
20717	23	25	14	10	18	7	63	83
20782	25	23.5	20	26	7	19	63	83
20802	11.9	16.2	8.5	7	8	8	63	83
20809	25	16.4	10	23	11	6	63	83
20850	16.5	19	19.5	9	13	10	63	83
20967	17	24	16.5	7	6	11	63	83
21116	18.5	21	24.9	11	9	17	63	83
21189	20	16.5	24	14	7	24	63	83
21227	35	33	23	29	21	9	63	83
21255	29.5	30	36	10	7	11	63	83
21468	18.5	22.4	35	19	17	26	63	83
21514	29	37	29	40	26	15	63	83

Table 02: Average Calculation of Morphological Data

Accession	Height(cm) Average	No of branches Average	Total days of flower initiation	50% flowering (total days)
20565	18.8	6	63	83
20579	22	16	63	83
20591	23.3	5	63	83
20613	43.9	9	63	83
20698	11.1	6	63	83
20717	20.6	12	63	83
20782	22.8	17.3	63	83
20802	12.2	7.6	63	83
20809	17.1	13.3	63	83
20850	18.3	10.6	63	83
20967	19.1	8	63	83
21116	21.4	12.3	63	83
21189	20.1	15	63	83
21227	30.3	19.6	63	83
21255	31.8	9.3	63	83
21468	25.3	20.6	63	83
21514	31.6	27	63	83

