



A Review on the Medicinal Potential of *Trachyspermum ammi* Found in Pakistan

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Numerous plants are used as a traditional medicine in herbal therapy and are well known for their effective potential in therapeutics for different ailments. Carom [*Trachyspermum ammi*] is one of the plants that are crucial in medicine. It was cultivated first time in different regions of Asia. It has been used in traditional medicine for a long time in a variety of pharmacological and medicinal aspects. It is renowned for its curative value in different miseries, especially infections. Like other fatal diseases, great advancements have been made by utilizing the therapeutic abilities of different medicinal plants regarding the cure and control of cancer progression. However still, there is a need for improvement in treatment strategies and meeting up significant deficiencies in cancer management. Carom may have some progressive role in controlling such types of ailments. Moreover, it may be anticipated as an immunotherapeutic agent and would dominate other infection-controlling agents and cancer-treating strategies due to its higher efficiency as well as biosecurity free of adverse side effects.

Keywords: Carom; *Trachyspermum ammi*; phytochemicals; cancer; nano-silver.

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1. INTRODUCTION OF CAROM

The botanical name of carom is *Trachyspermum ammi* and is commonly known as ajwain [1,2]. It is related to the botanical family named *Apiaceae* (*Umbelliferae*). Members of the family *Umbelliferae* are known as *Umbellifers*. They are linked with a family of carrot or parsley, including spices and herbs such as dill, caraway, anise seed, and fennel. This plant can grow upto 3 feet in height annually. Their leaves are green and narrow with light brown colored seeds. Flowers are delicate to touch [2,3]. It can be grown widely in arid or semi-arid areas where high levels of salts are present in soils [1]. It is believed to originate from the region of the Middle East. However, they are widely grown in other regions [3], including Egypt, Iran, Iraq, India, Pakistan, and Afghanistan [1,2]. For their cultivation, well-drained loam soil (pH 6.5-8.2) is ideal. They show ideal growth in colder temperatures (15-25°C). Direct or indirect sunlight has no effect on the growth of the plant, but appropriate moisture content in the air is necessary. They are harvested in late winter or early spring. They are also used in different food items as a spice and displayed medicinal value. Seeds of plants are pungent in smell and aromatic in taste [3,4].

1.1 Chemical Composition of Carom

The gas chromatography analyses via flame ionization detector (GC-FID) and mass spectrometry (GC-MS) were performed on essential oil of Iranian *T. ammi*, which constituted thymol and non-thymol fractions such as γ -terpinene, α - & β -pinenes, dipentene, α -terpinene, and carvacrol and para-cymene. Analysis of ajwain seed revealed the presence of fiber (11.9%), mineral matter (7.1%), flavone, saponins, glycosides, tannins, moisture (8.9%), carbohydrates (38.6%), fat (18.1%), and proteins (15.4%). In spite of them, slight amounts of α -3-carene, myrcene, and camphene are also found in the plant. Fruits contain glucoside and 6-O- β -glucopyranosyloxythymol. Dillapiole (9%), limonene (38%), and carvone (46%) are also found in its oil [1,4].

1.2 Traditional Uses of Carom

Carom has been used commonly as an Ayurvedic as well as Persian medicine traditionally. The essential oil extracted from the carom seeds is utilized in Afghanistan, India, Iran, Iraq, and Pakistan as a food flavor, perfumery, and preservative as well as in

preparing curry, a traditional dish. This oil also possesses certain therapeutic applications such as treatment for bronchial problems, gastrointestinal ailments, and lack of appetite. Roots are used for diuretic purposes and seeds for aphrodisiac properties. 2-4.4% brown-colored oil is found in its seeds. Its oil contains thymol (35-60%) which is used for the treatment of bronchial problems, lack of appetite, and other gastrointestinal ailments. Thymol is also used in perfumery and toothpaste. Despite them, oil also exhibited anti-aggregatory and anti-microbial effects in humans. Its fruit displayed carminative, antispasmodic, and stimulant properties. Traditionally, it was used for various ailments of animals as well [1,4].

