Antioxidant And Anticancer Activity Of Root Extract Of Ocimum Tenuiflorum L. (Tulsi)

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***Abstract* - Indian medicinal plants known for its various medicinal properties. The present investigation was to study the antioxidant and anticancer activity from the roots of Ocimum tenuiflorum. The preliminary phytochemical screening of root extract of ocimum tenuiflorum revealed the presence of flavanoids, alkaloid, saponins, tannins and polyphenols. In the present study the root extract of plant were investigated with various antioxidant systems. The results indicated that, the plant possessed abundant phenolic and flavonoids contents and exhibited excellent antioxidant activities. The effect of root extract of breast cancer cells MCF-7 are expressed as % cell viability. The results indicated that root extract of Ocimum tenuiflorum showed 87.39% cell viability.**

***keywords* - Ocimum tenuiflorum, Antioxidant, Anticancer activity,MCF-7**

INTRODUCTION

Antioxidants occur naturally in various foods and are essential for our health. The antioxidants, both natural and synthetic antioxidants are widely used as food additives. Antioxidants are mostly added to edible fats and fat containing foods, which prolongs the shelf life of foodstuffs by protecting them against deterioration such as fat rancidity, unpleasant odors and color change caused by oxidation. Naturally occurring antioxidants include tocopherols (vitamin E), bioflavonoids (citrin), retinoids (vitamin A), polyphenols (hydroxytyrosol), and ascorbic acid (vitamin C). These natural antioxidants play a major role in the prevention of cancer, heart disease, ageing and immune deficiency diseases. Researchers reported that the synthetic antioxidants such as Tertiary Butyl Hydroquinone (TBHQ), Propylgallate, Butylated hydroxytoluene (BHT), Butylated hydroxyanisole (BHA), causes severe liver cirrhosis and carcinogenesis (1). The synthetic antioxidants due to their high volatility, instability at elevated temperature, carcinogenic nature, and the consumer choices have made the manufacturers to shift their attention towards natural antioxidants (2).

Recently there has been worldwide attention towards the usage of natural substance in therapeutic antioxidants especially in medicinal and dietary plants due to the increased presence of many deadly disease risk factors to the human population. The industries use 90% medicinal plants collected from the wild. The pharmaceutical industry utilizes 800 species for drug production and for commercial cultivation only less than 20 plant species are used. About 70 percent of the plant collections comprise of destructive harvesting as the plant's roots, bark, wood, stem, and in herbs, the whole plants are used (3).

Breast cancer is the second leading cause of death following heart diseases, representing about 23% of all deaths. The breast is the most leading sites of cancer in females. In India, 19-34% of women have breast cancer, and in recent years, researchers assumed that cervical cancer as a leading site of cancer (4). Breast cancer, a malignant growth starts within the tissues of the breast primarily from the epithelial component of the gland where it proliferates at high rate, and resulting necrosis of surrounding tissues (5)

Ocimum tenuiflorum (Tulsi) family: Lamiaceae (Holy Basil) has a various medicinal activities. The potential therapeutic properties of *Ocimum tenuiflorum* are wide ranging and include the treatment and prevention of diabetes, cardiovascular diseases and cancer.(6)

Collection and authentication plant material

The fresh roots of *Ocimum tenuiflorum* were collected from the market at Mylapore at Chennai and the samples were identified, authenticated by Dr. S. Sankaranarayanan, Head of the Department, Department of Medicinal Botany, Government Siddha Medical college, Arumbakkam, Chennai.

Processing of Plant material and Solvent extraction

The roots of *Ocimum tenuiflorum* were collected, washed under running tap water followed by distilled water and shade dried for 5 days .The dried roots were ground to fine powder and sieved. 50 g of finely grounded roots was weighed and soaked in70% methanol at room temperature for 24 hrs. Then the extract was filtered using Whattmann filter paper No.1 and then concentrated to dryness in vacuum at 40°C-50°C (overnight) using a rotary evaporator. The residue was thoroughly mixed with methanol and used for further studies.