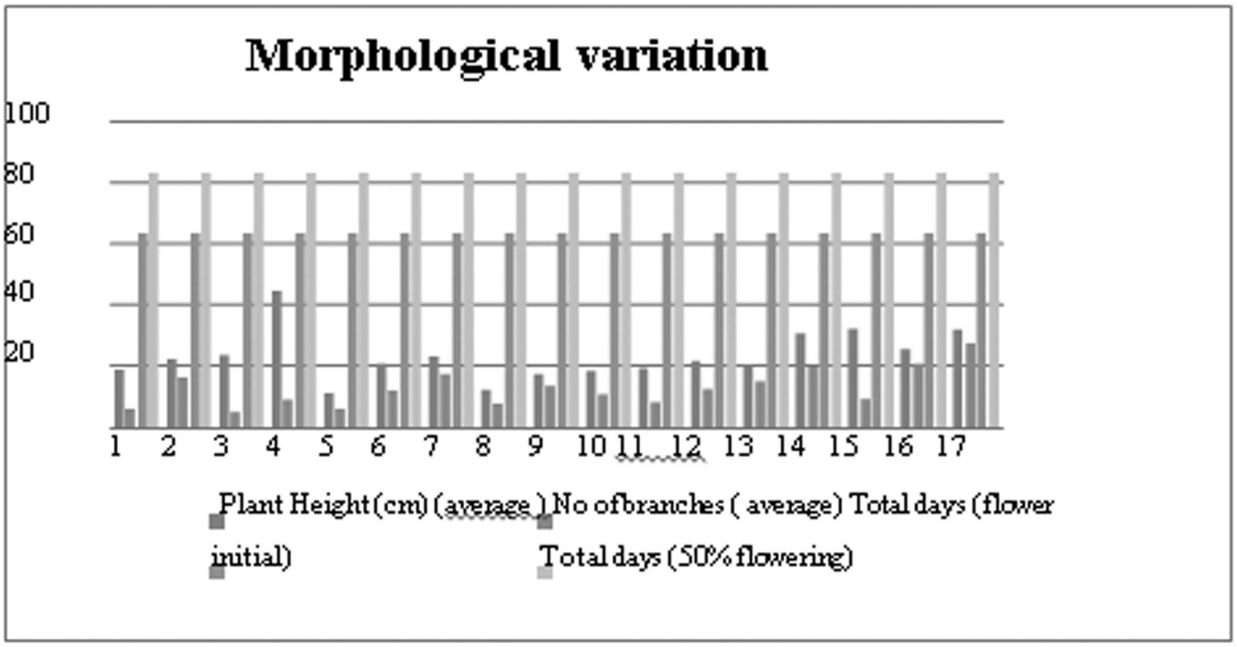

Fig. 1a: Morphological Variation Among 17 Accessions of Carom

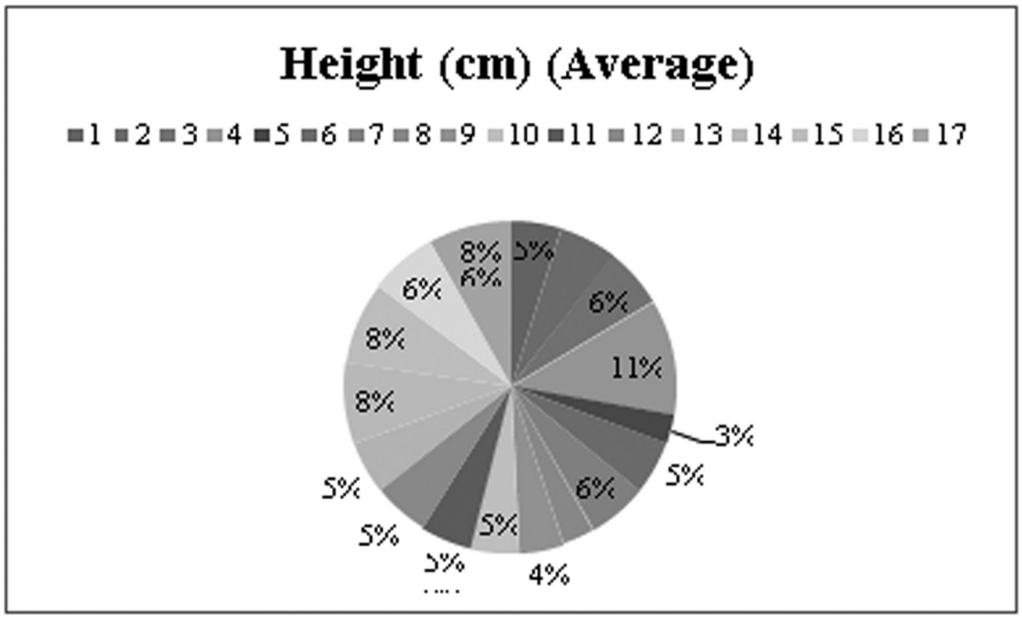

Fig. 1b: Pie Chart Showing Variation in Height Among Carom Accessions

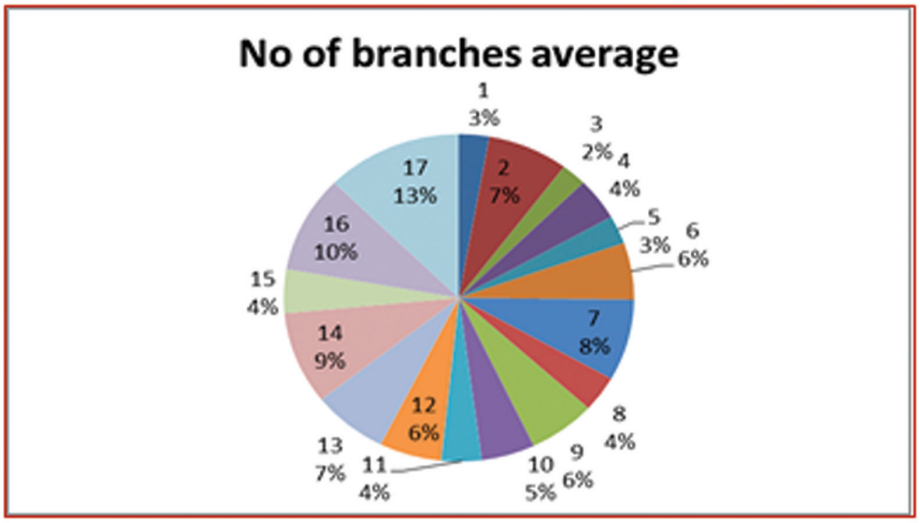

Fig. 1c: Pie Chart Showing Variation in the Average Number of Branches Among Carom Accessions

Table 03: Descriptive Statistics for the Morphological Variation in Seventeen Accessions of *Trachyspermum ammi*.

