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# *In vitro* antioxidant and anticancer potential of intra-cellular ethyl acetate extract of marine-derived fungus *Talaromyces tratensis SS10*

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## ABSTRACT

Marine fungi are well-known for producing structurally distinct secondary metabolites, making them potential sources of novel therapies. The present investigation aims to study the *in vitro* antioxidant and anticancer potential of intra-cellular crude ethyl acetate extracts of *Talaromyces tratensis* SS10. In the present study, qualitative and quantitative phytochemical studies of various solvent extracts of *T. tratensis* have been carried out using standard protocols. Later, ethyl acetate extract of *T. tratensis* was analyzed for phytochemicals using Gas Chromatography Mass Spectrometry (GC-MS). Further, the antioxidant properties of the *T. tratensis* ethyl acetate extract have been done by Ferric reducing antioxidant power assay (FRAP). Further, the anticancer potential of this extract has been carried out by MTT assay against human cancer cells such as MDA MB 231, HeLa, and HT-29. Ethyl acetate exhibited a higher yield of chemical extraction than the other solvents used. The GCMS analysis of *T. tratensis* ethyl acetate extract revealed major phytoconstituents such as N-(1,1-Dimethylpropyl)-2,2,3-trimethylaziridine-1-carboxamide, 1-Undecanol, 5,5 Dimethyl-3-vinyl cyclohex-2-en-1-one, 1,2-Benzenedicarboxylic acid, bis (2-methyl propyl) ester. *T. tratensis* ethyl acetate extract showed the highest percentage of $Fe^{3+}$ reduction (48.093±1.469%) at 120 µg/mL, with an $IC_{50}$ value of 157.26 µg/mL concentration. Furthermore, 100 µg/mL of the extract showed significant cell death rates in cytotoxic assays, indicating a low percentage of viable cells for all three examined cell lines. The *T. tratensis* ethyl acetate extract has shown a dose-dependent cytotoxic effect against all tested cancer cell lines. The better $IC_{50}$ value (6.25 µg/mL) was recorded in the case of HeLa cell lines followed by 12.5 µg/mL for both MDA MB 231 and HT-29 cell lines. The presence of bioactive compounds such as Benzeneethanamine, N-[(pentafluorophenyl)methylene]-beta.,3,4-tris[(trimethylsilyl) oxy]-, 1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester, and cyclononasiloxane, octadecamethyl- may have contributed to the ethyl acetate extracts' strong antioxidant and anticancer properties. The current study's findings show that *T. tratensis* SS10 has the potential for drug development due to its chemical constituents, which possess various biological activities.

**KEYWORDS:** *Talaromyces tratensis* SS10, Ethyl acetate extract, GC-MS, Antioxidant, Anticancer

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## INTRODUCTION

Marine fungi inhabit marine environments including saltwater and estuarine ecosystems. Presently, about 767 genera and 1898 species of marine fungi are distributed worldwide (Calabon *et al.*, 2023). These organisms contribute to decomposing organic matter, nutrient cycling, and other ecological processes in marine environments. They also hold great potential as a source of novel bioactive compounds. Since the isolation of cephalosporin (β-lactam antibiotic) from *Acremonium chrysogenum*, many bioactive compounds have been isolated from marine fungi (Gonçalves *et al.*, 2022). Marine fungal genera such as *Penicillium*, *Aspergillus*, *Fusarium*, and *Cladosporium* have been extensively studied by several researchers for the production of secondary metabolites (Imhoff, 2016).

Generally, marine fungi produce a variety of secondary metabolites such as alkaloids, peptides, polyketides, polysaccharides, terpenes, and steroids. Almost 3500 secondary metabolites from marine fungi have already been documented; however, a significant number are undoubtedly still unknown (Carroll *et al.*, 2019). A thorough review of the literature reveals that bioactive compounds from marine fungi have a significant therapeutic potential (Shabana *et al.*, 2021).

Free radicals and reactive oxygen species (ROS) are the fundamental causes of many human diseases. Oxidative stress results from an imbalance in prooxidant generation and neutralization (Rahamtulla *et al.*, 2023). Several investigations have shown that they induce oxidative damage to lipids, proteins, and DNA. This damage can cause a variety of chronic problems in humans, including diabetes, cancer, ageing, and other degenerative disorders (Gangwar *et al.*, 2015).

Cancer is a group of diseases characterized by uncontrolled cell division, which can result in over 100 life-threatening conditions. The most common cancers are lung, breast, and colon cancer. Marine fungal extracts exhibit anticancer properties and could be used to treat a variety of malignancies. Sun *et al.* (2019) reported the cytotoxic compound N-glutarylchaetoviridins C from *Chaetomium globosum* (HDN151398), a deep-sea sediment fungus from the South China Sea. This compound showed anticancer properties against MGC-803 and HO8910 cell lines. Studies of Niu *et al.* (2022) reported that *Aspergillus sydowii* MCCC 3A00324 fermented broth produced acremolin D, which was cytotoxic to K562, Hela-S3, A549, HepG2, and K562 cell lines.