Phytochemical Screening

The methanol extract roots of *Ocimum tenuiflorum* of was subjected to phytochemical screening to determine the presence of secondary metabolites such as alkaloids, flavonoids, terpenoids, tannins, glycosides, saponins and polyphenols using standard procedures (7&8).

Determination of total phenolics and total flavonoids content

The total phenolics content was determined according to the Folin-Ciocalteu method [9] and the results were expressed as mg gallic acid equivalent/g of dry weight of extract. While the total flavonoids content of the aqueous extract were measured by the method of [10] and the results expressed as mg quercetin equivalent/g dry weight. All tests were performed in triplicate and mean was centered.

Reducing capacity assessment

The reducing power activity was determined by the spectrophotometric method of (11). An amount of 20-120µg of methanol extract from the roots of *Ocimum tenuiflorum* was thoroughly mixed with 2.5 ml of 200 mM phosphate buffer (pH 6.6) followed by the addition of 2.5 ml of 30 mM potassium ferricyanide. The mixture was incubated at 50 °C for 20 min. To the mixture, 2.5 ml of trichloroacetic acid (600 mM) was added and centrifuged at 3000 rpm for 10 min. 2.5ml of supernatant solution was mixed thoroughly with 2.5 ml of distilled water and $FeCl_3$ (0.5 ml, 6 mM). The antioxidants present in the extract results in the reduction of Fe^{3+} to Fe^{2+}. The Perls Prussian blue colour was observed and read at 700nm. Vitamin C was used as a positive control.

Superoxide radical Scavenging assay

Superoxide radical scavenging assay was based on the extract's inhibition capacity of the photochemical reduction of nitroblue tetrazolium (NBT) (10) in riboflavin-light-NBT system presence, as mentioned previously (12 & 13). In short, respective 3 ml reaction mixture comprise of 50 mM phosphate buffer with pH 7.8, 13 mM methionine, 2 µM riboflavin, 100 µM EDTA, 75 µM NBT along with methanol extract of *Ocimum tenuiflorum* at varied concentrations. Mixture placed in front of fluorescent light and after 6 min using an ELICO (SL150) UV–Vis Spectrophotometer absorbance was measured at 560 nm. Similarly, matching tubes filled with reaction mixture were placed in the dark and these tubes used as blanks. Percentage of superoxide generation inhibition was estimated by relating the control's absorbance with that of the reaction mixture comprising test sample solution.

$$\text{Percentage (\%) of Super oxide radical scavenging capacity} = [(A_0 - A_1)/A_0] \times 100$$

Here, A_0 is the control's absorbance and A_1 is the organic solvent extract or standard's absorbance.

ABTS Radical Scavenging assay

ABTS (2,2'-azino-bis-3-ethyl benzothiazoline-6-sulphonic acid) radical scavenging assay of aqueous methanol extract of petals four different medicinal plants was determined according to the standard procedure Re *et al.* (1999). ABTS radical cation ($ABTS^{+}$) was prepared freshly by dissolving 14 mM ABTS solution (5ml) to 4.9 mM potassium persulfate solution (5ml) and placed in the dark for 12-16 hours at room temperature before use. This reaction mixture was diluted with distilled water to obtain an absorbance of 0.70 ±0.05 at 734 nm and it is used for the antioxidant assay. To the test tubes, both test and standard, 950 µl of ABTS solution was added. The different concentration of methanol extract of *O. tenuiflorum* (20-120 µg) was added to the test group and 50 µl of Vitamin - C was added to the standard. The reaction mixture of both test and standard was vortexed thoroughly for few minutes and incubate at room temperature for 6 minutes. The absorbance was read at 734 nm by using ELICO (SL150) UV–Vis Spectrophotometer. Ascorbic acid was used as reference antioxidant compound. The percentage of ABTS scavenging activity was calculated as;

$$\text{\% ABTS radical scavenging activity} = (A_0 - A1)/A_0 \times 100$$

ANTICANCER ACTIVITY

Cell line and Culture

From National Centre for Cell Science (NCCS), Pune, MCF-7 cell lines were obtained. These cells were kept in Minimal Essential Media added with 10% FBS, 100 U/ml of penicillin, and 100 µg/ml streptomycin and maintained in 5% moistened CO2 incubator at a temperature of 37°C. The cells were cultured in a sterilzed condition and exponentially growing cells were used for experimental studies.