Statistic	Height (cm)	Number of Branches
Mean	22.92	12.62
Standard Error	1.93	1.46
Median	21.40	12.00
Standard Deviation	7.96	6.02
Sample Variance	63.28	36.22
Range	32.80	22.00
Minimum	11.10	5.00
Maximum	43.90	27.00
Count (n)	17	17
Confidence Level (95.0%)	4.09	3.09

Biochemical Analysis Results

Total Phenolic Content in Different Accessions of Ajwain:

The biochemical analysis revealed the presence of phenolic compounds in all seventeen accessions ofÊ*Trachyspermum ammi*. The total phenolic content (TPC), quantified using the Folin-Ciocalteu method and expressed as gallic acid equivalents per gram of dry weight (mg GAE/g DW), exhibited considerable variation across the accessions. The values ranged from 25.93 to 30.12 mg GAE/g DW. The methanolic extract of accession 20809 contained the highest TPC, while accession 20967 showed the lowest concentration. This variation is presented numerically in Table 4 and is further illustrated graphically in Fig. 2a and Fig. 2b, which clearly corroborate the superior phenolic content in accession 20809.

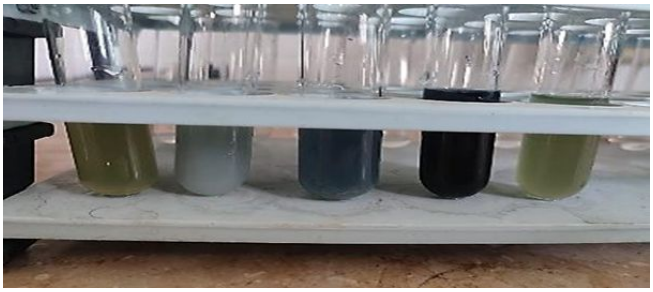

Fig. 2a: Determination of the Presence and Absence of Phenol Compounds

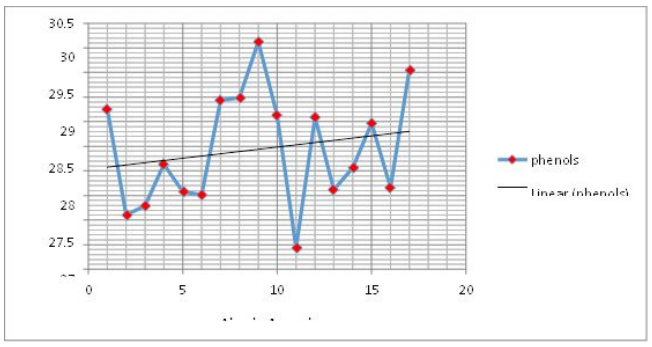

Fig.2b: Total Polyphenols in Methanolic Extracts of All 17 Ajwain Accessions

Table 04: Total Phenolic Contents of Methanolic Extracts of Different Accessions of *Trachyspermum ammi*

Accession No.	Plant part	Solvent	Total phenols (mg of GAE/g of dry weight)
20565	Leaves	Methanol	28.75
20579	Leaves	Methanol	26.60
20591	Leaves	Methanol	26.79
20613	Leaves	Methanol	27.63
20698	Leaves	Methanol	27.07
20717	Leaves	Methanol	27.01
20782	Leaves	Methanol	28.93
20802	Leaves	Methanol	28.98
20809	Leaves	Methanol	30.12
20850	Leaves	Methanol	28.63
20967	Leaves	Methanol	25.93
21116	Leaves	Methanol	28.59
21189	Leaves	Methanol	27.12
21227	Leaves	Methanol	27.56
21255	Leaves	Methanol	28.47
21468	Leaves	Methanol	27.15
21514	Leaves	Methanol	29.55