2. PHARMACOLOGICAL ACTIVITIES OF CAROM

Ramaswamy et al. reported that bioactive phytochemicals present within herbs and spices play a vital role in providing protection from chronic diseases. Three spices, ginger, cinnamon, and ajwain were evaluated to see if they had a synergistic effect on cancer cell lines. The qualitative and quantitative analyses of the extracted spices from their powdered forms in equal proportions were performed. The assessment of synergistic effects of these extracts on human lung cancer cell lines was made based upon different cell cytotoxicity and viability assays. It was revealed by the phytochemical screening that these spices constitute various contents like alkaloids, anthraquinones, carbohydrates, cardiac glycosides, coumarins, flavonoids, phlobotannins, saponins, steroids, tannins, and terpenoids. The calculated total phenolic content (TPC), tannin and antioxidant contents were 0.00693g%, 0.0072g% and 2.42% respectively. The results of the cell viability test have shown that the optimum effective concentration for inhibition of cancer cell proliferation ranges from 25-50 $\mu\text{g mL}^{-1}$. Whereas the increase in concentration from 50 $\mu\text{g mL}^{-1}$ had not shown any concomitant incentive in the inhibition process [5,6].

Carom seeds are comprised of volatile oil constituents that are rich in phenolic compounds present in acetone and methanol extracts which act as natural antioxidants such as thymol, butylated hydroxyl anisole (BHA), ellagic acid, and butylated hydroxyl toluene (BHT) [5,7]. The anti-cancer or anti-tumor activity of these phenolics has been demonstrated due to their

antioxidant mode of action against cancerous cell lines [8].

The anti-microbial and antioxidant activities, induction of lymphocyte proliferation, and cytotoxicity of tumor cells are the major known biological effects reported by its essential oil. The new target for the development of novel antibiotics, *i.e.*, inhibition of Nicotinate mononucleotide adenylyltransferase [NadD] has also been evaluated. Agar disc diffusion method was used for the measurement of anti-microbial effects of essential oil of carom, which were found to be relevant with the inhibition zones, *i.e.*, higher as compared to reference antibiotics, more specifically on *Candida albicans* and *Staphylococcus aureus* (54.3 mm and 34.7 mm respectively). The observed effect was independent of any involvement of the NadD enzymatic inhibition process. The inhibition on radical cation (ABTS⁺) was ajwain oil dose-dependent and had an C_{50} value of $22.4 \mu\text{g mL}^{-1}$. It was revealed by the cell proliferation (MTT) assay that essential oil of ajwain possesses specifically cytotoxic effects on carcinoma cells and exhibited an C_{50} value of $9.6 \mu\text{g mL}^{-1}$. While peripheral blood mononuclear cells (PBMC) proliferation assay has indicated the involvement of ajwain oil in cellular network interacting immune system [9].

Goswami and Chatterjee examined the traditional spices, *i.e.*, *Foeniculum vulgare* Mill. (Fennel) and *T. ammi* L. (Carom) for finding out their free radical scavenging potential along with the preventive activity against DNA damage via oxidative stress, which usually leads to various degenerative diseases. Acetonic, aqueous, and methanolic extracts of the seeds of these spices were prepared with the help of Soxhlet extraction assembly, and their phytochemical constituents were estimated quantitatively as well as qualitatively. The potential of free radical scavenging was evaluated by using standard methods such as ferric-reducing antioxidant power (FRAP) assay and DPPH radical scavenging assay. The outcomes obtained by acetonic seed extracts of both spices have revealed their relatively high total phenolic contents (TPCs) than that of the methanolic seed extracts, where total flavonoids contents (TFCs) were found to be highest. The highest activity of DPPH radical scavenging was obtained by 1 mg mL^{-1} concentration of methanolic seed extract of *F. vulgare*, and acetonic seed extract of *T. ammi* have shown the highest FRAP value of $2270.27 \pm 0.005 \mu\text{mol L}^{-1}$ [10].

