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ANTICANCER ACTIVITY OF OIL OF THE SEEDS OF TRACHYSPERMUM AMMI

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ABSTRACT

Trachyspermum ammi L. (Apiaceae) commonly known as ajwain is an important medicinal, aromatic and spice plant. Ajwain seeds yield 2-5% brownish essential oil, consists of *p*-cymene (37.18%), γ-terpinene (35.36%) and thymol (20.51%). Due to presence of numerous chemical constituents in ajwain, various biological and pharmacological properties have been reported. In the present invention cytotoxic activity of volatile oil of *T. ammi* was evaluated against three cancer cell lines *viz.*, Human alveolar lung adenocarcinoma cell line (A549), Human cervix adenocarcinoma cell line (HeLa) and Human gastric adenocarcinoma cell line (AGS) using MTT assay method. The cell lines were treated with different concentrations of volatile oil (12.5, 25, 50, 100 and 200 ug/ml) and the cytotoxicity assay was done by MTT assay. The volatile oil showed effective toxicity against A549, Hela and AGS cells with IC_{50} concentration of 26.78, 34.24 and 171.02 ug/ml respectively after the incubation period of 24hrs. Cisplatin with 15ug/ml was used as a standard control for the study. The results showed that the volatile oil may have satisfactory toxic potential against the tested cell lines.

INTRODUCTION

Cancer is a deadly disease that remains one of the leading causes of mortality worldwide due to uncontrolled cell proliferation, invasion, and metastasis. Despite considerable advances in biomedical research, cancer continues to pose major therapeutic challenges.[1,2] Natural products and bioactive compounds derived from medicinal plants have attracted considerable attention owing to their diverse pharmacological activities, including anticancer potential.[3,4]

In developing countries, nearly 70–80% of the population depends on traditional herbal medicines for primary healthcare needs. Herbal remedies are increasingly gaining acceptance because of their affordability, accessibility, and comparatively lower incidence of adverse effects.[5]

Trachyspermum ammi L. (Apiaceae), commonly known as ajwain, is an important medicinal and aromatic plant widely used in traditional Persian and Ayurvedic medicine. The plant is believed to have originated in Egypt and is presently cultivated in several countries including India, Iran, Iraq, Afghanistan, and Pakistan.[6,7]

The hydrodistilled essential oil obtained from *T. ammi* seeds, commonly referred to as ajwain oil, is extensively utilized as a flavoring agent, food preservative, and ingredient in perfumery. Ajwain seeds typically yield 2–5% essential oil, predominantly containing p-cymene, γ-terpinene, and thymol as major constituents.[8,9]

Due to its rich phytochemical composition, *T. ammi* exhibits a wide range of biological activities including antioxidant, antimicrobial, antifungal, anti-inflammatory, antinociceptive, antiviral, cytotoxic, antihypertensive, hypolipidemic, bronchodilatory, antispasmodic, antitussive, diuretic, anthelmintic, and antifilarial activities.[10-12]

In the present study, essential oil from *T. ammi* seeds was extracted using the conventional hydrodistillation

method employing a Clevenger apparatus, and its cytotoxic activity was evaluated against human alveolar lung adenocarcinoma (A549), human cervical adenocarcinoma (HeLa), and human gastric adenocarcinoma (AGS) cell lines using the MTT assay method.

METHODOLOGY

Chemicals and drugs

Fetal Bovine Serum (#RM10432, Himedia), Antibiotic-Antimycotic solution (100x) (Cat No: 15240096, Gibco, Invitrogen), MTT Reagent (5 mg/ml) (# 4060 Himedia) DMSO (#PHR1309, Sigma), Camptthecin (#C9911, Sigma), Cisplatin (#PHR1624, Sigma), DMSO (#TL1006, Himedia). All other chemicals and reagents used were of analytical grade.

Collections, identification and preparation of plant materials

The high quality of seeds of *T. ammi* are collected from the retail store (herbal store) at Hyderabad, Telangana, India. The collected seeds were dried and powdered coarsely for the present investigation. The grounded seed powder (100 g) and distilled water (400 mL) was mixed in a 1 L round bottom flask fitted with clavenger apparatus and then was heated for 3 h to extract the essential oil. The residual water droplets present in the extracted essential oil were removed using sodium sulphate. The oil was kept at 4°C until further analysis and the percent yield was calculated.

Cell Lines

The following cell lines were obtained from NCCS, Pune. a) A549 – Human alveolar lung adenocarcinoma cell line, b) HeLa-Human cervix adenocarcinoma cell line and c) AGS-Human gastric adenocarcinoma cell line.