Total Flavonoid Contents

The total flavonoid content (TFC) across the seventeen ajwain accessions displayed significant variation, with values ranging from 18.45 to 252.45 mg QE/g of dry weight. Accession 21189 was found to contain the highest flavonoid concentration, while the lowest value was recorded in another accession (18.45 mg QE/g). The complete quantitative data are presented in Table 5, and the comparative variation among all accessions is graphically illustrated in Fig. 3a and Fig. 3b, which clearly confirm the superior TFC in accession 21189

Fig. 3a: Determination of Flavonoid Content in 17 Accessions of Ajwain

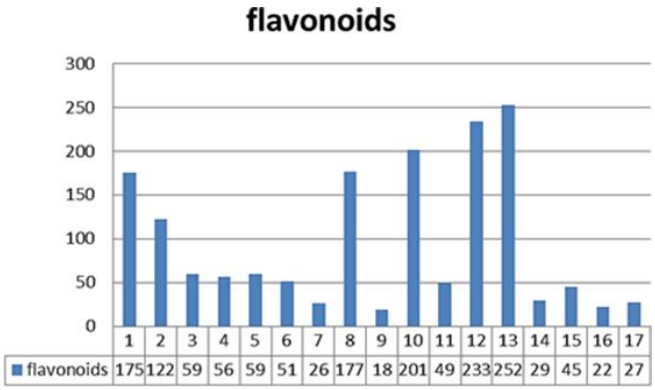

Fig. 3b: Total Flavonoid Content in Methanolic Extracts

Accession no	Plant part	Solvent	Total Flavonoid (CE/g DW)
20565	Leaves	Methanol	175.45
20579	Leaves	Methanol	122.45
20591	Leaves	Methanol	59.45
20613	Leaves	Methanol	56.45
20698	Leaves	Methanol	59.45
20717	Leaves	Methanol	51.45
20782	Leaves	Methanol	25.95
20802	Leaves	Methanol	176.95
20809	Leaves	Methanol	18.45
20850	Leaves	Methanol	201.45
20967	Leaves	Methanol	49.45
21116	Leaves	Methanol	233.45
21189	Leaves	Methanol	252.45
21227	Leaves	Methanol	29.19
21255	Leaves	Methanol	45.37
21468	Leaves	Methanol	21.53
21514	Leaves	Methanol	27.22

Table 05: Total Flavonoid Content

Antioxidant Potential

The DPPH radical scavenging assay confirmed the presence of antioxidant activity in all seventeen ajwain accessions, with considerable variation in potency (Table 6). The methanolic extract of accession 20809 exhibited the highest antioxidant activity at 55% inhibition, which aligns with its previously noted high phenolic content. In contrast, accession 20967 demonstrated the lowest activity at 12% inhibition. The full spectrum of variation in free radical scavenging potential among the accessions is presented graphically in Fig. 4.

Table 06: Total Antioxidant Potential

Accession no	Plant part	Solvent	Antioxidant potential (%)
20565	Leaves	Methanol	37.38%
20579	Leaves	Methanol	27.33%
20591	Leaves	Methanol	12.36%
20613	Leaves	Methanol	26.83%
20698	Leaves	Methanol	33.46%
20717	Leaves	Methanol	38.89%
20782	Leaves	Methanol	18.59%
20802	Leaves	Methanol	43.31%
20809	Leaves	Methanol	54.87%
20850	Leaves	Methanol	51.15%
20967	Leaves	Methanol	15.0%
21116	Leaves	Methanol	30.95%
21189	Leaves	Methanol	36.38%
21227	Leaves	Methanol	28.64%
21255	Leaves	Methanol	28.34%
21468	Leaves	Methanol	30.55%
21514	Leaves	Methanol	30.45%