Reagents: Minimal Essential Medium (MEM), Trypsin, Fetal Bovine Serum (FBS), Dimethyl Sulfoxide (DMSO), Methylthiazolyl diphenyl- tetrazolium bromide (MTT), Hank's Balanced Salt Solution (HBSS) are the reagents used in the study. All the reagents were purchased from 'Hi media & Sigma Aldrich', Mumbai.

***In Vitro* assay for Cytotoxicity actvity (MTT ASSAY)**

MTT assay was done to determine the anticancer activity of samples on MCF7 (14). Cells about 1×10^5 per well were plated in 0.2 ml of medium per well in 96-well plates. For the next 72 hours' cell plates were incubated at 5 % CO_2 incubator. The samples were added with varied concentrations in 0.1% DMSO at 5% CO_2 incubator for 24 hours. The sample solution was removed and followed by adding 20µl/well (5mg/ml) of 0.5% 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-tetrazolium bromide (MTT) in phosphate buffered saline. 1ml of DMSO was added after 4hrs of incubation. The viable cells were obtained and the absorbance was read at 540nm. The IC50 values of the compound were determined through graph where the increase in concentration reduced the cell viability percentage by 50%. The percentage (%) of cell viability, which is represented by the given formula:

$$\text{Percentage of cell viability} = A_{540} \text{ of treated cells} / A_{540} \text{ of control cells} \times 100\%$$

RESULTS AND DISCUSSION

Preliminary phytochemical screening

The phytochemical analysis of root extract of *O. tenuiflorum* showed the presence of alkaloids, terpenoids, phenolic compounds, flavonoids, glycosides and saponins (Table 1)

S. No	Phytochemicals	Results
1	Alkaloids	+
2.	Terpenoids	-
3	Steroid	+
4.	Phenols	+
5.	Flavanoids	+
6.	Tannins	+
7.	Glycosides	-
8.	Saponins	+

Table 1: Preliminary Phytochemical screening of root extract of *O. tenuiflorum*

Total phenols and flavonoids

The total phenol and flavonoid compounds quantified in the root extract of *O. tenuiflorum* seemed to be responsible for the antioxidant activity. The total phenol content was 350.2 µg/mg of GAE and the total flavonoid content was 6.38 µg/mg of QE in the extract. These results provide a comprehensive profile of the antioxidant activity *of O. tenuiflorum* with respect to their phenols and flavonoids content (Table 2).

Medicinal plant	Total phenolic content (GAE) µg/mg	Total Flavanoid content (QE) µg/mg
O. tenuiflorum	350.2	6.38

Table 2: Total phenol and flavanoid content of root extract of *O. tenuiflorum*

Reducing power activity

The reducing power assay was carried out by the reduction of Fe^{3+} to Fe^{2+} by the root extract of *O. tenuiflorum* and the subsequent formation of ferro-ferric complex. The reduction ability increases with increase in concentration of the extract. The maximum Fe^{3+} reduction was 85.29±5.97% at 30 µg/mL concentration and was compared with the standard The IC50 value of 38.03µg/mL Ascorbic acid. (Table 3)

S. No.	Concentration (µg/mL)	Fe^{3+} reducing power @ 700nm
		Root extract
1	5	39.52±2.76
2	10	52.58±3.68
3	15	65.87±4.61
4	20	77.56±5.42
5	25	81.66±5.71
6	30	85.29±5.97