Maintenance of Cell Lines

The A549 (Human alveolar lung adenocarcinoma cell line) was purchased from NCCS, Pune, India. The cells were maintained in DMEM/F12 HEPES media supplemented with 10 % FBS along with the 1% antibiotic-antimycotic solution in the atmosphere of 5% CO_2, 18-20% O_2 at 37^0C temperature in the CO_2 incubator and sub-cultured for every 2 days. Passage number 50 was used for the present study.

The AGS (Human gastric adenocarcinoma cell line) was purchased from NCCS, Pune, India. The cells were maintained in DMEM/F12 HEPES media supplemented with 10 % FBS along with the 1% antibiotic-antimycotic solution in the atmosphere of 5% CO_2, 18-20% O_2 at 37^0C temperature in the CO_2 incubator and sub-cultured for every 2days. Passage number 59 was used for the present study.

The HeLa (Human cervix adenocarcinoma cell line) was purchased from NCCS, Pune, India. The cells were maintained in Alpha MEM media supplemented with 10 % FBS along with the 1% antibiotic-antimycotic solution in the atmosphere of 5% CO_2, 18-20% O_2 at 37^0C temperature in the CO_2 incubator and sub-cultured for every 2days. Passage number 42 was used for the present study.

Cell Line Study

The *in vitro* cytotoxic activity of the extracted essential oil of *T. Ammi* was evaluated against Human alveolar lung adenocarcinoma cell line (A549), Human cervix adenocarcinoma cell line (HeLa) and Human gastric adenocarcinoma cell line (AGS) using MTT colorimetric assay according to the method Mosmann., 1983.

For anticancer activity, 200 µl cancer cell suspension was seeded in a 96-well plate at required cell density (20,000 cells per well), without the test agent. Cells were allowed to grow for about 24 hours. Appropriate concentrations (12.5, 25, 50, 100 and 200 µg/ml) of the isolated volatile oil were added and the plates were incubated for 24 hrs at 37°C in a 5% CO_2 atmosphere. After the incubation period, the plates were removed from incubator, and the spent media was removed. MTT reagent to a final concentration of 0.5 mg/ml of total volume was added. The plates were wrapped with aluminium foil to avoid exposure to light and returned to the incubator and incubate for another 3 hours. After incubation, the culture medium was then replaced with 100 µl of solubilisation solution (DMSO). Gentle stirring in a gyratory shaker will enhance dissolution. Occasionally, pipetting up and down may be required to completely dissolve the MTT formazan crystals especially in dense cultures. The absorbance of each well was measured on an ELISA reader at 570 nm. Cisplatin (20 mg/ml) was used as a positive control for cytotoxicity. For each compound, the concentration causing 50% cell growth inhibition (IC_{50}) was compared to the control and standard.[13]

The % cell viability is calculated using the below formula:

% Cell viability = [OD of treated cells / OD of Untreated cells] X 100

The IC_{50} value was determined by using linear regression equation *i.e.* Y = mX+C.

Here, Y = 50, m and C values were derived from the viability graph.

STATISTICAL ANALYSIS

The results are expressed as mean ± standard error of means (SEM). Data was analyzed with SPSS version 21.0. One-way analysis of variance (ANOVA) was used to determine the differences between treated and untreated groups. Tukey's post-hoc multiple comparisons method was used to determine the source of significant differences where appropriate. The results

were considered significant when $p < 0.05$.

RESULTS

Extraction of Volatile Oil

Hydrodistillation of *Trachyspermum ammi* seeds yielded 3.2 mL of volatile oil from 100 g of dried seeds, corresponding to a yield of 3.2% (v/w). The obtained yield is consistent with previous reports, which have documented essential oil yields ranging from 2–5% depending on geographical origin, harvesting conditions, and extraction methodology. The major constituents of *T. ammi* volatile oil are reported to be thymol, p-cymene, γ-terpinene, and carvacrol, compounds known for their antimicrobial, antioxidant, and anticancer activities.

Cytotoxic Activity Against A549 Cell Line

The cytotoxic activity of VOTA against the human lung cancer cell line (A549) was evaluated using the MTT assay after 24 h of treatment. The percentage cell viability decreased progressively with increasing concentrations of VOTA, indicating concentration-dependent cytotoxicity. The viability data and IC_{50} value are presented in **Table 1**.

Table 1: Cytotoxic effect of VOTA against A549 cells.

