Antioxidant, anticancer and molecular docking activity of tulsi plant

C.M. Noorjahan and T. Saranya

P.G. and Research Department of Zoology, Justice Basheer Ahmed Sayeed College for Women, Teynampet, Chennai-600 018, Tamil Nadu, India.

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ABSTRACT
An investigation was carried out to study the antioxidant, anticancer and docking activities of medicinal plant, Tulsi – Ocimum sanctum. The results of antioxidant activity of Tulsi leaves extract showed that tulsi leaves has good free radical scavenging ability and IC₅₀ value was found to be 54.23% at 500 µg/ml. The results of anticancer activity of Ocimum sanctum on normal VERO cell and MCF-7 cell line has showed good anticancer activity having the IC₅₀ concentration at 51.1 µg/ml against MCF-7 cell lines – Human Breast cancer cell line thereby indicating that the percentage of cell viability increases with the increased concentration of Ocimum sanctum (Tulsi) whereas 24.4% cell viability was recorded in the normal VERO cell line. DNA laddering assay was performed on agarose gel electrophoresis. A clear fragmented DNA ladders were observed in tulsi treated MCF-7– Human Breast cancer cell lines. But the untreated normal VERO cell lines did not show any DNA fragmentation. Thereby confirms that Ocimum sanctum induced cell death on breast cancer cell line MCF-7 cancerous cell through apoptosis. Molecular docking was carried out (AutoDock tools), Docking simulations was performed using Lamarckian genetic algorithm of Solis and Wests local search method. Interaction between HDAC6 and Cirsilileon possessing binding energy showed +15.91kcal for interaction studies. It was found that most of the hydrogen bonding is with the residues TYR 76, TRP 35, SER 67, TYR 81, GLU 33, TYR 48, ILE 69 and MET 53 present in the binding pocket. The ligand was docked with the target protein, and the best docking poses were identified and the binding poses of the cirsilieon was shown. This act as the best docking poses shows how the ligand molecule fits into the binding region of the target protein.

Key words: Anticancer normal, Antioxidant, Breast cancer cell line, MCF, Molecular docking, Tulsi plant leaves, VERO cell line.

INTRODUCTION
Medicinal plants and herbs have been proved to be of great importance to the health of the individuals and communities. In recent years, many scientific investigations of traditional herbal remedies for several diseases have been carried out and this has lead in the development of alternative drug and therapeutic strategies. Since the consumption of medicinal plants is increasing, it is interesting to use these plants as a supplement in food taking into account that these plants can present a significant amount of trace elements (Alves et al., 2000 and Andrade et al., 2005) and other nutrients.

Thus, such plants should be investigated to better understanding for their properties, safety practices in addition to usefulness (Nascimento et al., 2000). Numerous plants have been reported to have anticancer effects or to complement conventional therapeutics by targeting various hallmarks of cancer. Phytochemicals have been shown to interfere with stabilization of the microtubule structure, thereby inhibiting mitosis and cancer cell propagation. Based upon the above literature cited related to biological activities of medicinal plants, an investigation was carried out to study antioxidant, anticancer and molecular docking activities of medicinal plant, tulsi – Ocimum sanctum.

Aims and Objectives of the study are to determine antioxidant property of tulsi plant (Ocimum sanctum), to determine anticancer activity of Tulsi plant (Ocimum sanctum) and to study the molecular docking of tulsi plant.

MATERIALS AND METHODS
Tulsi plant was collected from local nursery located in Chennai, Tamil Nadu, India. The fresh leaves were collected and they were washed carefully under cool running water and then with sterilized distilled water. The leaves were then dried for 7 to 15 days, dried leaves were homo genized to a fine coarse powder using mortar and pestle and then stored in fine air tight container for further process.