Hassanshahian et al. evaluated the anti-microbial potential of the essential volatile oil from *T. ammi* against different microbes through the microtiter plate method. Thirty-six isolates of *Escherichia coli*, *Klebsiella pneumoniae*, and *Staphylococcus aureus* (12 each) were extracted from the urine culture samples of patients having urinary tract infections. The carom oil was obtained by the hydro-distillation method, and the minimal inhibitory concentrations were found out for the characterization of anti-microbial effects of this essential oil. The results obtained from *E. coli* have shown resistance against four different antibiotics viz cefixime (41.6%), ceftazidime (50%), erythromycin (58.3%), and tetracycline (75%). While isolates of *K. pneumoniae* showed resistance against three antibiotic agents involving cefixime, ceftazidime, and erythromycin, *i.e.*, upto 58.3%, 33.3%, and 75% respectively and isolates of *S. aureus* were found resistant to six antibiotics such as cefixime (33.3%), ceftazidime (66.6%), oxacillin (3.3%), penicillin (50%), trimethoprim-sulfamethoxazole (41.6%) and vancomycin (8.3%). The minimum inhibitory concentration (MIC) value of all the bacteria that were tested was also determined, and the highest MIC values obtained from the essential oil of *T. ammi* against *E. coli* and *K. pneumoniae* were 100 ppm and 250 ppm, respectively. Hence, the inhibition of bacterial growths was reported by the anti-microbial activity of essential oil extracted from *T. ammi* [11].

Vijayaraghavan et al. established a unique biosynthesis method to prepare nanoparticles of silver (Ag-NPs) by utilizing aqueous extracts from *Papaver somniferum* and *T. ammi*. P-cymene, α -terpinene and thymol are the major constituents of *T. ammi* while codeine and morphine are significant ingredients of *P. somniferum*. In order to form biocompatible silver nanoparticles (Ag-NPs), essential oil from *T. ammi* has been proved as a better reducing agent than alkaloids of *P. somniferum*. The effectiveness of both of these extracts was evaluated by using same extract dosage while synthesizing silver nanoparticles. It was concluded that the triangular-shaped nanoparticles of variable size ranging from 87 nm to 998 nm were synthesized by *T. ammi* while the same dosage of *P. somniferum* extracts had synthesized $3.2 \mu\text{m}$ to $7.6 \mu\text{m}$ of spherical shaped particles [12].

Kaur and Arora conducted an experiment for the screening of antibacterial properties as well as phytochemicals present within the aqueous seed

extracts of *Anethum graveolens* Linn., *Foeniculum vulgare* Mill., and *T. ammi* L. in order to provide a scientific basis to these traditional plants being used for treating ailments. MIC, viable cell count studies, and agar diffusion assay helped in the assessment of aqueous as well as organic seed extracts. Later on, the obtained results of antibacterial activity were compared with the effect of some standard antibiotics, which were found relatively better than the antibiotics. The best antibacterial activity was observed by acetone and hot water seed extracts against all the bacteria other than a strain of *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*. The MIC required from aqueous and acetone seed extracts varied from 20 to 80 mg mL⁻¹ and 5 to 15 mg mL⁻¹, respectively. The presence of alkaloids (2.80 to 4.23%), flavonoids (8.58 to 15.06%), saponins or cardiac glycosides (0.55 to 0.70%), and tannins (19.71 to 27.77%) were analyzed during the screening of phytochemicals [13].

The carom oil poses certain anti-aggregatory, anti-microbial and fungicidal effects upon human health; hence, used for the cure of various bronchial as well as gastrointestinal anomalies [2,14]. Carom is also involved in the treatment of atonic dyspepsia, flatulence, diarrhea, and tumors of different types. Besides this, the carom seeds have antispasmodic, anthelmintic, carminative, laxative, stimulant, and stomachic properties as well [2,15,16]. The phenolic compounds like tannins and flavonoids are putative antioxidants and carry anti-inflammatory, anti-carcinogenic, and radical scavenger capabilities. Their presence within dietary antioxidants plays a crucial role in the prevention of cancer as well as certain other cardiovascular diseases [17].

Essential oil of *T. ammi* was analyzed through gas chromatography in order to get the major constituent, thymol, which was about 153.8% as per spectroscopic studies and was extracted out from the oil. Various other phytochemicals such as beta-sitosterol-3-O-b-D-glucoside, eicosanoic acid, linolenic acid, lupeol, and stearic acid from the ethyl acetate, hexane, and methanol extracts were obtained by the hydro-distillation process. Nuclear magnetic resonance (NMR) was used for the elucidative study of structures of these compounds by using 1D and 2D spectral methods with the help of electron ionization mass spectrometry (EI-MS), infrared (IR), and fast atom bombardment mass spectrometry (FAB-MS). The obtained outcomes were compared

with previously reported values and authentic samples. Certain chemicals were investigated for the first time in this study of *T. ammi*, which were then utilized in biological activity tests for the evaluation of thymol showing inhibitory activity in response to barnyard grass (*Echinochloa crusgalli*) and radish seeds (*Raphanus sativus*). Whereas the germination and growth of shoot, as well as root length, was completely suppressed. A slight inhibitory effect (15-33%) at the concentration of 500 ppm was also posed by lupeol against both of these plant seeds. On the contrary, no inhibition effect was observed over barnyard grass by the eicosanoic acid, linolenic acid, and stearic acid [18].