Drug conc (µg/ml)	% cell viability	IC50 conc (ug/ml)
Untreated	100.00	26.78
Cisplatin-15ug	50.93	
VOTA-12.5ug	67.28	
VOTA-25ug	53.26	
VOTA-50ug	32.46	
VOTA-100ug	21.16	
VOTA-200ug	8.41	

The concentration-dependent reduction in cell viability is further illustrated in **Figure 1**.

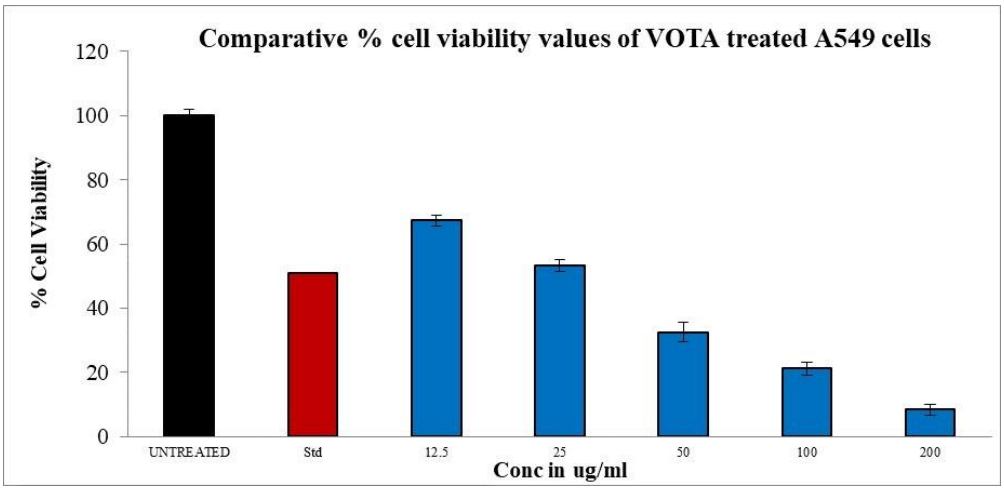


Figure 1: Percentage cell viability of A549 cells treated with VOTA

The IC_{50} value of VOTA against A549 cells was found to be 26.78 µg/mL, indicating potent cytotoxic activity. Cell viability decreased from 67.28% at 12.5 µg/mL to 8.41% at 200 µg/mL.

Cytotoxic Activity Against AGS Cell Line

The cytotoxic activity of VOTA against the human gastric cancer cell line (AGS) was assessed using the MTT assay. The results showed a gradual decrease in cell viability with increasing concentrations of the test compound. The percentage cell viability and IC_{50} value are presented in **Table 2**.

Table 2: Cytotoxic effect of VOTA against AGS cells.

Drug conc (µg/ml)	% cell viability	IC50 conc (ug/ml)
Untreated	100.00	171.02
Cisplatin-15ug	45.09	
VOTA-12.5ug	98.18	
VOTA-25ug	92.78	
VOTA-50ug	81.88	
VOTA-100ug	70.78	
VOTA-200ug	42.14	

The concentration-response relationship is depicted in **Figure 2**.

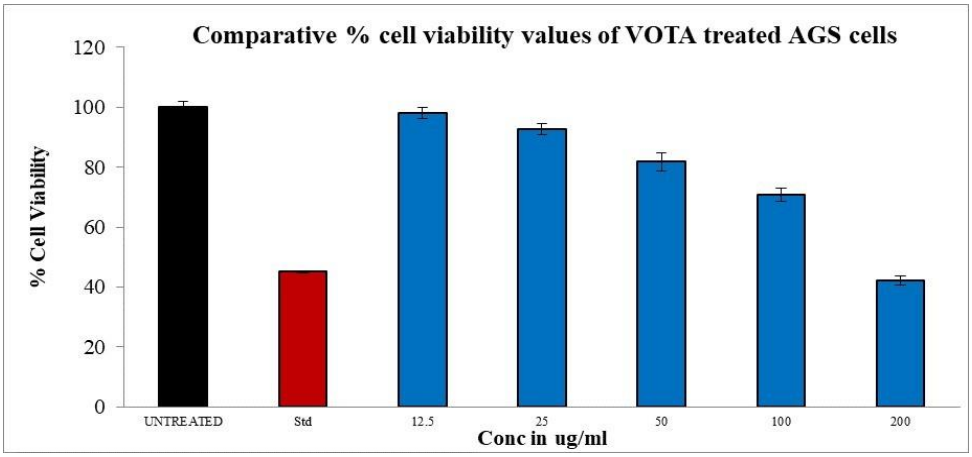


Figure 2: Percentage cell viability of AGS cells treated with VOTA.