Table 3: Reducing power reduction activity root extract of *O. tenuiflorum*

Superoxide radical scavenging activity

Superoxide can also reduce certain iron complexes such as cytochrome C. Superoxide anions are thus precursors to active free radicals that have potential for reacting with biological macromolecules and thereby inducing tissue damage. Also, superoxide has been observed to directly initiate lipid peroxidation. It has also been reported that antioxidant properties of some flavonoids are effective mainly via scavenging of superoxide anion radical. Superoxide radicals are normally formed first, and their effects can be magnified because they produce other kinds of free radicals and oxidizing agents. it has maximum absorbance in 560nm. The maximum supueroxide reduction was 58.06±4.06% at 30 µg/mL concentration and was compared with standard ascorbic acid (IC_{50} = 50.95µg/mL concentration).(Table 4)

S. No.	Concentration (µg/mL)	Superoxide radical scavenging assay @ 590 nm
		Root extract
1	5	14.19±0.99
2	10	22.58±1.58
3	15	30.32±2.12
4	20	40.64±2.84
5	25	49.03±3.43
6	30	58.06±4.06

Table 4: Superoxide radical scavenging activity root extract of *O. tenuiflorum*

$ABTS^{\cdot+}$ radical cation scavenging assay

$ABTS^{\bullet+}$ is a blue chromophore produced by the reaction between ABTS and potassium persulfate and in the presence of the plant extract or ascorbic acid, preformed cation radical gets reduced and the remaining radical cation concentration was then quantified. The maximum $ABTS^{\bullet+}$ radical cation scavenging activity was 79.37% at 30 µg/mL concentration and compared with standard Ascorbic acid (IC_{50} = 19.08 µg/mL concentration).(Table 5)

S. No.	Concentration (µg/mL)	$ABTS^{\cdot+}$ radical cation scavenging assay @735nm
		Root extract
1	5	12.41±0.84
2	10	28.96±2.02
3	15	48.96±3.42
4	20	52.41±3.66
5	25	68.96±4.82

