Anticancer and antioxidant activity study along with GC-MS analysis of the essential oil of Ocimum tenuiflorum

Sribatsa Lanchhana Dash[1] Pranav Gupta[1*] Virendra Pratap Singh Rathore[1] Vikram Kumar Sahu[2]

[1]*Maharana Pratap College of Pharmacy, Kothi, Mandhana, Kanpur, Uttar Pradesh – 209217*

[2]*Maharana Pratap College of Pharmaceutical Sciences, Kanpur, Uttar Pradesh, India*

**Corresponding author :* Pranav Gupta

ABSTRACT

Objective: The recent work was carried out to evaluate the presence of bioactive compounds and anticancer activities of selected plant Ocimum Tenuiflorum essential oil.

Methods: The essential oil was prepared by using Clevenger apparatus. The presence of phytochemicals was determined using standard protocols. The anticancer activities were evaluated by sulforhodamine B (SRB) dye assay method.

Results: The qualitative analysis of plant essential oil showed the presence of bioactive compounds, namely, protein, alkaloids, steroids, phenolics, and flavonoids. The essential oil was tested for in vitro anticancer activity against human lung cancer (A-549) cell lines using SRB assay. The plant essential oil showed significant results against A-549 cancer cell lines.

Conclusion: The study concluded that the extract of O. tenuiflorum can be further carefully used in the treatment for cancer therapy.

Keywords: Synthetic, Anticancer, Ocimum tenuiflorum, Herbal, Flavonoids

INTRODUCTION

A diverse range of illnesses that may afflict individuals of all ages and spread to nearby tissues is known as cancer. According to data from the American Cancer Society, 2-4 percent of fatalities globally each year are attributable to cancer[1]. A benign or malignant tumour can result from disruptions in the closely controlled mechanism that normally governs the ratio of cell division to cell death[2]. A comprehensive analysis of research papers and publications revealed that herbal medicine demonstrates a range of therapeutic benefits and enhances the health security of primary care patients[3]. The WHO estimates that over 80% of people worldwide receive their main medical treatment from ayurvedic medicines. Previous reviews of the literature have demonstrated that Ayurveda, Unani, and Siddha have employed different portions of the plant to cure a variety of human and animal ailments. Regarding herbal remedies, *O. tenuiflorum* is a rich source of proteins, phenolics, alkaloids, flavonoids, steroids, and other compounds that may be used to cure a variety of illnesses, including cancer[4-8]. Conventionally, a variety of illnesses have been treated with herbal plants[9]. The study of herbal remedies in complementary and alternative medicine that address the prevention and treatment of cancer has received a lot of attention[10]. Bioactive chemical compounds found in medicinal plants are a broad collection that are both highly valued and easily accessible. Among the substances that have long been utilized in medicine are proteins, flavonoids, alkaloids, tannins, essential oils, and proteins [11–13]. Human leukaemia cell line (A-549) was the cancer cell lines used in the in vitro screening for the anticancer activity of the plant extract *Ocimum tenuiflorum* using the sulforhodamine B (SRB) assay, which produced noteworthy results [14-15].

O. tenuiflorum belongs to family Labiateae and the plant is very important for their therapeutic potentials. O. tenuiflorum (Labiatae) is a strongly scented small annual herb, up to 18 inches tall, grows into a low bush and is commonly known as holy basil[16]. O. tenuiflorum or tulsi essential oil are used in ayurvedic remedies for common colds, headaches, stomach disorders, inflammation, heart disease, various forms of poisoning and malaria. Traditionally, the plant is taken in many forms, as herbal tea, dried power or fresh leaf[17]. Several recent investigations using these extracts have indicates anti-inflammatory, antioxidant and immune-modulatory and antistress properties. In addition, it has been reported to have radioprotective and anti-carcinogenic property[18].

Our research was therefore designed to assess the presence of bioactive compounds and anticancer potential of *O. tenuiflorum* essential oil using two chosen human cancer cell lines, namely the lung cancer cell line (A549)[19].

MATERIALS AND METHODS:

Collection of plant material,and preparation of essential oil :

The plants were collected from Maharana college of Pharmacy, Mandhana, Kanpur UP India. The botanical name of selected plant is *O. tenuiflorum* which belongs to the family Labiateae. The plant leafs were washed with tap water and dried at room temperature in the laboratory. The essential oil was obtained by Clevenger apparatus and stored at 4°C.