2.1 Plants as an Alternative Source for Treatment of Cancer

Plants play a crucial role in the treatment of numerous diseases and are being in use for centuries [19]. Several medicines have been developed from terrestrial plants in the past, while modern drugs have also been derived from them currently. Plants were used for the first time for medicinal purposes in 2600 BC [20]. According to the world health organization (WHO) estimation, the traditional plant-derived medicines are being consumed for treatment purposes by approximately 80% of the population [21].

Phytochemicals give aroma, color, and taste to the plant fruit; besides this, these also play a vital role in the enhancement of DNA repair mechanisms, defense against ingested carcinogens, and affecting metastasis as well as the progression of cancer. The phytochemicals combat cancer with the help of antioxidant enzymes presents naturally within the body [22].

A wide range of phytochemicals such as carotenoids, coumarins, curcumins, flavonoids, lignans, phthalides, polyphenolics, saponins, sterols, sulfides, and terpenoids have been discovered so far within different species of herbs and spices that are reported for their extraordinary pharmacological and physiological characteristics [23,24]. This treatment strategy is entirely natural and is free of all the unnecessary drawbacks, *i.e.*, mutations and metabolic aberrations. Besides this, the therapy is inexpensive and easily available to all classes of society [25].

Significant advancements regarding cure and control of cancer progression have been made, but still, there is a need for improvement in

treatment strategies and meeting up significant deficiencies as cancer is the 2nd most leading cause of death across the globe. Chemotherapy is a mode of cancer treatment that, of course, leaves a series of undesired side effects eventually. Hence, there is an extreme need for natural therapies in order to cure cancer which involves plant-derived products that tend to cause a reduction in the adverse side effects and perform much better. A few plant-derived products have been equipped for cancer treatment so far, but there is room for much more. Whereas a myriad of plant-derived products exhibiting promising anti-cancer potential has been tested *in vitro* are yet to be examined in humans. Before moving forward with their application on people, a complete comprehensive investigation is required to determine the efficacy of all of these plant compounds with anti-cancer potential [26].

Camptothecin and paclitaxel were commercially utilized for cancer treatment and occupied around 1/3rd of the global anti-cancer market [27]. Betulinic acid is one of the most common secondary metabolites of the plant taken from *Betula* and *Zisypus* species for the treatment of melanoma [28-30]. Roscovitine, another synthetic agent, has been obtained from *Raphanus sativus* (Brassicaceae) also shows an effective response against grade II cancer at clinical trials [31,32]. Similarly, flavopiridol, a secondary metabolite of medicinal plant, was derived, and the anti-cancer potential of the compound was examined for the cure. The activity of the compound was remarkable against combating certain tumors of grades I and II involving lymphomas and leukemia that are the most common of cancer types [33,34].

The antioxidants are responsible for the increment of antioxidative capacity within the plasma, which ultimately leads to the reduction of risk of various diseases like cancer hence, known as 'Potential Modifiers' of cancer [35]. Anthocyanins, catechins, coumarins, flavones, flavonoids, iso-catechins, isoflavones, lignans, and phenolics are fundamental phytochemicals for carrying out the antioxidant activity. It has been reported that the higher concentration of spices favors the lowering of cell damage as well as prevention of cancer cell growth.