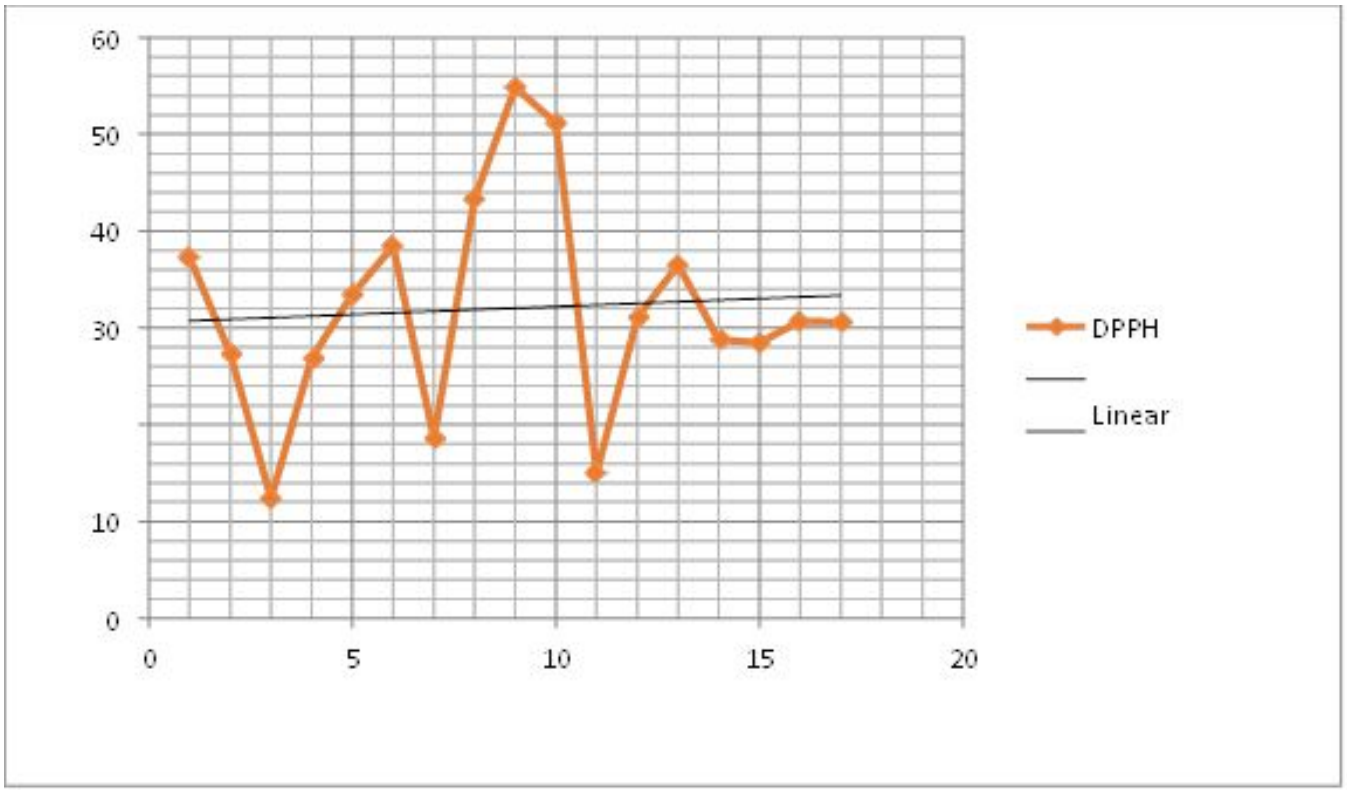

Fig. 4: Total Antioxidant Potential in Methanolic Extracts of All Accessions

A comparative analysis of the biochemical profiles revealed a pronounced relationship between phytochemical composition and antioxidant activity. Accession 20809, which exhibited the highest antioxidant potential (55%), also contained the greatest concentration of total phenolics. Interestingly, its flavonoid content was not the highest among the accessions, suggesting that its potent antioxidant capacity may be primarily driven by its polyphenolic constituents. This could indicate that the specific phenolic compounds present in this accession possess a high degree of bioactivity, or that synergistic effects between different phytochemicals are enhancing the overall antioxidant effect. In contrast, accession 21189 recorded the highest flavonoid content but demonstrated a lower antioxidant activity than accession 20809. Correlation analysis further substantiated a positive relationship between phenolic content, flavonoid content, and antioxidant activity across the germplasm (Table 7 and 8, Fig. 5), underscoring the collective contribution of these metabolites to the plant's radical scavenging potential. Future studies focusing on the isolation and identification of the specific phenolic compounds in accession 20809 are warranted to elucidate the precise mechanisms behind its superior bioactivity.

Table 07: Comparison of Total Polyphenol, Flavonoid and Antioxidant Potential Between Methanolic Extracts of Ajwain