A variety of biological activities such as antibacterial, antiviral, antifungal, antiparasitic, anticancer, and anti-inflammatory properties are exhibited by several secondary metabolites derived from marine fungi. The creation of novel medications and therapeutic agents may benefit greatly from these bioactive molecules. Therefore, the present investigation focused on the antioxidant and anticancer properties of intracellular crude extracts of the marine-derived fungus *Talaromyces tratensis* SS10.

## MATERIALS AND METHODS

### Marine Fungal Strain Used

In the present study, the marine fungus *Talaromyces tratensis* SS10 (Figure 1a & b) (Accession number OQ476225.1) previously isolated from the seawater of Manginapudi beach, Machilipatnam, India was used (Shabana *et al.*, 2023).

### Preparation and Extraction of *T. Tratensis* Intracellular Metabolites

*T. tratensis* was cultured on optimized Rose Bengal Agar media [glucose (10 g/L), yeast extract (5 g/L), monopotassium phosphate (1 g/L), magnesium sulphate (0.25 g/L), 2% (w/v) NaCl, rose bengal dye (0.025 g/L), Agar (20 g/L), pH 6.9, sterilized distilled water] for about ten days at 28 ± 2 °C. The liquid state fermentation was initiated in 1 L Erlenmeyer flasks containing sterilized 500 mL of Rose Bengal Broth (RBB) by inoculating small blocks of mycelia from the agar plate. The flasks were incubated for ten days at 28 °C at 120 rpm on a rotary shaker. The mycelia grown after 10 days were collected by filtration, washed thoroughly with sterile distilled water, dried at 40 °C for two days, and weighed using an analytical scale.

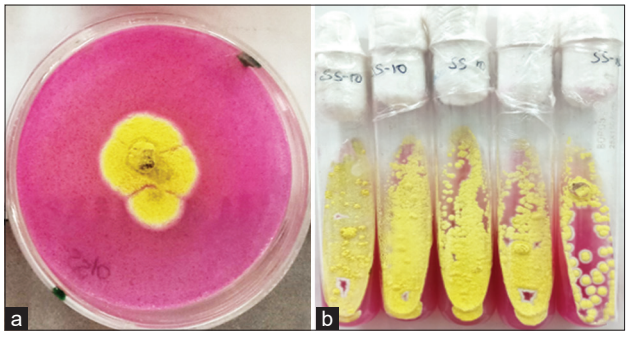


**Figure 1:** *Talaromyces tratensis* SS10 a) Pure strain on Rose Bengal Agar (RBA) and b) Pure strain on RBA slants

The dried mycelia were mechanically ground (pestle and mortar) with various solvents (chloroform, petroleum ether, ethyl acetate, and methanol) separately. In the current investigation, 5 g of dried mycelia is extracted with 50 mL of solvents separately, then transferred to four conical flasks and placed on a rotatory shaker for 24 hours at 120 rpm. Later, the extracts were centrifuged at 10,000 rpm for five minutes, and the supernatants were collected and kept at 4 °C for further analysis.

### Qualitative Phytochemical Screening

Ethyl acetate and methanol extracts of *T. tratensis* were qualitatively analyzed for secondary metabolites. The preliminary phytochemical screening was carried out using the standard protocols formulated by Harborne (1973) and Trease and Evans (1989, 1996). All the phytochemical tests were done in triplicate.

### Quantitative Analysis of Secondary Metabolites

1. Estimation of alkaloids

Approximately 5 g of the powdered *T. tratensis* crude extract was transferred to a 250 mL beaker containing 200 mL of 10% acetic acid in ethanol and left to stand for 4 hours. Later, the sample was filtered, and the filtrate was reduced to one-fourth of its original volume in a water bath. A few drops of concentrated $NH_4OH$ were added to the extract until precipitation was complete. The precipitate formed in the solution was washed with dilute $NH_4OH$, then transferred to petri plates, dried, and weighed. The weight was expressed as %g alkaloids on a dry weight basis (Mir *et al.*, 2016).

2. Determination of total phenols

The Folin-Ciocalteau reagent method was used to estimate the total phenolic content of crude extracts of *T. tratensis*, with minor adjustments (Baba & Malik, 2015). 100 mL of crude extract (1 mg/mL) was mixed with 900 mL of methanol and 1 mL of Folin-Ciocalteau reagent. The above mixture was then treated with 1 mL of a 20% (w/v) $Na_2CO_3$ solution. The mixture was incubated at room temperature in the dark for 30 minutes. The absorbance was then measured at 765 nm. The total phenolic content was expressed in terms of gallic acid equivalent (mg/mg extract).