2.2 Anti-cancer Potential of Carom

Breast cancer is one of the most abundant cancer types across the globe, and it accounts

for twenty-three percent of all the cancers occurring in women. Patients with this cancer type shows adverse side effects, including toxicity to the chemotherapeutic drugs. In contrast to this, the plant-derived source of anti-cancer drugs has been proved more significant. The anti-cancer potential of *Trachyspermum ammi* was analyzed by using its extract over the MCF-7 cell line [36-38]. Various phytochemicals were observed to be present within this plant extract. MTT assay of the ethanolic extract was performed for the demonstration of IC₅₀ concentration. The optimum cytotoxicity was recorded at the concentration of 25 µg mL⁻¹ *T. ammi* extract, which was considered as IC₅₀ as well. The morphological changes such as cellular viability by using fluorescent microscopy and DNA fragmentation with the help of gel electrophoresis technique. Significant apoptotic signs such as cell shrinkage, nuclear DNA fragmentation, and membrane blebbing were observed due to the activity of ethanolic extract of ajwain. Gene expression of p35 was analyzed with the help of RT-PCR, and a significant increase (P<0.001) was observed when compared with the normal MCF-7 cell line whereas, mRNA levels of anti-apoptotic Bcl-2 gene were found significantly reduced (P<0.01) as compared to normal MCF-7 cell line [37].

Abdel-Hameed et al. characterized the chemical composition of oil and n-hexane extract of *T. ammi* along with the anti-cancer and anti-microbial potential by using GC-MS analysis, sulphorhodamine method, and disc diffusion method. Around 23 mono-terpenoids were identified in volatile oil, out of which β-pinene, p-cymene, γ-terpinene, and thymol were the four major constituents with a quantity of 38.49, 194.91, 266.28, and 201.97 mg g⁻¹, respectively. In contrast, the quantity of the rest of the 19 compounds was less than 10 mg g⁻¹ of oil. Similarly, 12 mono-terpenoids were found in n-hexane extract that exhibits three major constituents, *i.e.*, p-cymene, γ-terpinene, and thymol, with a quantity of 32.69, 56.41, and 138.85 mg g⁻¹ of extract, respectively, while the other nine compounds had less than 10 mg g⁻¹ quantity. Both of these oil and extract possess anti-microbial potential against five microbes and anti-cancer potential against HepG2 cell lines. The effects observed by the essential oil were relatively higher than that of the n-hexane extracts [39]. Moreover, Abdullah et al. did the GC-MS-analysis of ajwain seed extracts and found out that the phytochemicals present in ajwain seeds possesses anti-cancer, anti-tumor,

anti-microbial, anti-inflammatory, anti-oxidant and anti-trypansomal activities [40].

National cancer institute (NCI) is working on developing certain topical as well as systemic formulations for a synthetic agent that exhibit the anti-cancer potential to confront clinical trials [32]. The antioxidant compounds, as well as enzymes, are naturally present within several plants, including the medicinal ones that play a significant role in cancer therapeutics. *T. ammi* contains both antioxidant compounds, thymol, and antioxidant enzymes, *i.e.*, glutathione reductase and superoxide dismutase. The collective impact of the activity of all these bioactive compounds favors the cure of cancerous cells [41]. The antioxidants neutralize the free radicals present in high concentrations within cancer cells due to uncontrolled and continuous cell growth.

3. PHARMACOLOGICAL ROLE OF CAROM IN TRADITIONAL AND MODERN MEDICINE

Carom or ajwain has been used in traditional medicine for along time in a variety of pharmacological and medicinal aspects [42, 43]. In traditional Persian medicine, carom is well-known for centuries. Persian practitioners called carom seeds the most useful part of the herb. Ajwain, with regards to its nature, is considered bitter, acrid, dry and hot. Oral use of seeds was reported to be useful for paralysis, tremor, palsy, and other neural disorders in the field of neurology. Persian practitioners also utilized the eye and ear drops formulated from seeds of ajwain in order to control the infection and improve auditory weakness. In the field of respiratory, ajwain was said to be effective for cough, pleurisy, and dysphonia. The fruit was widely administered for liver spleen and gastrointestinal disorders such as nausea, vomiting, reflux, abdominal cramps, and loss of appetite. They are also supposed to help with gastrointestinal issues and have carminative and stimulant qualities [2, 4, 5, 43]. It is reported as an anthelmintic medicine and also an antidote for various natural toxic agents. It is also believed to be beneficial for dissolving the calculi and stones if taken with wine. The seeds were also used as a diuretic, galactagogue, and aphrodisiac by Persian healers. In addition to treating gripes and chronic fever, Persians also make use of ajwain seeds as a fumigant to treat female genital diseases. It has also been utilized in medicine for leukoderma, ecchymosis, and pityriasis. It is

plastered with honey to cure all types of ecchymosis. In cosmetics, local administration of ajwain as a paint results in a yellowish complexion on the skin. The application of ajwain seeds decoction is reported in the field of toxicology. It is revealed that washing the scorpion's bitten area with its decoction alleviates pain. It is also suggested that its use also decreases opioid withdrawal-associated adverse effects. Ajwain was also introduced as a potent analgesic and anti-inflammatory agent. Hence, it was also applied alone or with honey or albumin (egg white) at the infected part.

