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Evaluation of the Anti-Oxidant Activity and Screening of Secondary Metabolites Using the FTIR Technique in a Methanolic Seed Extract of Ajwain seeds (Trachyspermum ammi L.)

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Abstract:
Ajwain, or *Trachyspermum ammi*, is a spice with a lot of antioxidant power thanks to the flavonoids and polyphenols it contains in its seeds. Research has revealed that the seeds have strong free radical scavenging capabilities against many types of oxidative stress, mainly due to the presence of thymol, carvacrol, and γ-terpinene. Screening for secondary metabolites and assessing anti-oxidant activity were the goals of this FTIR study. Free alcohol, intermolecularly and intramolecularly bound alcohol, imine, oxime, ketone, or alkene, stretch phenol, and amine were all identified based on the experimental data. Also, Amin, reach your limits. The peak values for benders, =C-H alkenes, strong C-F alkyl halides, 738.74 strong =C-H alkenes, 813.96 strong =C-H alkenes, 1018.41 strong C-F alkyl halides, and 1415.75 strong C=C aromatic compounds were recorded. The fruit extract (Ethyl acetate, Ethanol, and standards) of Ajwain seeds (*Trachyspermum ammi* L.) and its antioxidant activities, including peroxynitrite, hydroxyl, and nitric oxide radical scavenging. Crude, ethyl acetate fraction, ethanol fraction, and standard extracts were among the several varieties recorded (725.08±36.07, 617.00±30.41) and Gallic acid (standard) (862.00±38.07) respectively of Peroxynitrite scavenging. While recorded (315.00±28.09, 223.00±21.07), and Mannitol (standard) (618.09±29.14) respectively Hydroxyl radical scavenging potential. At the same time record (48.00±3.04, 21.89±2.05), and Curcumin (standard) (78.00±4.96) respectively Nitric oxide radical scavenging potential. Findings from this study point to *Trachyspermum ammi* L. as a potential plant source of active chemicals with medicinal use for the treatment of certain fungal diseases.

Keywords: Anti-Oxidant Activity, Secondary Metabolites, FTIR, *Trachyspermum ammi* L.

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INTRODUCTION

As a traditional folk cure and a flavoring ingredient used widely in many different industries, *Trachyspermum ammi* (Apiaceae family) plays an important role in pharmaceutics and the food industry. The plant possesses a number of useful qualities, including as anti-spasmodic, carminative, and stimulating effects. Traditional medicine has long recognized its value as a remedy for a wide range of gastrointestinal issues, including but not limited to: diarrhea, piles, atonic dyspepsia, abdominal tumors, bronchial difficulties, asthma, galactagogue, and amenorrhea [1-3]. For centuries, Persian healers have used an ointment made from *T. ammi* seeds to soothe ear infections and improve hearing loss. There is some evidence that *T. ammi* can help with coughing, pleurisy, and dysphonia in the field of respiratory medicine. The gastrointestinal system (including symptoms including nausea, vomiting, reflux, stomach cramps, and loss of appetite) and the liver and spleen were common targets for fruit administration. Studies in modern pharmacology have shown that this plant has a wide range of biological activities, including anti-fungal, anti-microbial, antioxidant, cytotoxic, abortifacient, antitussive [4], anthelmintic, anti-nociceptive, hypolipidemic, anti-hypertensive, anti-spasmodic, broncho-dilating action, anti-lithiasis [5], diuretic, and anti-filarial properties [6]. It is widely acknowledged that medicinal plants and their phytochemicals, particularly essential oils, have the ability to neutralize biological oxidants and radicals. These include superoxide anion, hydrogen peroxide, hydroxyl radical, nitric oxide, peroxynitrite, and lipid peroxides [7-9], as well as thiobarbituric acid reactive substances and their byproducts. Oxidative stress and cell damage can result from these extremely reactive radicals and oxidants modifying lipids, sugar, nucleic acids, proteins, enzymes, and metabolites [10]. It appears that a shift in the balance between oxidants and antioxidants, along with the cell damage that these unstable free radicals cause, is a key factor in aging and pathological degenerative diseases like cancer, heart disease, cataracts, immunodeficiency, brain dysfunction, and depression. An effective strategy for protecting cells from oxidative damage is to use natural antioxidants, which neutralize and deactivate oxidants and radicals [11, 12]. Atherosclerosis, cancer, diabetes mellitus, Parkinson's disease, immune dysfunction, and aging are all triggered by oxidative stress, which is caused by an imbalance in the antioxidant defence mechanisms and an overproduction of free radicals. By scavenging and stimulating the breakdown and suppression of such a disease, antioxidants—whether synthesized or found naturally—can be useful in preventing free radical production. Because of concerns that they may cause cancer, synthetic antioxidants including tert butylhydroquinone, butylated hydroxyanisole, and butylated hydroxytoluene are discouraged from being used in food [13-15]. Because of the restrictions imposed on synthetic antioxidants due to their carcinogenicity, there has been a surge in interest in natural antioxidant resources that are provided to humans and animals through diet or as targeted pharmaceuticals. The phenolic compounds found in plants, including flavonoids, phenolic acids, and tocopherols, are natural antioxidants that are gaining popularity in the food and medicine industries due to their perceived protective effects against harmful oxidation-induced damage and their relatively safe status. The phenolic chemicals, found in a wide variety of foods including fruits, vegetables, and tea, are thought to be primarily responsible for the antioxidant capacity of many plants, according to epidemiological and in vitro research on medicinal plants and vegetables [16-18]. The majority of current research on antifungal medications targets either synthetic or naturally occurring plant compounds. Research on traditional medicine focuses on characterizing the antifungal activity of various plants and herbs since their extracts have biological action in vitro and in vivo. When it comes to primary care, traditional medicine has long been the most practical and affordable option, especially in areas where access to contemporary medications is limited [19, 20]. People in poor countries have long-established practices that include the use of medicinal plants as part of their traditional medicine. Furthermore, as science and technology advance, more and more research are focusing on the phyto-active components of plants, what they do, and the healing effects they might have. Isolated plant compounds are supplied in a variety of pharmaceutical dosage forms and packaging in compliance with the pharmacopoeia. The Malvaceae family stands out among many others among leafy plants because of the high concentration of polyprenols, which serve as chemotaxonomic markers [21]. Furthermore, they are rich in cyclopropane acids, which are absent in plants belonging to other families.