3. Determination of total flavonoids

The total flavonoid content of *T. tratensis* crude extracts was determined using a slightly modified Aluminium chloride method (Sivaraj *et al.*, 2019). Approximately 500 mL of crude extract (1 mg/mL) was mixed with 0.5 mL of methanol and 1 mL of a 5% (w/v) sodium nitrite solution. Following that, about 1 mL of 10% (w/v) aluminium chloride solution was added to the above mixture and shaken thoroughly. Later, 100 mL of 1 M NaOH solution was added to the mixture, and its absorbance was measured at 510 nm. The total flavonoid content was expressed as (mg/mg extract) quercetin equivalent.

## Gas Chromatography-Mass Spectroscopy (GC-MS) Analysis

The GC-MS analysis was performed using an ethyl acetate extract of *T. tratensis*. The fungal extract was loaded onto an HP-5 column (30 m x 0.25 mm film thickness, Agilent Technologies 6890 NJEOL GC Mate II GC-MS model). The following chromatographic settings were employed when operating the equipment: helium as the carrier gas, flow rate of 1 mL/min, injector temperature of 200 °C, column oven temperature of 50-250 °C, and injection rate of 10°C/min. The Mass Spectroscopy copy conditions were as follows: 70 eV ionisation voltage, 250 °C ion source temperature, 250 °C interface temperature, and a mass range of 50-600 mass units (Noorjahan *et al.*, 2024)

The mass spectrum of GC-MS was analysed using the National Institute of Standards and Technology (NIST) database, which has over 62,000 patterns. The mass spectra of the unknown phytocomponents were compared to those of recognised components recorded in the NIST library. The phytocomponents obtained from the *T. tratensis* ethyl acetate extract were then identified by their name, molecular mass, and structure (Noorjahan *et al.*, 2023).

## Pharmacological studies

1. Ferric ($Fe^{3+}$) reducing power assay

The reducing power of *T. tratensis* ethyl acetate extract was determined by a slightly modified potassium ferricyanide method (Settharaksa *et al.*, 2018). One mL of crude extract (20-120 µg/mL) was combined with 1 mL of potassium ferricyanide [$K_3Fe(CN)_6$] (1%, w/v) solution and 1 mL of phosphate buffer (0.2 M, pH 6.6). The mixture was then incubated in a water bath at 50 °C for 20 minutes. Each mixture was treated with 500 µL of 10% w/v trichloroacetic acid. The absorbance at 700 nm was measured after adding 100 µL of freshly made $FeCl_3$ (0.1%, w/v) solution and shaking thoroughly (Sundaram *et al.*, 2021). Ascorbic acid was selected as the standard reference. The percentage of reduction was computed as follows:

$$\% \text{ of } Fe^{3+} \text{ reduction} = \frac{\text{Sample - Control} \times 100}{\text{Sample}}$$

2. *In vitro* cytotoxic assay

The anticancer activity of ethyl acetate crude extracts of *T. tratensis* was assessed using MDA-MB-231 (Breast), HT29 (Colon), and HeLa (Cervical) cancer cell lines.

### *Chemicals and reagents*

MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide) was purchased from Invitrogen (United States). Sigma, Aldrich was the source of all other fine chemicals utilized in the experiment.

### *Cell culture*

MDA-MB-231, HT29, and HeLa cancer cells were obtained from the National Centre for Cell Science (Pune). Rose-well Park Memorial Institute (RPMI) media was supplemented with 10% foetal bovine serum (FBS), penicillin/streptomycin (250 U/mL), gentamycin (100 µg/mL), and amphotericin B (1mg/mL) for cell culture. All cell cultures were kept at 37 °C in a humidified environment containing 5% $CO_2$. Cells were allowed to grow to confluence for 24 hours before use.

### *Cell growth inhibition studies using MTT assay*

Cell viability was assessed using the MTT reduction assay reported by Mosmann (1983) with minor modifications. MDA-MB-231, HT29, and HeLa cancer cells were seeded at $5\times10^3$ cells/well in 96-well plates for 24 hours in 200 µL of RPMI with 10% FBS. After removing the culture supernatant, RPMI medium with varying doses of crude extract (1.56-100 µg/mL) was added and incubated for 48 hours.