The hydrosol and oil derived from ajwain seeds were also employed in medicine. The combination of ajwain hydrosol, cinnamon, and borage was widely suggested as an excellent energizing remedy powerful energizing medicine. In Persian pharmaceutical and writings, ajwain hydrosol and oil extracts have been utilized in treatments of the palsy, paralysis management, tremors, chronic and neuropathic [43].

4. CAROM AND NANOTECHNOLOGY

Nanotechnology plays a vital role in engineering and manipulating the size of particles at the nano-level ranging from approximately 1-100 nm. Its importance in various areas like biomedical science, chemical industries, cosmetics, gene and drug delivery, health care and feed, environmental science, food, energy science, electronics, mechanics, and space industries has been already established [44-46]. It also has extensively been used for the treatments of diabetes, cancer, allergy, inflammation, and infection, *etc.* In recent years the trend of the green synthesis of NPs is growing due to the number of its advantages over chemical synthesis methods such as simplicity, mild reaction conditions, and cost-effectiveness. Moreover, it is compatible with biomedical and food applications, and this technique eliminates the use of high pressure, temperature, energy, and toxic chemicals. The growing need for environmental-friendly production of nanoparticles forced researchers to choose the greenway for their fabrication [47-49].

Various metallic nanoparticles, because of their remarkable properties over their bulk counterparts, are used in a variety of applications. Photochemical reduction, chemical reduction, electrochemical reduction, heat evaporation, and other commonly used methods are not as effective as biological methods. In the

biological method, the plant extract has been used as a reducing and capping agent for the production of nanoparticles due to their reducing properties. The change in the properties of the nanoparticles, such as size, distribution, and morphology of the nanoparticles, are clearly observed with biomaterial [50]. Various biochemicals are utilized to synthesize a variety of palladium, silver, quantum dots (CdS, ZnS), gold, zinc, iron, and copper nanoparticles. Silver nanoparticles possess the properties like photoelectrochemical, anti-microbial, antibacterial, magnetic, optical, chemical, and catalytic activities. Because of their anti-microbial properties, they are utilized in medical applications like coated capsules, blood collecting vessels, biological labeling, band aids silver is non-toxic to animal cells and highly toxic to bacteria and other microorganisms (*E-coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, etc.). Therefore, nano-silver is considered a safe, effective, and valuable bactericidal metal to be used for medical purposes [51].

At present, biogenic methods are being used for the synthesis of silver nanoparticles, which are usually non-toxic, low cost, usage less amount of chemicals, environmental friendly workable at mild temperature and pressure conditions. Ajwain consists of the fatty acids, proteins, flavonoids and alkaloids that promotes antioxidant, anti-microbial, anti-inflammatory and immune stimulant activity [52,53] The current investigation focused on the synthesise of AgNPs, using the aqueous seeds extract of ajwain at different experimental conditions and their application in antibacterial activity against the *Bacillus subtilis*, *Staphylococcus aureus* and *Escherichia coli* at room temperature [54]. Work will contribute in establishing the importance of plant sources and implementing green chemistry in synthesis of nano metal particles for the future research.

5. FUTURE PROSPECTS AND CONCLUSION

Such medicinal plants are always in focus for scientists to discover their therapeutic potential for different ailments. In the near future, *T. ammi* may be anticipated as an immunotherapeutic agent and would dominate other infection-controlling agents and cancer-treating strategies due to its higher efficiency as well as biosecurity free of adverse side effects. *T. ammi* has been commonly used for its medicinal significance in

infectious diseases and cancerous conditions. As evidenced by previous studies, carom may play a curative role in these ailments and unlocked a new dimension for the development of advanced cancer therapies.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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