Normal VERO cell line and MCF, breast cancer cell line were obtained from the laboratory of Royal Bio Research, Chennai, Tamil Nadu, India. The cells were maintained in Minimal Essential Medium supplemented with 10% Fetal Bovine Serum (FBS), Penicillin (100 µg /ml), and Streptomycin (100 µg/ml) in a humidified atmosphere of 50 µg/ml carbon-di oxide at 37°C.
Fresh leaves of tulsi plant were softly eroded in deionized water to remove the dust particles. Preparation of tulsi leaf powder was carried out by following the procedure of Tayyaba Naseem and Muhammad Akhyar Farrukh (2015).

50 gms of the powdered tulsi leaves was taken and soaked in 250 ml of aqueous solution (Distilled water), allowed to stand overnight and filtered to obtain aqueous extract of Tulsi leaves.

Antioxidant activity of tulsi was determined using 1,1, diphenyl-2-picrylhydrazyl-2-picrylhydrazyl (DPPH Assay). The ability of the sample to scavenge free radical was estimated according to the method of Blois (1958).

In vitro assay for cytotoxicity activity and anticancer activity (MTT assay) of Ocimum sanctum (Tulsi) leaves extract was carried out by following the procedure of Mosmann (1983). The % cell viability was calculated.

\[
\% \text{ cell viability} = \frac{A_{570} \text{ of treated cells}}{A_{570} \text{ of control cells}} \times 100
\]

Cell control and sample control were included in each assay to compare the full cell viability assessments.

DNA Fragmentation of normal VERO cell lines and MCF7 – Breast Cancer cell line were studied by DNA fragmentation assay by following the procedure of Su et al. (2005).

For molecular docking of tulsi plant extract, three dimensional crystal structure of HDAC -6 (PDB ID: 3PHD) was downloaded in pdb format from the protein data bank. After that, the structure was prepared and refined using the protein preparation wizard of Docking server, charges and bond orders were assigned; hydrogen were added to the methionines, and all waters were added using force field OPLS_2005, minimization was carried out setting maximum heavy atom RMSD (root-mean-square-deviation) to 0.30 Å. Docking calculations were carried out using Docking Server (Bikadi, Hazai, 2009).

The data obtained from the experiments was analysed and expressed as mean and Standard Deviation.

RESULTS AND DISCUSSION

Plants plays important roles in discovery associated with new beneficial therapeutic agents and have received significant focus because of their bioactive substances like antioxidants. Hence an investigation was carried out to study the antioxidant, anticancer and molecular docking activities of medicinal plant, Tulsi – Ocimum sanctum leaves.

The free radical scavenging assay is based on the reduction of 1,1 diphenyl-2-picrylhydrazl (DPPH). The ability of the sample and the standard ascorbic acid to scavenge the free radical at different concentrations from 100µg/ml to 500µg/ml of Tulsi leaves extract was depicted in Table - 1. From the results of the antioxidant study, it was observed that there was an increase in percentage of inhibition as the concentration of plant leaves extract increases. However, the highest percentage of inhibition was recorded as 52±1.93% in 500 µg/ml of sample and lowest percentage of inhibition was recorded as 12.98±3.57% in 100 µg/ml of sample. Thus the study showed that tulsi leaves extract has a good free radical scavenging ability and IC₅₀ value was found to be 54.23% at 500 µg/ml tulsi leaves extract. The values were significant at 0.5% level. This is supported by the work of Eshrat et al., 2001.

Tulsi leaves extract - MTT assay was carried out against normal VERO cell lines and MCF7– Human Breast Cancer cell line. The results of anticancer activity of tulsi leaves extract on normal VERO cell and MCF7 - Human Breast cancer cell line were shown in Plates - 1 and 2 and Table 2. The results of the anticancer study revealed that tulsi leaves extract showed good anticancer activity which has the IC₅₀ (51.1 %) at 25 µg/ml concentration of tulsi leaves extract against MCF7 – Breast cancer cells. The results of

### Table 1: Antioxidant activity of tulsi (Ocimum sanctum) leaves extract (DPPH Assay).

| Concentration (µg/ml) | % of Inhibition | Mean ± Standard Deviation | Chi-Square |
|-----------------------|-----------------|----------------------------|------------|
|                       | Standard        | Ocimum sanctum             |            |
| 100                   | 86.6            | 11.8                       | 12.98±3.57 | 0.5         |
| 200                   | 90              | 16.94                      | 17.40±0.80 |            |
| 300                   | 91.6            | 28.81                      | 29.83±0.901|            |
| 400                   | 95              | 35.59                      | 34.25±1.807|            |
| 500                   | 98.3            | 54.23                      | 52±1.93    |            |

Mean±St.Deviation
The values are significant at 0.5% level.