The cells were treated with MTT (10 µL, 5 mg/mL) at 37 °C for 4 hours, followed by 1 hour of DMSO at room temperature. The plates were read at 595 nm using a scanning multi-well spectrophotometer. The data were given as the mean of three independent experiments. The percentage growth inhibition was determined using the following formula, and the $IC_{50}$ values were obtained by linear regression analysis.

$$\text{Percentage of cell viabilty} = \frac{\text{Ab-As} \times 100}{\text{Ab}}$$

Ab = absorbance of control; As = absorbance of sample

## RESULTS AND DISCUSSION

*Talaromyces tratensis* has a unique yellow colour and a slow growth rate. After 10 days of incubation at 28±1 °C, it grew to 30-40 mm. This fungus formed aseptate conidia which have been associated with phialides and ascospores. *T. tratensis* conidiophores are recognized by their smooth surfaces and lateral branching (Shabana *et al.*, 2023). Crude extracts of *T. tratensis* were extracted by using different solvents such as chloroform, petroleum ether, ethyl acetate, and methanol. The percentage yield of extract was found high in ethyl acetate

(3.12%) followed by methanol (2.68%), chloroform (2.23%), and petroleum ether (1.86%) extracts.

## Qualitative Phytochemical Studies

Preliminary phytochemical screening of crude extracts of *T. tratensis* revealed the presence of various secondary metabolites (Table 1). The ethyl acetate fungal extract of *T. tratensis* showed the presence of all the phytochemicals tested where whereas the methanolic crude extract showed only the presence of alkaloids, flavonoids, and phenols. Chloroform extract showed a positive result for alkaloids and no other phytochemicals were detected. Similarly, petroleum ether extract of *T. tratensis* showed only the presence of steroids. The differences in the presence of phytochemical substances could be attributed to the compound's varied solubility in the solvent utilized (Bako *et al.*, 2005).

## Quantitative Phytochemical Studies

In the present study, ethyl acetate and methanol crude extracts of *T. tratensis* were selected for the quantitative phytochemical analysis. Ethyl acetate crude extract showed the highest alkaloid content (3.612 mg/g dry weight), highest phenolic content (297.38 µg/mg of Gallic Acid Equivalent (GAE)), and highest flavonoid content (75.07 µg/mg of quercetin equivalent (QE)) in comparison to methanol crude extract (Table 2).

## Bioactive Compounds of *T. tratensis* ethyl Acetate Extract

In the present study, GC-MS analysis of *T. tratensis* ethyl acetate extract was carried out due to its rich phytochemical content. The gas chromatogram (Figure 2) revealed the presence of 29 phytoconstituents. The retention time, molar mass, and peak area percentage of the phytoconstituents are displayed in Table 3. The major phytoconstituents such as N-(1,1-Dimethylpropyl)-2,2,3-trimethylaziridine-1-carboxamide, 1-Undecanol, 5,5 Dimethyl-3-vinyl cyclohex-2-en-1-one, 1,2-Benzenedicarboxylic acid, bis(2-methyl propyl) ester, 1-Propanol, 2-amino-, (.+/-.)- and Sulfurous acid, decyl 2-propyl ester are recorded in the *T. tratensis* ethyl acetate extract.

Tridecan-1-ol, a long-chain primary fatty alcohol recorded in *T. tratensis* ethyl acetate extract, has antibacterial properties (Garaniya & Bapodra, 2014). 1-Undecanol, a long-chain fatty alcohol, is detected in the same extract. This compound has a bactericidal action against *Staphylococcus aureus* (Togashi *et al.*, 2007) and a strong nematicidal activity against *Bursaphelenchus xylophilus*, the pinewood nematode that causes pine-wilt disease (Cavaco *et al.*, 2022).

3-Isopropoxy-1,1,1,7,7,7-hexamethyl-3,5,5-tris(trimethylsiloxy) tetrasiloxane is an organosilicon compound detected in *T. tratensis* ethyl acetate extract. According to studies by Singh *et al.* (2021), this phytocompound inhibits the growth of rice pathogens by targeting histone deacetylase of *Magnaporthe oryzae* and peptide deformylase of *Xanthomonas oryzae* pv. Oryzae.

1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester, an aromatic carboxylic acid found in the *T. tratensis* ethyl acetate extract. Earlier this compound was also reported from the chloroform extract of Rhizospheric *Actinomycetes* (AIA26 isolate) and possesses antibacterial and anticancer activity (Kumari & Menghani, 2021).

Benzeneethanamine, N-[(pentafluorophenyl)methylene]-beta.,3,4-tris[(trimethylsilyl) oxy]- is recorded in the *T. tratensis* ethyl acetate extract. This alkaloid is also recorded earlier in the crude extracts of *Penicillium chrysogenum* and has anti-HIV, antimicrobial, and antioxidant properties (Makhwitine *et al.*, 2023).