Accession no	Phenol's	Flavonoid	Antioxidant (DPPH)
20565	28.75	175.45	37.38%
20579	26.60	122.45	27.33%
20591	26.79	59.45	12.36%
20613	27.63	56.45	26.83%
20698	27.07	59.45	33.46%
20717	27.01	51.45	38.89%
20782	28.93	25.95	18.59%
20802	28.98	176.95	43.31%
20809	30.12	18.45	54.87%
20850	28.63	201.45	51.15%
20967	25.93	49.45	15.0%
21116	28.59	233.45	30.95%
21189	27.12	252.45	36.38%
21227	27.56	29.19	28.64%
21255	28.47	45.37	28.34%
21468	27.15	21.53	30.55%
21514	29.55	27.22	30.45%

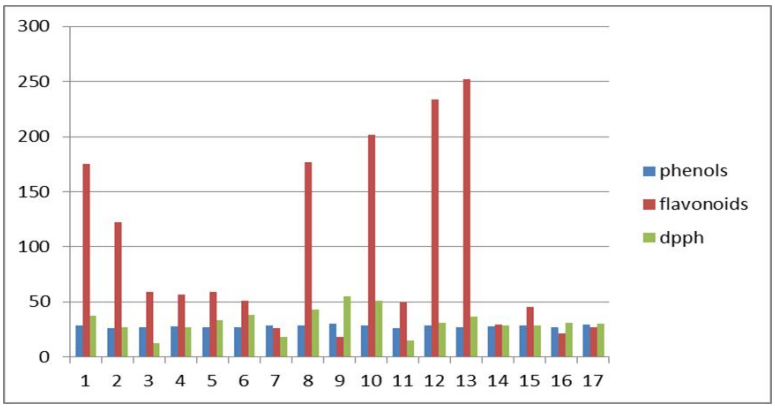

Fig. 5: Comparison of Phenol, Flavonoid and DPPH among Methanolic Extracts of Ajwain

Table 08: Correlation between Morphological and Biochemical Characters

	Phenols	*Flavonoid*	*DPPH*	*Height*	*Branches*
Phenols	1				
Flavonoid	0.05002	1			
DPPH	0.558255	0.338168	1		
Height	-0.00567	-0.36378	-0.36457	1	
Branches	0.226876	-0.26753	0.008137	0.34057	1

DISCUSSION

Trachyspermum ammi L. (ajwain) is recognized not only as a culinary spice but also as a significant reservoir of bioactive phytochemicals. These compounds, including phenolic acids, flavonoids, and other antioxidants, are increasingly associated with protective effects against a spectrum of chronic diseases, such as inflammation, diabetes, cancer, and cardiovascular disorders [23]. The present study affirms that ajwain, a member of the Apiaceae family a group renowned for its medicinal species [24] contains substantial levels of these health-promoting compounds.