Materials and Methods

Collecting Samples

Gathering botanical samples, the seeds of ajwain were bought at a nearby bazaar. Hydro distillation was employed with Clevenger's apparatus to extract the Ajwain seeds (*Trachyspermum ammi* L.). The 50 g of plant material was ground into a powder and then mixed with 250 ml of sterile water in 1 L of flasks and kept at 70°C. The oil was separated after three hours, dried using anhydrous sodium sulfate, and stored in screw-capped vials at 4°C until needed.

Artemisia annua FTIR analysis

These FTIR spectra for each GLV were obtained by experimentally running and laboratory processing a large amount of data already collected from an FTIR instrument, primarily using PC-based software. It was actually done so that the FTIR spectra of these two kinds of GLVs could be recorded. To set up the experiment for FTIR measurement, a tiny quantity of crushed leaf samples was turned into pellets using KBr. Simultaneously, a thin layer was physically compressed from the combination under study. Simultaneously, data was gathered in the wave number range of 4000 cm−1 to 500 cm−1 in order to gather reliable and researched information regarding the transmission of infrared light. Three independent tests were performed on all experimental samples in this case, with untreated KBr pellets acting as a control.

Hydroxyl radical scavenging as an Antioxidant activity

2-Deoxyribose condensation with TBA yields a quantitative degradation product, which forms the basis of the experiment. The system consisting of Fe3+, ascorbate, EDTA, and H2O2 (the Fenton reaction) was used to produce hydroxyl radicals. The reactions were conducted in a 1 ml container containing the following components: 2-deoxy-2-ribose (2.8 mM), KH2PO4-KOH buffer (20 mM, pH 7.4), FeCl3 (100 µM), EDTA (100 µM), H2O2 (1.0 mM), ascorbic acid (100 µM), and different quantities (0-200 µg/ml) of the reference or test sample or sample. After a one-hour incubation at 37°C, half a milliliter of the reaction mixture was added to one milliliter of 2.8% TCA. Then, one milliliter of 1% aqueous TBA was added, and the mixture was incubated at 90°C for fifteen minutes to produce the desired color. A suitable blank solution was used to measure absorbance at 532 nm after cooling. We ran each test six times. A typical OH scavenger, mannitol, was utilized as a positive control [22, 23]. In order to determine the percentage inhibition, the test and blank solutions were compared.