The compound cyclononasiloxane, octadecamethyl- is recorded in the *T. tratensis* ethyl acetate extract. Studies by Lutfia *et al.* (2021) also recorded the same compound in the ethyl acetate extracts of *Zingiber griffithii* rhizome and *Hypomontagnella monticulosa* Zg15SU mycelial extracts. Several research studies (Moustafa *et al.*, 2013; Al Bratty *et al.*, 2020; Muthuselvam & Kathick, 2021; El-Fayoumy *et al.*, 2021) reported this organosilicon compound has antifungal, antiparasitic, antioxidant anticancer activities.

Table 1: Phytochemical screening of the crude extracts of *T. tratensis*

| S. No. | Phytocomponents | Test/Reagent used | Chloroform extract | Ethyl acetate extract | Methanol Extract | Petroleum ether extract |
|---|---|---|---|---|---|---|
| 1 | Alkaloids | Dragendorff's test | + | + | + | - |
| 2 | Flavonoids | 1% $AlCl_3$ test | - | + | + | - |
| 3 | Phenols | 10% $FeCl_3$ test | - | + | + | - |
| 4 | Saponins | Frothing test | - | + | - | - |
| 5 | Steroids | Liebermann-Burchard test | - | + | - | + |
| 6 | Tannins | Iodine test | - | + | - | - |
| 7 | Terpenoids | Salkowski test | - | + | - | - |

Table 2: Quantitative phytochemical studies of *T. tratensis* crude extracts

| *T. tratensis* crude extract | Total alkaloid content (mg/g) dry weight | Total phenolic content (µg/mg of gallic acid equivalent) | Total flavonoid content (µg/mg of quercetin equivalent) |
|---|---|---|---|
| Ethyl acetate extract | 3.612 | 297.38 | 75.07 |
| Methanol extract | 1.523 | 64.33 | 21.03 |