The qualitative analysis of the oil

The qualitative analysis of the extracted oil was done using Gas Chromatography-Mass Spectrometry to identify the specific component.

SRB (Sulforhodamine B) assay

A quick and accurate colorimetric technique for assessing drug-induced cytotoxicity in adherent and suspended cell cultures is the SRB (Sulforhodamine B) test. The test, initially presented by Skehan and associates, was created for the National Cancer Institute's (NCI) large-scale, disease-focused drug development programme, which was initiated in 1985. Aminoxanthene dye SRB has two sulfonic groups and provides a vibrant pink colour. SRB provides a sensitive indicator of cellular protein in TCA-ixed (trichloroacetic acid) cells by binding to basic amino acid residues in proteins under moderately acidic conditions. The SRB test is also utilised to assess colony extinction and development[20].

1. Cell Culture:

Grow adherent cells to 80% confluency. Trypsinize and spin down the cells, add 5 ml of growth medium to disperse the cells. Determine the cell density by using a hemocytometer. Add growth

medium to the cells to adjust to an appropriate concentration. Add 200 microlitre of the cells with a recommended density between 5,000-20,000 cells/well to a 96 well clear flat bottom plate.

2. Compound Treatment :

Prepare serial dilutions of your testing compounds appropriately, using DMSO as solvent. Add compounds to the wells. Prepare a DMSO only well as vechicle control and another well containing culture medium only as background control.

3. Cell fixation :

Without removing the culture medium, add ¼ volume of the fixation solution to each well. Incubate the plate for 1 hr at 4ºC. Remove the solution and use 200 µl of dH2O to wash the 3 times. Washing should be done as gentle as possible to avoid disturbance of the cell monolayer. Remove wash solutions as much as possible by pipetting. After cell fixation, washing and drying steps are complete, the plate can be stored at room temperature for a month if desired.

4. SRB staining :

Add 45 µl of SRB Solution to each well and stain for 15 min at room temperature in the dark. Note that SRB should be protected from light as it is light sensitive. After incubation, remove the staining solution. Add 200 µl of 1X washing solution to each well 4 times. Washing should be done as quickly as possible to avoid bleaching. Remove wash solutions as much as possible by pipetting and air-dry the plate if necessary.

5. Solubilization :

Add 200 µl of 1X Solubilization to each well. Shake the plate occasionally or place the plate on a shaker for 10 min at room temperature.

6. Measurement :

Measure the O.D. at 565 nm. If intense color is observed (> O.D. 3.5) due ti cell overload you may use a suboptimal wavelength (eg. 490-530) to lower the reading back to the linear range of your instrument.

Calculation

$$\%\ \boldsymbol{Viable\ Cells} = \frac{\boldsymbol{A_{(test)}}}{\boldsymbol{A_{(control)}}} \times \mathbf{100}$$

Where,

$A_{(test)}$ = Absorbance of test sample

$A_{(control)}$ = Absorbance of control

Antioxidant activity:

DPPH assay (2,**2-Diphenyl-1-picrylhydrazyl free radical-scavenging capacity**)

In accordance with Karamać et al., the 2,2-diphenyl-1-picrylhydrazyl (DPPH)radical scavenging ability was measured.In brief, 100 µL of various essential oil concentrations (0.001, 0.01, 0.1, and 1.0 mg/mL) were combined with two millilitres of 0.5 millilitres per millilitre DPPH in methanol for the test. With an ultraviolet–visible (UV/VIS) spectrophotometer, the absorbance was measured at 517 nm following a 20-minute incubation period[21].

Using the following formula, the proportion of free radical-scavenging ability was determined:

$$Radical\ scavenging\ capacity\ \% = \frac{(Ablank - Asample)}{Ablank} \times 100$$

Where Asample is the absorbance of DPPH mixed with essential oil Ablank is the absorbance of DPPH in which sample has been replaced with methanol.