Our investigation of seventeen distinct ajwain accessions revealed considerable morphological and biochemical diversity. This variation, observed in traits such as plant height and branch number, is critical from a germplasm perspective, as it indicates a rich genetic base that can be exploited in targeted breeding programs to develop superior cultivars. The biochemical analysis further demonstrated a wide variation in total phenolic and flavonoid contents among the accessions. A key finding of this study is the strong positive correlation established between the total phenolic content and the antioxidant activity, as measured by the DPPH radical scavenging assay. This relationship is mechanistically plausible, as the redox properties of phenolic compounds allow them to act as hydrogen donors and free radical quenchers [25-27]. The methanolic extract of accession 20809, which exhibited the highest phenolic content, concurrently displayed the most potent antioxidant activity, underscoring the likely contribution of these polyphenols to its radical-scavenging capacity.

In contrast, the relationship between total flavonoid content (TFC) and antioxidant activity was less straightforward. While a positive trend was observed, the correlation was not as strong as that for phenolics. This finding aligns with several previous reports that found a weak or non-significant correlation between TFC and antioxidant power [28]. A plausible explanation is that antioxidant efficacy is not merely a function of the total quantity of flavonoids, but is highly dependent on their specific structural features, such as the pattern of hydroxylation and glycosylation, which influence their redox potential [29,30]. Therefore, the specific profile of flavonoid compounds in an accession, rather than the total concentration alone, may be the determining factor for its bioactivity.

The choice of extraction solvent is another critical factor influencing the yield and composition of bioactive compounds. Our results, showing varying extraction yields for different solvents, corroborate earlier findings [31]. The polarity of the solvent selectively dissolves different classes of compounds; while water yielded the highest extract mass, medium-polarity solvents like ethyl acetate are often more effective for concentrating specific phenolic antioxidants [32-34]. The use of methanol in this study proved effective in extracting a range of antioxidant compounds, as evidenced by the

significant DPPH scavenging activity across accessions.

In conclusion, this study validates the significant phytochemical potential ofÊ*T. ammi*Êand highlights the substantial intrinsic variation present across different accessions. The identification of accessions like 20809, with high phenolic content and potent antioxidant activity, is of particular value. These findings provide a solid foundation for the future conservation and utilisation of ajwain genetic resources. Subsequent research should focus on the detailed characterisation of the specific phenolic compounds in high-performing accessions and furtherÊ*in vivo*Êstudies to fully elucidate their health-promoting mechanisms.

CONCLUSION

This study successfully characterized the morphological and biochemical diversity of seventeenÊ*Trachyspermum ammi*ÊL. accessions from a conserved seed bank. Significant variation was observed in key agronomic traits such as plant height and branching architecture, as well as in critical biochemical markers, specifically total phenolic and flavonoid content and associated antioxidant activity. A strong positive correlation was established between phenolic content and antioxidant potential, identifying accession 20809 as a particularly promising candidate due to its high phenolic levels and potent DPPH radical scavenging activity. The findings confirm that carom is a rich source of natural antioxidants, underscoring its potential for nutraceutical and functional food applications. The documented diversity underscores the value of seed bank resources for selective breeding programs aimed at enhancing the medicinal quality of ajwain. To build upon these findings, future work should employ molecular markers to elucidate the genetic basis of this variation and conduct further phytochemical analysis to identify the specific active compounds responsible for the observed bioactivity. Such advanced research will be crucial for the full exploitation and conservation of this valuable medicinal species.

Ethical Approval & Ethical Consent

This study did not involve human participants or activities posing ecological risk; hence, ethical approval was not required.

Conflict of Interest: The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Author's Contribution

FN: Conceived and designed the study, performed statistical analysis, and drafted the manuscript also conducted experiments, collected and analyzed data.

SM & RT: Supervised the study, contributed to study design and interpretation of results. Sadia Malik critically revised the manuscript, approved the final version, and is for correspondence throughout the publication process.

AM: Revised the manuscript critically. **EF**: Assisted in manuscript revision, and approved the final version.

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