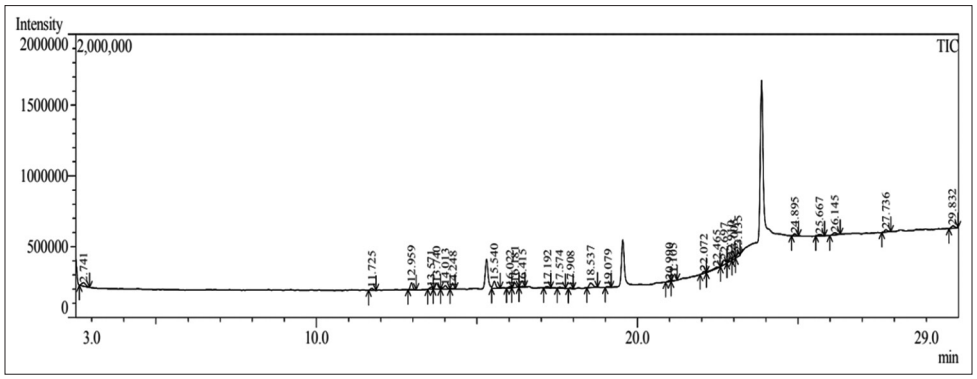


**Figure 2:** GC-MS chromatogram of *T. tratensis* ethyl acetate extract

Table 3: Phytochemicals recorded in the ethyl acetate extract of *T. tratensis*

| Peak | Retention Time | Name of the Phytoconstituents | Peak Area % |
|---|---|---|---|
| 1 | 2.741 | 1-Propanol, 2-amino-, (.+/-.)- | 6.12 |
| 2 | 11.725 | Benzamide, N-[4-(5-mercapto-4-methyl-4H-1,2,4-triazol-3-yl) phenyl]-2-methoxy- | 2.26 |
| 3 | 12.959 | 5,5 Dimethyl-3-vinyl cyclohex-2-en-1-one | 7.47 |
| 4 | 13.571 | 1,2-Hexanediol, 1,2-diphenyl- | 2.30 |
| 5 | 13.740 | 1-Undecanol | 8.03 |
| 6 | 14.013 | Sulfurous acid, decyl 2-propyl ester | 5.90 |
| 7 | 14.248 | 3-Fluoro-4-trifluoromethylbenzoic acid, neopentyl ester | 0.64 |
| 8 | 15.540 | N-(1,1-Dimethylpropyl)-2,2,3-trimethylaziridine-1-carboxamide | 8.64 |
| 9 | 16.022 | L-Lyxose | 1.15 |
| 10 | 16.181 | n-Tridecan-1-ol | 5.21 |
| 11 | 16.415 | Decane, 2,3,6-trimethyl- | 0.83 |
| 12 | 17.192 | Sulfurous acid, isohexyl pentyl ester | 1.63 |
| 13 | 17.574 | Borane, diethyl (decyloxy)- | 1.68 |
| 14 | 17.908 | 3-Isopropoxy-1,1,1,7,7,7-hexamethyl-3,5,5-tris (trimethylsiloxy) tetrasiloxane | 0.63 |
| 15 | 18.537 | 1,2-Benzenedicarboxylic acid, bis (2-methylpropyl) ester | 7.44 |
| 16 | 19.079 | Heptadecanoic acid, 10-methyl-, methyl ester | 1.04 |
| 17 | 20.990 | Benzeneethanamine, N-[(pentafluorophenyl) methylene]-.beta.,3,4-tris[(trimethylsilyl) oxy]- | 1.09 |
| 18 | 21.105 | 1,2,4-Triazol-5-acetic acid, 3-amino- | 0.55 |
| 19 | 22.072 | Cyclononasiloxane, octadecamethyl- | 2.02 |
| 20 | 22.465 | 3,5-Dihydroxy-3-methyl-pentanamide, N-benzhydryl- | 3.36 |
| 21 | 22.697 | 2-Methyl-5-(2,6,6-trimethyl-cyclohex-1-enyl)-pentane-2,3-diol | 6.05 |
| 22 | 22.910 | 5-Keto-9-tetradecenamide | 3.01 |
| 23 | 23.005 | Cyclononasiloxane, octadecamethyl- | 3.00 |
| 24 | 23.135 | 1,3-Dioxolo[4,5-c] pyran-7-ol, tetrahydro-2,2-dimethyl-4-methoxy-7-[5-(t-butyldimethylsilyloxy)-4-met | 4.38 |
| 25 | 24.895 | Tetracosamethyl-cyclododecasiloxane | 2.22 |
| 26 | 25.667 | 3-buten-2-one, 4-(5,5-dimethyl-1-oxaspiro[2.5]oct-4-yl) | 2.16 |
| 27 | 26.145 | Cyclononasiloxane, octadecamethyl- | 4.19 |
| 28 | 27.736 | Cyclononasiloxane, octadecamethyl- | 3.57 |
| 29 | 29.832 | Cyclononasiloxane, octadecamethyl- | 3.41 |

In the present study, Tetracosamethyl-cyclododecasiloxane is recorded in the *T. tratensis* ethyl acetate extract. This siloxane compound was found to have several biological activities such as hepatoprotective activity, antispasmodic, antirheumatic, and appetizing agent (Madhavan & Mahadevi, 2021).

## Pharmacological Studies

### *Antioxidant activity*

Antioxidants are substances that can prevent or postpone oxidation reactions by neutralizing free radicals, thereby preserving cells from oxidative damage. The widely used ferric reducing antioxidant power (FRAP) assay converts $Fe^{3+}$ to $Fe^{2+}$ through a redox-linked colorimetric reaction using antioxidants as reductants. At low pH, a colourless ferric-probe complex transforms into a coloured ferrous-probe complex through the reduction of ferric ($Fe^{3+}$) to ferrous ($Fe^{2+}$) ions. This test can measure antioxidant capabilities as low as 0.2 mM $Fe^{2+}$ equivalents (Bhandari & Kawabata, 2004).

In the present study, the FRAP assay of *T. tratensis* ethyl acetate extract revealed that different concentrations (20-120 µg/mL) showed different percentages of reduction (Table 4). An increase in the crude extract concentration was accompanied by a rise in the reducing power (dose-dependent reduction). At 120 µg/mL, ethyl acetate extract showed better antioxidant activities when compared to the other concentrations of the extract (Table 4).

The $IC_{50}$ value of ethyl acetate extract was calculated using linear regression analysis. Ethyl acetate extract of *T. tratensis*

showed an $IC_{50}$ value of 157.26 µg/mL (Table 4). This suggests that *T. tratensis* ethyl acetate extract can interact with free radicals and convert them into stable, non-reactive forms. Positive control ascorbic acid showed an $IC_{50}$ = 10.32 µg/mL concentration.

Phenolic substances are essential in the fight against oxidative stress (Pang *et al.*, 2018). The antioxidant property of ethyl acetate extract can be attributed to the high phenol and flavonoid content (Table 2). The extract has reducing power activity because of the phenolic components that donate electrons and/or hydrogen to perform the role of free radical scavenger.

### *Anti-cancer activity of T. tratensis ethyl acetate extract against human cancer cell lines*

Uncontrolled cell division is a hallmark of cancer-related illnesses. Human cells are subjected to free radicals and reactive oxygen species (ROS), which can lead to cancer and can be treated with chemotherapy, radiation therapy, or surgery, but all of these treatments have adverse effects. As a result, it is vital to isolate the bioactive components.