### Table 2: Anticancer activity of tulsi (Ocimum sanctum) leaves extract against Normal VERO Cell and MCF7 – Human Breast Cancer cell lines.

| Concentration (µg/ml) | % cell Viability of Control - VERO Cell line | % cell Viability of MCF7 – Cell line |
|-----------------------|---------------------------------------------|-------------------------------------|
| 100                   | 24.4                                        | 24.4                                |
| 50                    | 33.8                                        | 33.8                                |
| 25                    | 51.1                                        | 51.1                                |
| 12.5                  | 63.7                                        | 63.7                                |
| 6.25                  | 75.5                                        | 75.5                                |
| 3.12                  | 95.2                                        | 87.6                                |
| Control cells         | 100                                         | 100                                 |

The values are significant at 0.5% level.
Plate 1: Anticancer activity of tulsi (*Ocimum sanctum*) leaves extract on normal VERO cell lines.

Plate 2: Anticancer activity of Tulsi (*Ocimum sanctum*) leaves extract on MCF<sub>7</sub> – Human Breast cancer cell lines.

Plate 3: DNA Fragmentation of Tulsi (*Ocimum sanctum*) leaves extract on MCF<sub>7</sub> – Human Breast cancer cell lines.

Fig 1a: Three dimensional structure of HDAC-6 of tulsi (*Ocimum sanctum*) leaves extract on MCF<sub>7</sub> – Human Breast cancer cell lines.

Fig 1b: Molecular interaction of HDAC-6 with Cirsilineol of tulsi (*Ocimum sanctum*) leaves extract on MCF<sub>7</sub> – Human Breast cancer cell lines.
the study also showed that normal VERO cell line has 95.2% cell viability recorded in 3.12 μg/ml concentration of tulsi leaves extract. Similar results were observed by Lam et al., 2018.

DNA laddering assay was performed on agarose gel electrophoresis, to determine tulsi leaves extract cell death of MCF, – Breast cancer cell line via apoptosis. A clear fragmented DNA ladders were observed in MCF, – Breast cancer cell lines treated with tulsi leaves extract and untreated normal VERO cell lines did not show any DNA fragmentation. DNA laddering assay was performed on agarose gel electrophoresis (Plate - 3). Therefore the data obtained from this study confirms that Ocimum sanctum leaves extract induced cell death in MCF, – Breast cancer cell line. This work was supported by Mangala Gowri and Priya, (2017).

The results of molecular docking were shown in fig- 1a and 1b which shows the binding poses of the cirsilieon. The binding energy for Cirsilileon was estimated by Docking server. Interaction between HDAC6 and Cirsilileon possessing binding energy shows +15.91kcal for interaction studies. The figure displays the binding poses for the presence of hydrogen bonds. It was found that most of the hydrogen bonding were with the residues TYR 76, TRP 35, SER 67, TYR 81, GLU 33, TYR 48, ILE 69 and MET 53 present in the binding pocket. The ligand was docked with the target protein, and the best docking poses were identified. This best docking poses shows how the ligand molecule fits into the binding region of the target protein. Similar results were obtained by Lee et al., (2011).

CONCLUSION
Thus it can be concluded from the above study that tulsi plant leaves extract act as therapeutic agent which is of great interest in both pharmaceutical and industry. The present investigation reveals that the tulsi leaves extract has medicinal applications, especially for anticancer profiling. tulsi plant leaves have shown promising results in vitro studies, signifying that they are potential as therapeutic carriers. This exploration creates the new avenue to a new standard where the extract can be a powerful weapon against cancer. This encouraging results provides useful information for designing a much better anticancer compound using a tulsi plant leaves extract with minimal side effects.

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