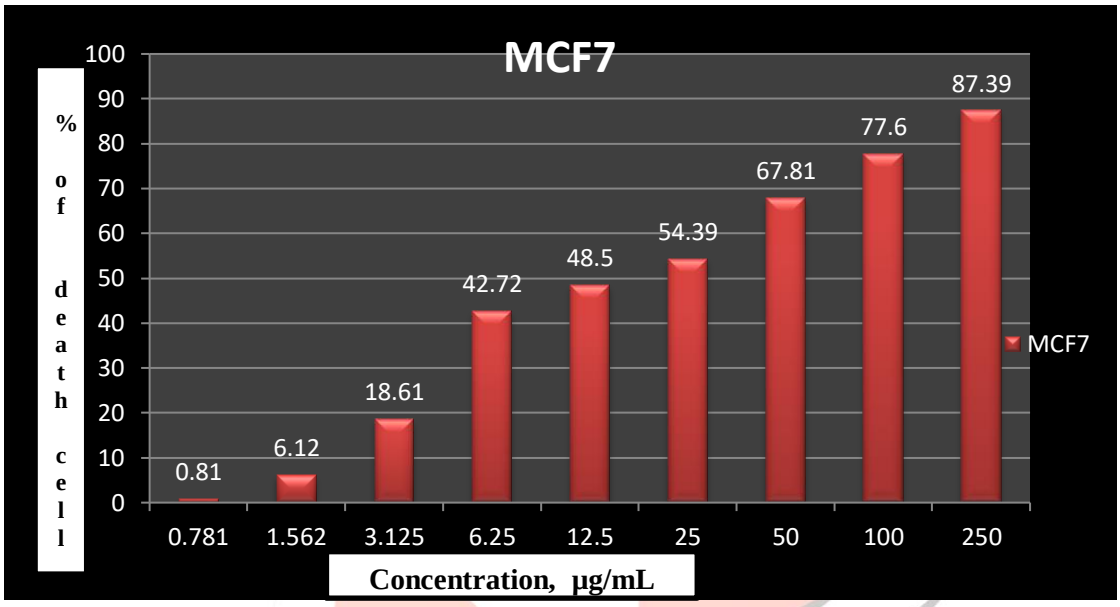
6	30	79.37±4.82

Table 5: ABTS radical scavenging activity root extract of *O. tenuiflorum*

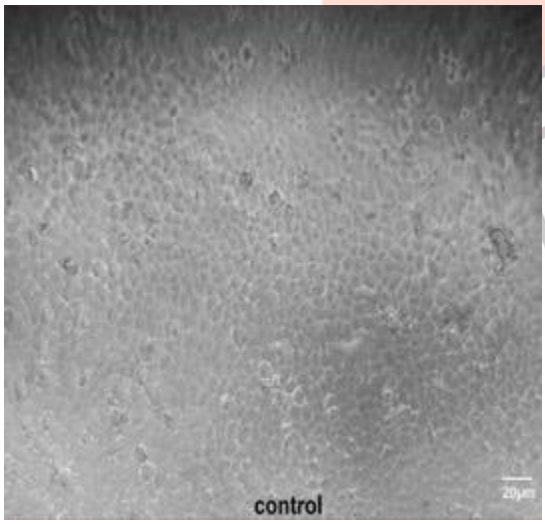
ANTICANCER ACTIVITY OF ROOT EXTRACT OF *OCIMUM TENUIFLORUM*

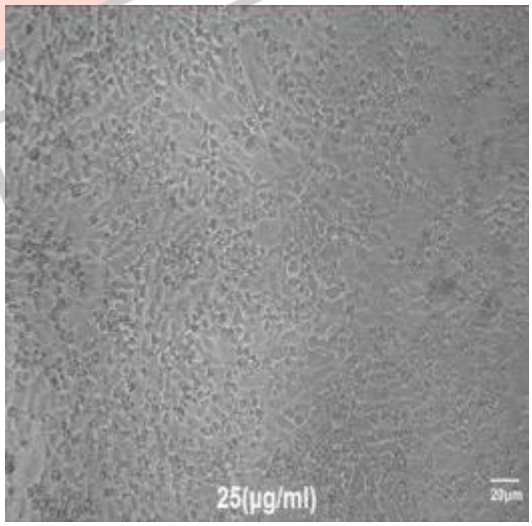
The cytotoxic effect of root extract of *ocimum tenuiflorum* against breast cancer cell lines MCF-7 was determined by a rapid colorimetric assay using MTT (methyl-thiazolyl-tetrazolium bromide) assay.. Measurements were performed and the concentration required for a 50% inhibition of viability (IC_{50}) was determined graphically (Graph-1 Fig-1). The effect of the root extract of MCF cells was expressed as the % cell viability.

The results indicated that the maximum MCF7 cell death was 87.39 ± 0.62% at 250 µg/mL concentration **of** root extract of *Ocimum tenuiflorum* and the IC_{50} was 12.88 µg/mL concentration.The previous studies reported the antiproliferative activity of phenolics (15) but the mechanism of action have not been clearly understood yet. The various mechanisms include metabolite activation of promutagen (16),acts as blocking agents and results in formation of adducts with the help of mutagens, free radical scavenging, suppression of tumour cell invasiveness and finally inhibition of matrix metalloproteinase-2/-9 activity (17).