In the present investigation, the anti-cancer activity of *T. tratensis* ethyl acetate extract against three different human cancer cell lines HeLa, MDA-MB 231, and HT-29 was determined using the MTT assay. The use of *T. tratensis* ethyl acetate extract to treat various cancer cell lines caused morphological changes such as cell size and volume reduction, cell shrinkage, cell membrane blebbing, chromatin condensation, nuclear fragmentation, and the formation of apoptotic bodies, indicating that the extracts induced apoptosis.

At 100 µg/mL of ethyl acetate extract, all three cell lines experienced substantial cell death rates, indicating a low percentage of viable cells. The results also showed that the extract exhibited cytotoxic activity in a dose-dependent manner against all the cancer cell lines tested (Table 5). When the concentration of *T. tratensis* ethyl acetate extract is increased the percentage of cell death also increased. The current findings are consistent with the cytotoxic investigations of Majoumouo *et al.* (2020) and Bhat *et al.* (2023). The better $IC_{50}$ value (6.25 µg/mL)

Table 4: FRAP assay of ethyl acetate extract of *T. tratensis*

| Crude extract used | Concentration µg/mL | Absorbance | | | % of $Fe^{3+}$ reduction | | | | $IC_{50}$ values µg/mL |
|---|---|---|---|---|---|---|---|---|---|
| | | I | II | III | %-I | %-II | %-III | Mean±SD | |
| Ethyl acetate extract | Control | 0.123 | 0.126 | 0.121 | - | - | - | - | 157.26 |
| | 20 | 0.135 | 0.137 | 0.13 | 8.88 | 8.02 | 6.92 | 7.94±0.982 | |
| | 40 | 0.176 | 0.172 | 0.171 | 30.11 | 26.74 | 29.23 | 28.69±1.747 | |
| | 60 | 0.19 | 0.194 | 0.196 | 35.26 | 35.05 | 38.26 | 36.19±1.795 | |
| | 80 | 0.214 | 0.216 | 0.219 | 42.52 | 41.66 | 44.74 | 42.97±1.589 | |
| | 100 | 0.23 | 0.235 | 0.232 | 46.52 | 46.38 | 47.84 | 46.91±0.805 | |
| | 120 | 0.233 | 0.239 | 0.241 | 47.21 | 47.28 | 49.79 | 48.093±1.469 | |

Table 5: Effect of *T. tratensis* ethyl acetate extract on human cancer cell lines

| Cell lines | Control OD Mean | Concentration (µg/mL) | Absorbance at 570 nm | | | % of cell death | | | % of cell death Mean±SD | % Live cell | $IC_{50}$ µg/mL |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | OD 1 | OD 2 | OD 3 | | | | | | |
| MDA MB 231 | 0.431 | Control | 0.458 | 0.412 | 0.425 | - | - | - | - | - | 12.5 |
| | | 100 | 0.122 | 0.096 | 0.102 | 71.69 | 77.73 | 76.33 | 75.25±3.16 | 24.75 | |
| | | 50 | 0.158 | 0.114 | 0.125 | 63.34 | 73.55 | 71.00 | 69.30±5.31 | 30.70 | |
| | | 25 | 0.165 | 0.158 | 0.152 | 61.72 | 63.34 | 64.73 | 63.26±1.51 | 36.74 | |
| | | 12.5 | 0.258 | 0.225 | 0.232 | 40.14 | 47.80 | 46.17 | 44.70±4.03 | 55.30 | |
| | | 6.25 | 0.269 | 0.256 | 0.269 | 37.59 | 40.60 | 37.59 | 38.59±1.74 | 61.41 | |
| | | 3.125 | 0.312 | 0.302 | 0.315 | 27.61 | 29.93 | 26.91 | 28.15±1.58 | 71.85 | |
| | | 1.56 | 0.352 | 0.345 | 0.325 | 18.33 | 19.95 | 24.59 | 20.96±3.25 | 79.04 | |
| HeLA | 0.387 | Control | 0.385 | 0.388 | 0.389 | - | - | - | - | - | 6.25 |
| | | 100 | 0.015 | 0.014 | 0.016 | 96.12 | 96.38 | 95.87 | 96.12±0.26 | 3.88 | |
| | | 50 | 0.058 | 0.045 | 0.047 | 85.01 | 88.37 | 87.86 | 87.08±1.81 | 12.92 | |
| | | 25 | 0.089 | 0.096 | 0.102 | 77.00 | 75.19 | 73.64 | 75.28±1.68 | 24.72 | |
| | | 12.5 | 0.155 | 0.145 | 0.168 | 59.95 | 62.53 | 56.59 | 59.69±2.98 | 40.31 | |
| | | 6.25 | 0.198 | 0.201 | 0.212 | 48.84 | 48.06 | 45.22 | 47.37±1.90 | 52.63 | |
| | | 3.125 | 0.205 | 0.256 | 0.288 | 47.03 | 33.85 | 25.58 | 35.49±10.82 | 64.51 | |
| | | 1.56 | 0.263 | 0.296 | 0.321 | 32.04 | 23.51 | 17.05 | 24.20±7.52 | 75.80 | |
| HT-29 | 0.652 | control | 0.635 | 0.632 | 0.689 | - | - | - | - | - | 12.5 |
| | | 100 | 0.147 | 0.147 | 0.154 | 77.45 | 77.45 | 76.38 | 77.10±0.62 | 22.90 | |
| | | 50 | 0.258 | 0.255 | 0.245 | 60.43 | 60.89 | 68.42 | 61.25±1.04 | 38.75 | |
| | | 25 | 0.296 | 0.289 | 0.312 | 54.60 | 55.67 | 52.15 | 54.14±1.81 | 45.86 | |
| | | 12.5 | 0.354 | 0.352 | 0.351 | 45.71 | 46.01 | 46.17 | 45.96±0.23 | 54.04 | |
| | | 6.25 | 0.398 | 0.412 | 0.421 | 38.96 | 36.81 | 35.43 | 37.07±1.78 | 62.93 | |
| | | 3.125 | 0.455 | 0.455 | 0.425 | 30.21 | 30.21 | 34.82 | 31.75±2.66 | 68.25 | |
| | | 1.56 | 0.598 | 0.555 | 0.563 | 8.28 | 14.88 | 13.65 | 12.27±3.51 | 87.73 | |