The IC_{50} value obtained for AGS cells was 171.02 µg/mL, suggesting comparatively lower sensitivity towards VOTA treatment.

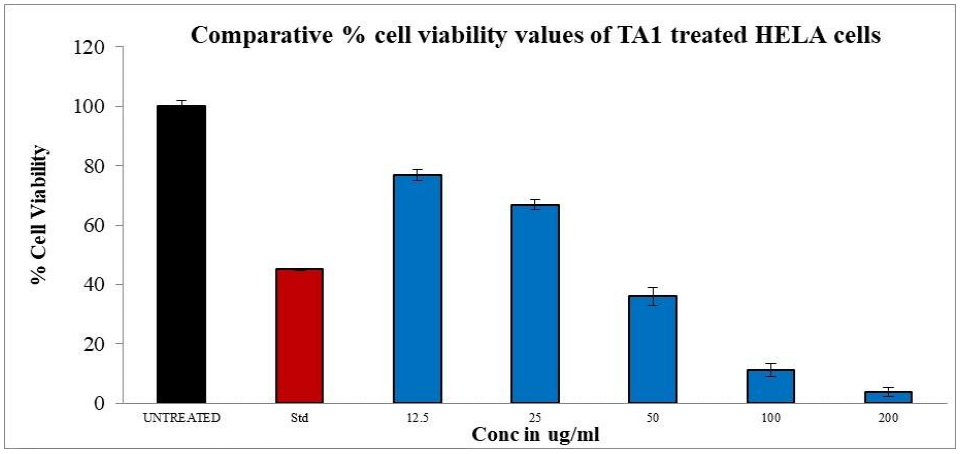
Cytotoxic Activity Against HeLa Cell Line

The cytotoxic activity of VOTA against the human cervical cancer cell line (HeLa) was evaluated after 24 h of treatment. A significant decrease in cell viability was observed with increasing concentrations of VOTA. The detailed results are shown in **Table 3**.

Table 3: Cytotoxic effect of VOTA against HeLa cells.

Drug conc (µg/ml)	% cell viability	IC50 conc (ug/ml)
Untreated	100.00	34.24
Cisplatin-15ug	45.09	
VOTA-12.5ug	76.95	
VOTA-25ug	66.90	
VOTA-50ug	36.09	
VOTA-100ug	11.25	
VOTA-200ug	3.73	

The graphical representation of percentage cell viability is shown in **Figure 3**. **Figure 3. Percentage cell viability of HeLa cells treated with VOTA.**



The IC_{50} value of VOTA against HeLa cells was determined to be 34.24 µg/mL, indicating substantial cytotoxic activity.

Comparative Analysis of IC_{50} Values

A comparison of IC_{50} values obtained for the three cancer cell lines is shown in **Figure 4**.

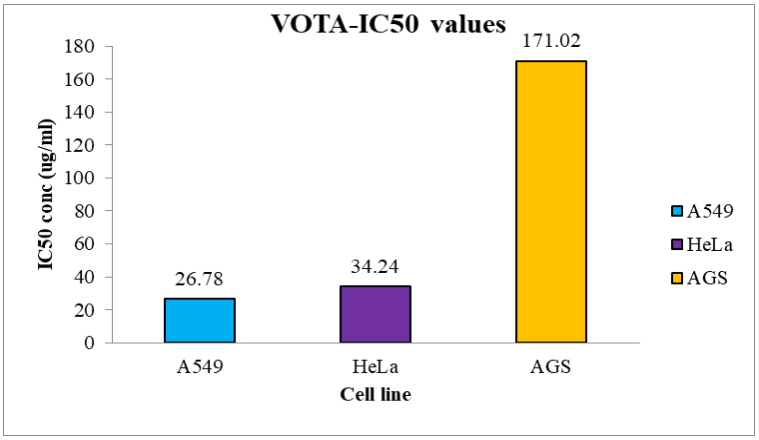


Figure 4: Comparative IC_{50} values of VOTA against A549, HeLa, and AGS cell lines.

As evident from Figure 4, the lowest IC_{50} value was observed for A549 cells (26.78 µg/mL), followed by HeLa cells (34.24 µg/mL), whereas AGS cells exhibited the highest IC_{50} value (171.02 µg/mL). This indicates that A549 cells were the most susceptible to VOTA treatment.

DISCUSSION

The present study evaluated the anticancer potential of the volatile oil of *Trachyspermum ammi* (VOTA) against three human cancer cell lines, namely A549 (lung adenocarcinoma), HeLa (cervical adenocarcinoma), and AGS (gastric adenocarcinoma), using the MTT assay. The results demonstrated a concentration-dependent reduction in cell viability in all tested cell lines, indicating the cytotoxic potential of the essential oil.