Graph 1: **Anticancer effect of root extract of ocimum tenuiflorum against MCF-7 breast cancer cell line.**





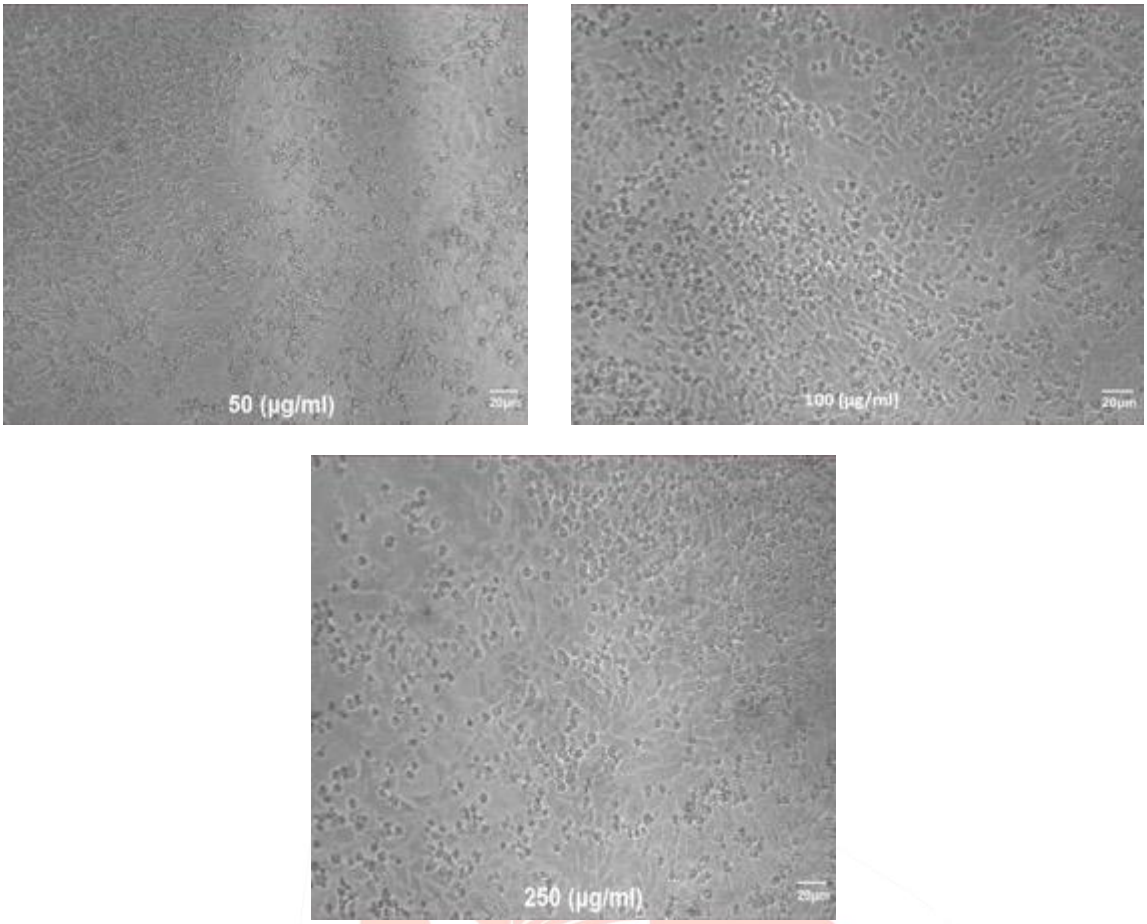


Fig 1: Microscopic view of MCF -7 Breast cancer cells of control and treated cells with root extract of *Ocimum tenuiflorum*. The morphology of MCF-7 cells progressively changed from 7.8 µg/ml to 250 µg/ml concentration of the extract and was compared with control

CONCLUSION

Phytochemical screening of root extract of *Ocimum tenuiflorum* had showed the presence of flavonoids, terpenoids, phenols, carbohydrates and proteins. The results of the present study indicate that root extract of *Ocimum tenuiflorum* exhibited the highest antioxidant activity in all the assays . The effect of root extract of breast cancer cells are expressed as % cell viability. The results indicated that root extract of ocimum tenuiflorum showed 87.39% cell viability. In future study, the bioactive compounds from *ocimum tenuiflorum* can be identified for the development of the anticancer drug.

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