ABTS assay (2,2'-Azinobis-3-ethylbenzothiazoline-6-sulfonic acid cation radical-scavenging capacity)

Using the procedure developed by Rufino et al., the 2,2'-azinobis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS) test was used to measure the essential oil's ability to scavenge radicals. The ABTS radical solution was made by combining 2.6 mmol/L potassium persulfate with 7.4 mmol/L ABTS. Following the addition of 1900 µL of ABTS radical solution to 100 µL of sample, the mixtures' absorbance was measured at 737 nm using a UV/VIS spectrophotometer after 7 minutes[22]. Using the following formula, the free radical-scavenging capability was determined:

$$Radical\ Scavenging\ (\%) = 100 - (Asample - Ablank\ /Acontrol\ \times 100)$$

Where Asample is the absorbance of the ABTS mixed with the sample,
Acontrol is the absorbance of the ABTS mixed with deionized water
Ablank is the absorbance of the sample mixed with deionized water.
Acontrol is absorbance of the control (Trolox) mixed with deionized water act as positive control.

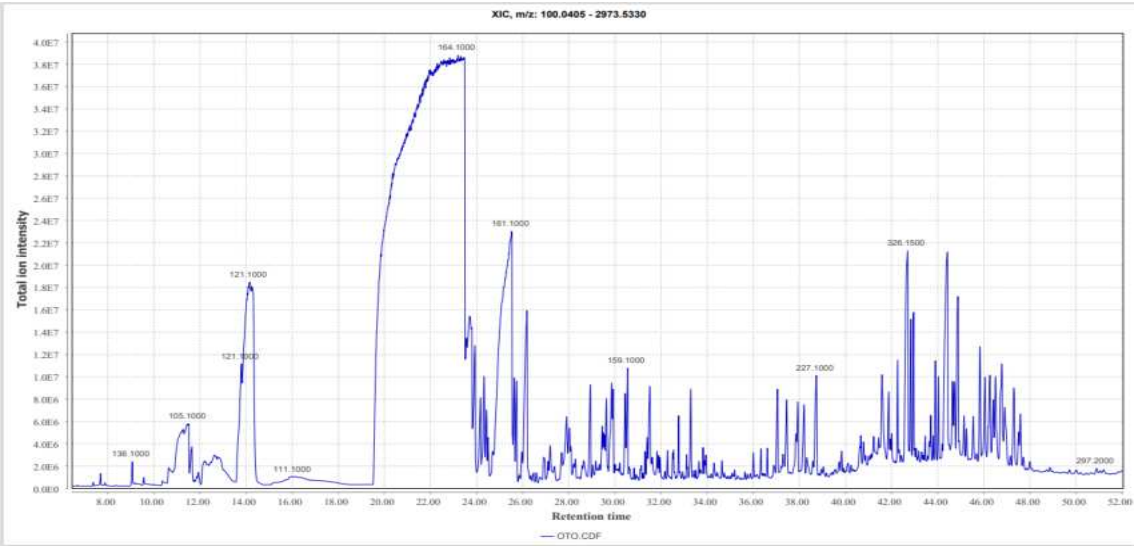
RESULT:

Identification of plant material by Gas chromatography mass spectroscopy (GC-MS) :

Sample was dissolved in Methanol and injected in an GC-MS QP2010 model (Shimadzu®), Column, GC, SH-I-5Sil MS Capillary, 30m x 0.25mm x 0.25um, injection mode: Splitless. The operating conditions of the GC–MS set for the analysis were as follows: oven temperature 45 °C for 2 min then 140 °C at 5°C/ min and finally increased to 280 °C and held isothermally for 10 min. The sample injection was 2 µL and the carrier gas was helium at 1 mL/min. The ionization of the sample components was carried out 70 eV. The running time of the GC was from 9.10 min – 52.0 min. 22 major compound were identified in essential oil of *O. tenuiflorum* which are:

Compound Name and Retention time

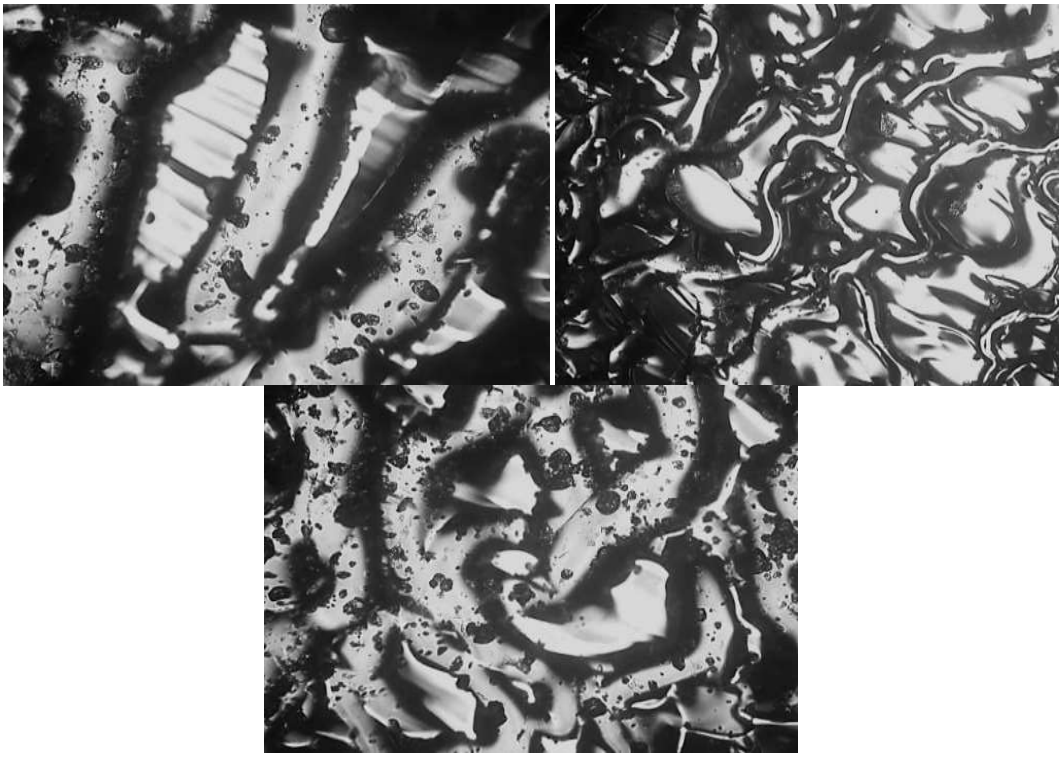
Compound Name	Retention time
Alpha.-Pinene	7.885
Beta.-Myrcene	9.568
Gamma.-Terpinene	11.931
Caryophyllene	23.768
Gamma.-Muurolene	24.167
14-Hydroxycaryophyllene	27.189
Isoaromadendrene epoxide	27.368
Caryophyllene oxide	28.027
Junenol	29.033
Spathulenol	29.326
Alpha.-Cadinol	29.554
Epizonarene	30.001
Aristolene epoxide	31.217
Ylangenol	31.617
Jaeskeanadiol	33.512
Muurola-4,10(14)-dien-1.beta.-ol	33.644
Valtrate	33.706
Hexadecanoic acid, methyl ester	33.899
Salvial-4(14)-en-1-one	33.940
Aromadendrene oxide-(2)	34.640
Phytol	36.487
Delta.9(11)-Methyltestosterone	38.325



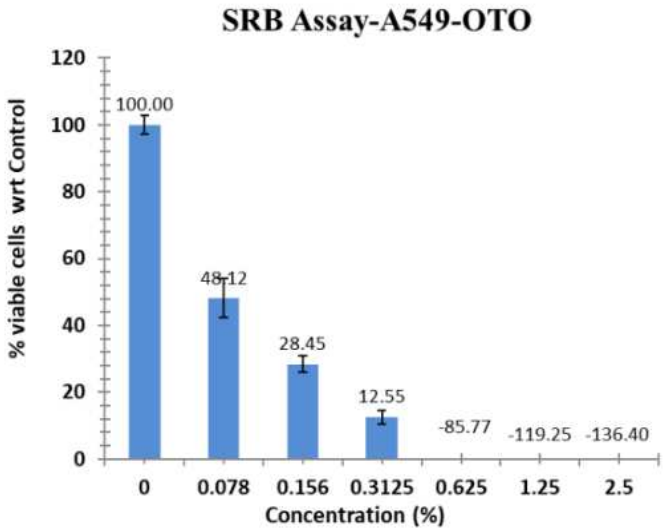
GC-MS Chromatogram

In vitro cytotoxicity (Sulphorhodamine B assay SRB) Study:

The A549 cell line (purchased from NCCS Pune) was used to test the offered substances' cytotoxicity using the SRB Assay. The 96-well plate was grown with 8000 cells/well for 24 hours at 37°C with 5% CO2 in DMEM (HIMEDIA-AT149-1L) medium supplemented with 10% FBS (HIMEDIA-RM 10432-500M) and 1% antibiotic solution (Penicillin-Streptomycin-Sigma-Aldrich P0781). The following day, cells received treatment at various dosages and concentrations (as per indicated in the excel sheet). Samples were diluted in incomplete cell culture medium (without FBS) to obtain varying concentrations.Treatment-free cells were referred to as Control cells, while SRB-free cells were referred to as Blank cells. Following a 24-hour incubation period, 100 µL of Tri Chloro Acetic Acid (TCA, 10%-Fisher Scientific-28444) was introduced into each well and allowed to incubate for an additional hour. Subsequently, the plate was cleaned using DM water and allowed to air dry at room temperature. Each well received one hour of SRB Solution (Ottokemi3520-42-1) at a final concentration of 0.04%. After a one-hour incubation period, the plate was allowed to air-dry at room temperature and then cleaned with 1%(v/v) acetic acid (SRL Chem-64-19-7) to get rid of any unbound dye. After adding the Tris base solution (pH=10.5 to the well, it was agitated for ten minutes on an orbital shaker to dissolve the protein-bound dye, which was then measured at 510 nm using an Elisa plate reader (iMark, Biorad, USA). The IC50 was computed using the Graph Pad Prism -6 programme. Pictures were taken with an AmSope digital camera (10 MP Aptima CMOS) under an inverted microscope (Olympus ek2).



Statistical Analysis:



***IC*$_{50}$ value = 0.038%**

Interference:

The A549 cell line was treated to several concentrations of the sample, and cytotoxic activity was evaluated for sample OTO and 50% inhibitory concentration, as indicated in table 1, based on the SRB test findings. The concentration of an inhibitor, sample, or formulation at which the number of viable cells is halves is known as the IC50. It was discovered that the sample was very cytotoxic to the A549 cell line.

Antioxidant assay:

DPPH assay:

The resultant OD value and %scavenging for sample using DPPH Assay method

S.No.	Concentration of sample (mg/ml)	Sample	Blank	%scavenging activity
1.	0.001	0.2	0.214	**6.54**
2.	0.01	0.19	0.214	**11.21**
3.	0.1	0.15	0.214	**29.91**
4.	1	0.09	0.214	**57.94**

ABTS assay:

The resultant OD value and %scavenging for sample using ABTS Assay method

S.NO.	Concentration of sample (mg/ml)	Sample	%scavenging activity
1.	0.001	0.1	**1.61**
2.	0.01	0.24	**24.12**
3.	0.1	0.31	**35.37**
4.	1	0.55	**73.95**
5.	Control	0.622	
6.	Blank	0.09	

Discussion

The qualitative test results showed that *O. tenuiflorum* essential oil contains bioactive compounds, namely, proteins, phenolics, alkaloids, flavonoids, and steroids. The anticancer potential was determined by the cytotoxic potential of the test material using liver and colon cancer cell lines (A549), which were grown on tissue culture plates in the presence of test material. The cell growth is measured after staining with SRB dye that binds to basic amino acid residues in trichloroacetic acid fixed cells. The *O. tenuiflorum* have rich source of bioactive organic compounds such as proteins, phenolics, alkaloids, flavonoids, and steroids that possess unique medicinal activities. Therefore, plant containing these bioactive organic compounds may serve as a potential source in the treatment of lung cancer. The possible cause of cancer activity is due to the free radical formation. Hence, plants containing phenolics, alkaloids, flavonoids, steroids, etc., are constantly being screened for anticancer activity. Some of the active compounds present in this plant are reported to be antioxidant. The activity of compounds should be depended more likely to the type and concentration of cancer cell lines. The major challenge is to design new drugs that will be more effective for the treatment of cancerous cells and have lesser side effect. Many plant extracts kill and reduce the growth of cancerous cell through activating apoptosis and affecting growth regulators[23].

CONCLUSION

The work authenticated the *O. tenuiflorum* plant contains bioactive compounds that have a potential to reduce cancer cell growth in vitro by different inhibitory pathways. Many plant extracts destroy cancer cells through activating apoptosis and effecting growth regulators. The research work was very interesting to study the anticancer potential, biochemically identification of compounds, and mechanism of action of the phytoconstituents. Finally, it can be concluded that the phytoconstituents of this plant can be further used in the treatment of lung cancer.

Acknowledgements

Authors are thankful to the college authority for providing necessary facilities to conduct the work.

Conflict of interest:

The authors declare no conflict of interest.

Funding

None

Ethical approvals

This study does not involve experiments on animals or human subjects.

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