Picking up free radicals of Nitric Oxide

Aqueous sodium nitroprusside (SNP) solutions produce nitric oxide, which, at physiological pH, reacts with oxygen to form nitrite ions. The Griess Illosvoy reaction can be used to measure these ions. In a final volume of 3 ml, the reaction mixture included phosphate buffered saline (pH 7.4), different dosages of the test solution (0-70 µg/ml), and 10 mM SNP. After 150 minutes of incubation at 25°C, 1 ml of the incubated solution was mixed with 1 ml of sulfanilamide (0.33% in 20% glacial acetic acid) and left to stand for 5 minutes. The mixture was then incubated at 25°C for 30 minutes after adding 1 ml of napthylethylenediamine dihydrochloride (NED) (0.1% w/v). A blank sample was used for spectrophotometric measurement at 540 nm of the pink chromophore that was produced by diazotizing nitrite ions with sulphanilamide and then coupling with NED. We ran each test six times [24, 25]. As a reference, curcumin was utilized.

Reduction of peroxynitrite

The process outlined by Beckman et al. [26] was used to manufacture peroxynitrite (ONOO). Following a brief period on an ice bath, 5 milliliters of 0.6 M KNO2 and 5 milliliters of 0.7 M H_2O_2 were combined with 5 milliliters of ice-cold water. 12.2 M NaOH was introduced. The reaction mixture was left at -20°C overnight after being treated with granular MnO2 that had been prewashed with 1.2 M NaOH to eliminate excess H_2O_2. We measured the concentration spectrophotometrically at 302 nm (ε = 1670 M-1 cm-1) after collecting the peroxynitrite solution from the top of the frozen mixture. To assess the peroxynitrite scavenging activity, an Evans Blue bleaching assay was utilized. A standard method was used to conduct the assay, with a small modification. The reaction mixture came to a final volume of 1 ml and included the following components: 50 mM phosphate buffer (pH 7.4), 0.1 mM DTPA, 90 mM NaCl, 5 mM KCl, 12.5 µM Evans Blue, different concentrations of plant extract (0-200 µg/ml), and 1 mM peroxynitrite. The absorbance was measured at 611 nm after 30 minutes of incubation at 25°C. Through comparing

the test and blank sample data, the percentage scavenging of ONOO-was computed. We ran each test six times. The substance that served as the reference was gallic acid.

Data Analysis by Statistic

Using SPSS 19.0 (IBM, New York, NY, USA) and Tukey's honestly significant differences (HSD) test, we compared the average mean values using the ANOVA analysis of variance, with a confidence interval of 95% or 99%. For statistical purposes, a p-value below 0.05 was considered significant.

RESULTS AND DISCUSSION

Ajwain seeds, scientifically known as *Trachyspermum ammi*, are a kind of fruit that looks like thyme but has a bitter, oregano-anise flavor. They are commonly fried or dry-roasted and have a long history of use as a digestive aid because of the thymol they contain. Free alcohol, intermolecularly and intramolecularly bound alcohol, imine, oxime, ketone, or alkene, stretch phenol, and amine were all identified based on the experimental data. Also, Amin, reach your limits. The peak values for benders, =C-H alkenes, strong C-F alkyl halides, 738.74 strong =C-H alkenes, 813.96 strong =C-H alkenes, 1018.41 strong C-F alkyl halides, and 1415.75 strong C=C aromatic compounds were recorded. Alcohols bound to micromolecules, free alcohols, alkanes, aromatic compounds, imines, oximes, ketone or alkene, and phenol were all detected in the laboratory analysis using FTIR. The acquired data made it quite evident that the bands in the infrared region are mostly caused by the chlorophyll molecule. The chlorophyll molecule serves to mask other parts, which is the primary source of this phenomenon.