The hydrodistillation process yielded 3.2 mL of volatile oil from 100g of *T. ammi* seeds (3.2% v/w). This yield falls within the range of 2–5% reported for ajwain seeds in previous studies, suggesting efficient extraction of volatile constituents. The biological activity of ajwain oil has been largely attributed to the presence of thymol, γ-terpinene, p-cymene, and carvacrol, which constitute the major components of the essential oil. These compounds have been reported to possess antioxidant, antimicrobial, anti-inflammatory, and anticancer properties.[14,15]

Among the three cell lines studied, A549 cells exhibited the highest sensitivity to VOTA, with an IC_{50} value of 26.78 µg/mL. The percentage cell viability decreased from 67.28% at 12.5µg/mL to 8.41% at 200µg/mL, indicating potent cytotoxic activity. According to the criteria proposed by the National Cancer Institute, crude plant extracts and natural products exhibiting IC_{50} values below 30 µg/mL are generally considered promising candidates for further anticancer investigations. Therefore, the observed activity against A549 cells suggests significant anticancer potential.

Similarly, VOTA demonstrated pronounced cytotoxic activity against HeLa cells, with an IC_{50} value of 34.24 µg/mL. Cell viability decreased markedly from 76.95% at 12.5µg/mL to only 3.73% at 200µg/mL. The sensitivity of HeLa cells to ajwain oil may be associated with the ability of thymol-rich essential oils to induce apoptosis through mitochondrial dysfunction, generation of reactive oxygen species (ROS), activation of caspase-dependent pathways, and DNA fragmentation. Previous studies have reported that thymol exhibits selective cytotoxicity toward cancer cells while causing comparatively lower toxicity to normal cells.[16]

In contrast, AGS gastric cancer cells showed comparatively lower sensitivity to VOTA, with an IC_{50} value of 171.02 µg/mL. Although a concentration-dependent decrease in cell viability was observed, the effect was less pronounced than that observed in A549 and HeLa cells. At 200 µg/mL, AGS cells retained 42.14% viability, indicating relative resistance to the treatment. Differences in sensitivity among cancer cell lines may arise from variations in membrane permeability, cellular metabolism, expression of drug transporters, antioxidant defense mechanisms, and genetic background of the tumor cells.

The observed cytotoxicity may be attributed primarily to thymol, which is reported to be the major constituent of *T. ammi* essential oil. Thymol has been shown to induce cell cycle arrest, mitochondrial membrane depolarization, oxidative stress, and apoptosis in various cancer cell models. Furthermore, p-cymene and γ-terpinene may contribute synergistically to the overall anticancer effect by enhancing membrane permeability and facilitating intracellular accumulation of active compounds.[17] Such synergistic interactions among phytoconstituents are often responsible for the superior biological activity of whole essential oils compared with isolated constituents.

The present findings are consistent with previous reports describing the cytotoxic and anticancer activities of *T. ammi*. Abdel-Hameed et al reported significant anticancer activity of *T. ammi* essential oil against various human cancer cell lines and attributed the effect

to its phenolic monoterpene content.[18] Similarly, Alshammari et al demonstrated that bioactive constituents isolated from *T. ammi* possess considerable cytotoxic activity against cancer cells through apoptosis-mediated mechanisms.[19]

Overall, the results of the present investigation demonstrate that the volatile oil of *Trachyspermum ammi* possesses significant in vitro anticancer activity, particularly against lung (A549) and cervical (HeLa) cancer cell lines. The order of cytotoxic sensitivity was found to be A549 > HeLa > AGS, based on the obtained IC_{50} values. The observed activity may be attributed to the combined action of thymol, γ-terpinene, p-cymene, and other bioactive constituents present in the oil. However, further studies involving apoptosis markers, cell cycle analysis, molecular signaling pathways, and in vivo models are required to elucidate the precise mechanisms underlying the anticancer activity of VOTA and to establish its therapeutic potential.

CONCLUSION

The volatile oil of *Trachyspermum ammi* demonstrated potent cytotoxic activity against A549 and HeLa cell lines with IC_{50} values of 26.78 and 34.24 µg/mL, respectively, while exhibiting moderate activity against AGS cells (IC_{50} = 171.02 µg/mL). The observed concentration-dependent reduction in cell viability and characteristic apoptotic morphological changes suggest that the thymol-rich volatile oil possesses promising anticancer potential warranting further mechanistic and *in vivo* investigations.

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