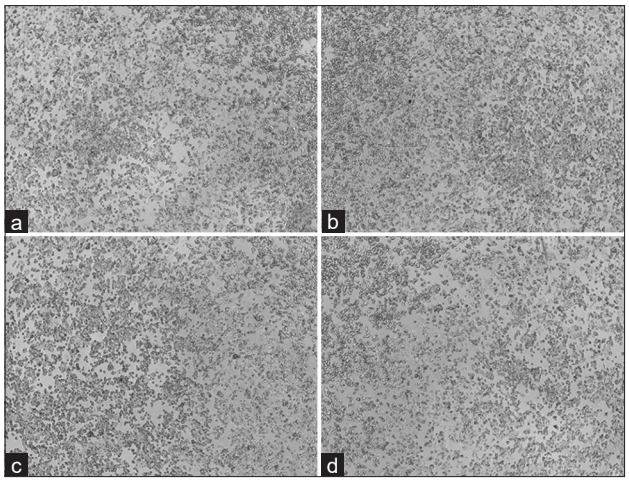


**Figure 3:** *In vitro* cytotoxic effect of *T. tratensis* ethyl acetate extract on HeLa cell line. a) Control, b) 3.125 µg/mL, c) 12.5 µg/mL and d) 100 µg/mL

was recorded in the case of HeLa cell lines (Table 5 & Figure 3). This extract also showed an $IC_{50}$ value of 12.5 µg/mL for both MDA MB 231 and HT-29 cell lines (Table 5).

The cytotoxic property of *T. tratensis* ethyl acetate extract can be attributed to compounds such as 1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester, and cyclononasiloxane, octadecamethyl- as these compounds are already reported to possess anticancer properties (Kumari & Menghani, 2021; Ganie *et al.*, 2024).

Antioxidant-rich substances scavenge free radicals and inhibit the radical chain reaction of oxidation, causing the oxidation process to be delayed or repressed. The antioxidant activity of phenols and their derivatives adds to their anticancer effects (Dai & Mumper, 2010). The present investigation found a positive relationship between the antioxidant and anticancer properties of *T. tratensis* ethyl acetate extract. The extract's antioxidant properties may be responsible for its anticancer potential. Furthermore, the extract has antioxidant action due to its high phenol and flavonoid content. The presence of phenolic chemicals and their derivatives has been demonstrated to cause cascade-based apoptosis in cancer cells, resulting in cytotoxicity (Robinson *et al.*, 2017). The synergistic effect of all bioactive substances in crude extracts enhances cytotoxicity. The increased speed of reaction and bioavailability of active molecules causes a rise in the combined effect of active compounds (Yaacob *et al.*, 2014).

## CONCLUSION

*Talaromyces tratensis* SS10 ethyl acetate extract exhibited potent antioxidant and anticancer properties. This is due to the presence of numerous bioactive compounds in the extract, including 1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester, Benzeneethanamine, N-[(pentafluorophenyl)methylene]-beta.,3,4-tris[(trimethylsilyl) oxy]- and cyclononasiloxane, octadecamethyl-. Further research on this extract could help determine how it might be used to treat cancer and other disorders brought on by oxidative stress. To confirm the biological activity of active principles found in *T. tratensis*, more studies using animal models are required.

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