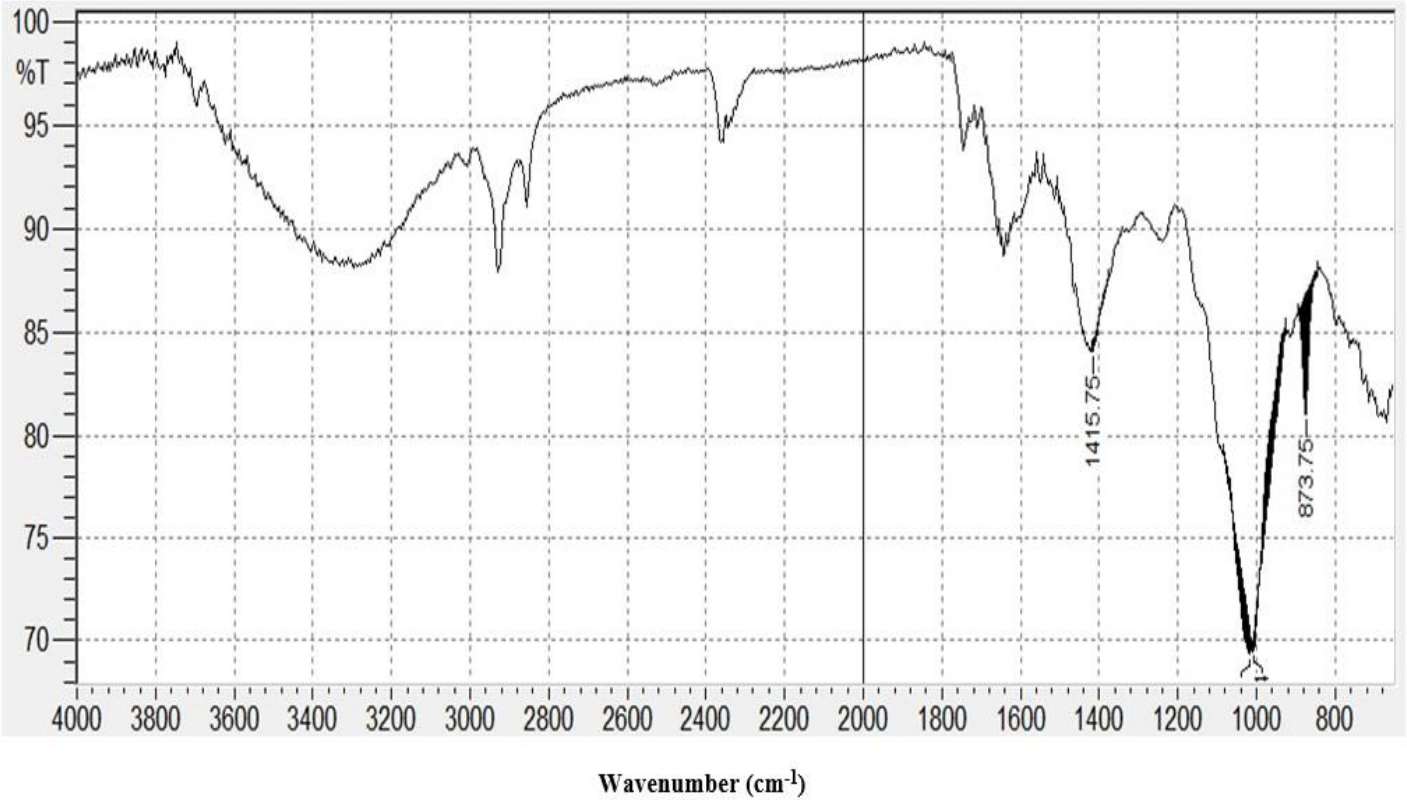

Figure 1. Fourier-transform infrared spectroscopic profile solid analysis of *Trachyspermum ammi* L

Table 1. FT-IR peak values of solid analysis of *Trachyspermum ammi* L.

No.	Peak (Wave number cm-1)	Intensity	Corr. Intensity	Base (H)	Base (L)	Area	Corr. Area	Type of Intensity	Bond	Type of Vibration	Functional group assignment	Group frequency
1.	873.75	81.078	5.786	893.04	844.82	3.396	0.505	Strong	=C–H	Bending	Alkenes	650-1000
2.	1006.84	69.585	0.730	1010.70	925.83	8.901	0.045	Strong	C-F	Stretch	alkyl halides	1000-1400
3.	1018.41	69.358	1.260	1083.99	1010.70	9.798	0.336	Strong	C-F	Stretch	alkyl halides	1000-1400
4.	1415.75	84.086	0.332	1417.68	1375.25	2.778	0.026	Medium	C=C	Stretch	Aromatic	1400-1600

Thymol, polyphenols, flavonoids, and carotenoids are the main antioxidants found in ajwain seeds (*Trachyspermum ammi*). These compounds have several potential uses, including as a food preservative, a nutraceutical, and a medicinal remedy for gastrointestinal problems, inflammation, and microbial infections [28]. The fruit extract (Ethyl acetate, Ethanol, and standards) of Ajwain seeds (*Trachyspermum ammi* L.) and its antioxidant activities, including peroxynitrite, hydroxyl, and nitric oxide radical scavenging. The documentation of various extract types includes crude, ethyl acetate fraction, ethanol fraction, and standard samples of peroxynitrite scavenging, with respective measurements of (725.08±36.07, 617.00±30.41) and Gallic acid (standard) (862.00±38.07). Hydroxyl radical scavenging potential was determined as (315.00±28.09, 223.00±21.07) for Mannitol (standard) and (618.09±29.14) for other substances. Concurrently, the Nitric oxide radical scavenging potential of Curcumin (standard) was 78.00±4.96, whereas that of record was 48.00±3.04 and 21.89±2.05.

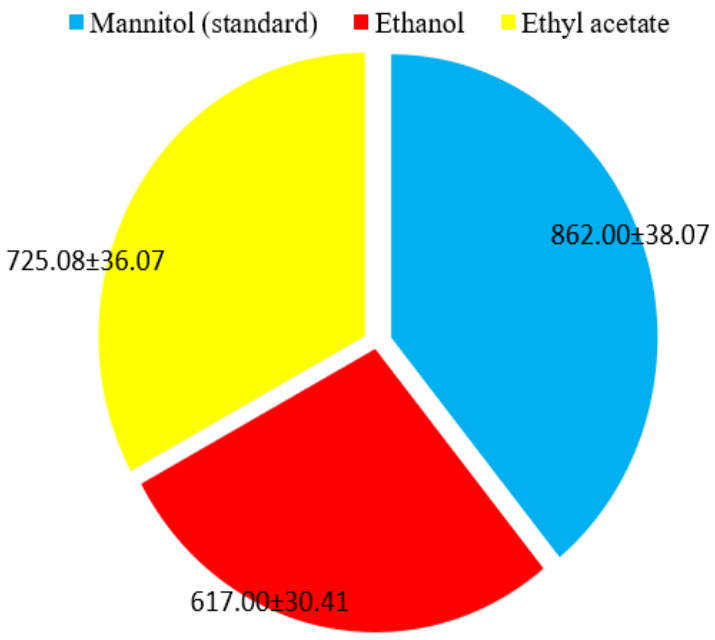

Figure 2. Antioxidant activity (Peroxynitrite scavenging) of seed extract (Ethyl acetate, Ethanol fraction and Mannitol (standard) of Ajwain seeds *(Trachyspermum ammi L.)*

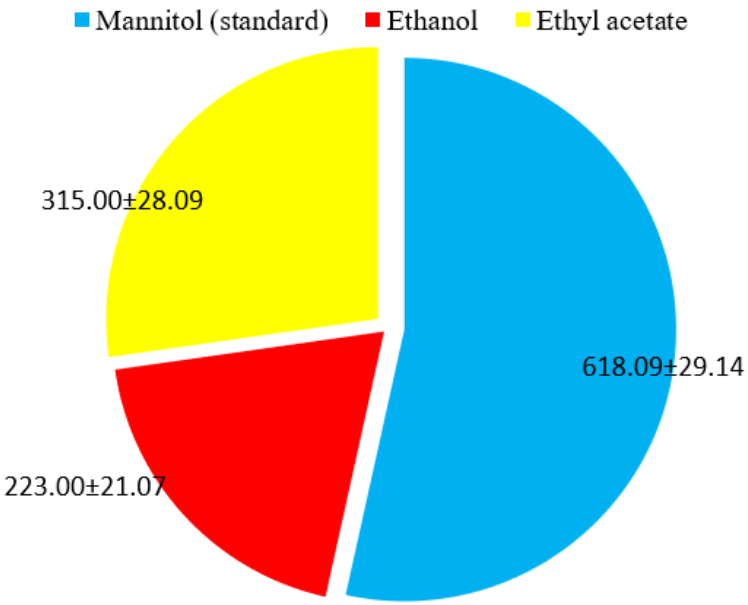

Figure 3. Antioxidant activity (Hydroxyl radical scavenging) of seed extract (Ethyl acetate, Ethanol fraction and Mannitol (standard) of Ajwain seeds *(Trachyspermum ammi L.)*

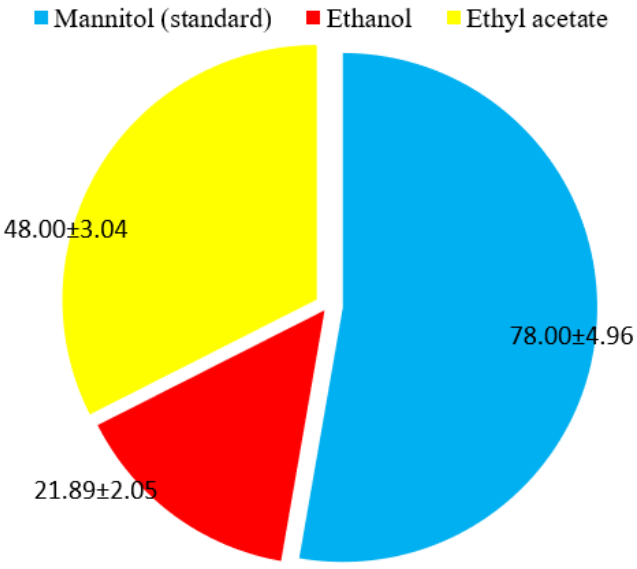

Figure 4. Antioxidant activity (Nitric oxide radical scavenging) of seed extract (Ethyl acetate, Ethanol fraction and Mannitol (standard) of Ajwain seeds *(Trachyspermum ammi L.)*

An abundance of colorful fruits, vegetables, herbs, and spices (such as oregano, rosemary, berries, spinach, etc.) contain plant antioxidants, which are natural compounds (such as flavonoids, vitamins C & E, polyphenols) that plants produce to protect themselves from environmental stress. These compounds are also vital for human health, reducing oxidative stress associated with chronic diseases. Plants utilize them to deal with stress, and people can benefit from them by eating them to fight off dangerous free radicals; various plant sources provide different kinds of antioxidants. Many environmentally friendly alternatives to traditional solvent extraction processes have been developed to enhance the efficiency of extracting antioxidant components from plant materials. These include methods that utilize enzymes to aid in the extraction process, pressurized liquid extraction, supercritical fluid extraction, high hydrostatic pressure

extraction, pulsed electric field extraction, high voltage electrical discharges extraction, and microwave-assisted extraction [29, 30]. Additionally, various evaluation assays have been developed to further assess the antioxidant capacities of natural product extracts, particularly those that people frequently consume. These assays include the following: cellular antioxidant activity assay, oxygen radical absorbance capacity (ORAC), ferric ion reducing antioxidant power (FRAP) assay, the Trolox equivalence antioxidant capacity (TEAC) assay, and many more. Use of these tests has led to recommendations for the healthiest antioxidant foods and rankings of antioxidant plants. A free radical that voluntarily seeks stability is DPPH. Hence, DPPH free radicals capture the antioxidant's hydrogen radical when they react with it. Consequently, a reduction in free radicals was noted. Compared to the extract from Alcea rosea leaves, shows a better scavenging capacity at varying concentrations of the standard in this experiment. With increasing concentrations of both the standard and leaf extracts, a higher percentage of inhibition was seen. The pharmacological activity of medicinal plants is greatly enhanced by their secondary metabolites, the majority of which are phenolic compounds. Being a prominent constituent, its redox characteristic and scavenging activities provide it high antioxidant activity [31]. More radical scavenging is likely attributable to the plant's high phenolic component content. Since galic acid is mass-equivalent to other phenolic compounds and is a major phenolic compound [32-33], it was used as a reference in this test. Flavonoid molecules, which are secondary metabolites of plants and have the antioxidant capacity of plants owing to their antiradical function, are also present [34, 35]. The results show that the plant contains flavonoids, which have anti-radical properties and are used to treat a variety of ailments.

CONCLUSION:

Ultimately, this study's findings suggest that Ajwain seeds, a kind of *Trachyspermum ammi* L., have medicinally active chemicals that may be useful in controlling some fungal diseases. Diet, lifestyle, and human diseases are closely related, according to the studies. The study's intended result is the creation of a polyherbal medication with the goal of selecting and formulating the optimal dosage for therapeutic use. To determine the effectiveness in vivo, the study can be further expanded.

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