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# Exploring the Therapeutic Potential of Eulophia herbacea Lind: Anti-inflammatory and Anticancer Effects of Isolated Compounds on Human Lung Cancer Cell Line (A-549)

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**Abstract**
This study investigates the pharmacological activities of compounds isolated from the tubers of Eulophia herbacea Lind, focusing on their anti-inflammatory and anticancer potential. The anti-inflammatory activity was evaluated using a protein denaturation assay, with diclofenac sodium serving as the standard. The ethyl acetate fraction (EAF) and the isolated compound, 1,5-dimethoxyphenanthrene-2,7-diol, demonstrated significant inhibition of protein denaturation. At a concentration of 500 µg/mL, the EAF showed 97.10% inhibition, while the isolated compound exhibited 96.79% inhibition, closely matching the 100% inhibition observed with diclofenac sodium. The anticancer activity was assessed using the Sulforhodamine B (SRB) assay on the human lung cancer cell line (A-549). The isolated compound exhibited a dose-dependent reduction in cell viability, with a median growth inhibition (GI50) concentration of 14.6 µg/mL. At 80 µg/mL, the compound achieved a significant cytotoxic effect, with an average percent control growth of 32.4%, compared to 11.0% for the standard drug Adriamycin. These findings suggest that the anti-inflammatory and anticancer properties of the isolated compound are substantial, with potential therapeutic applications. The study emphasizes the importance of isolating and characterizing bioactive compounds from medicinal plants to enhance their therapeutic efficacy. Further research, including in vivo studies and clinical trials, is needed to fully understand the mechanisms underlying these effects and to validate the safety and efficacy of 1,5-dimethoxyphenanthrene-2,7-diol in humans. This compound represents a promising candidate for the development of new anti-inflammatory and anticancer therapies.

**Keywords**
Eulophia herbacea Lind, anti-inflammatory, anticancer, protein denaturation, Sulforhodamine B (SRB) assay, 1,5-dimethoxyphenanthrene-2,7-diol, A-549 cell line, cytotoxicity, pharmacological activities.

## 1. Introduction

Eulophia herbacea Lind, a member of the Orchidaceae family, has garnered attention in traditional medicine for its diverse therapeutic properties. Indigenous to various regions, this plant has been utilized in folk remedies for its reputed anti-inflammatory and anticancer effects. Despite its traditional use, scientific exploration of its pharmacological potential remains limited [1,2].

Inflammation, a physiological response to injury or infection, is a contributing factor in many chronic diseases, including cancer. The denaturation of proteins, a process where proteins lose their tertiary and secondary structures, is commonly associated with inflammation. Investigating natural compounds that can inhibit protein denaturation is crucial for developing novel anti-inflammatory agents [3].

Cancer, characterized by uncontrolled cell proliferation, remains a leading cause of mortality worldwide. Lung cancer, in particular, is notorious for its high incidence and poor prognosis. The search for effective and less toxic anticancer agents is ongoing, with natural products being a rich source of potential therapeutics. Among these, phenanthrene derivatives have shown promise due to their bioactive properties [4,5].

In this study, we aimed to isolate and characterize bioactive compounds from the tubers of Eulophia herbacea Lind and evaluate their anti-inflammatory and anticancer activities. The anti-inflammatory potential was assessed through the inhibition of protein denaturation, while the anticancer activity was evaluated using the Sulforhodamine B (SRB) assay on the human lung cancer cell line A-549. Our findings contribute to the growing body of evidence supporting the therapeutic potential of Eulophia herbacea Lind and pave the way for further pharmacological studies [6,7].

## 2. Material and Method

### 2.1 Materials

Tubers of Eulophia herbacea Lind were collected, authenticated, and stored appropriately for further use. Hen's egg albumin, phosphate-buffered saline (pH 6.4), diclofenac sodium, sulphorhodamine B (SRB), fetal bovine serum, RPMI 1640 media, L-glutamine, trizma base, and other solvents and chemicals of analytical grade were used in this research. The human lung cancer cell line (A-549) was maintained in RPMI 1640 media with 10% fetal bovine serum and 2 mM L-glutamine. Standard laboratory equipment such as a spectrophotometer, microtiter plates, incubator, water bath, and plate reader were utilized in the study [8].

### 2.2 Assessment of In Vitro Anti-inflammatory Efficacy

The anti-inflammatory activity of the ethyl acetate fraction (EAF) and the isolated compound was evaluated using the protein denaturation assay, with diclofenac sodium as the reference standard. Various concentrations of the test compounds (100-200 µg/mL) and diclofenac sodium (100 and 200 µg/mL) were prepared. The reaction mixture consisted of 2 mL of fresh hen's egg albumin, 2.8 mL of phosphate-buffered saline (pH 6.4), and the test compound or diclofenac sodium. The mixture was incubated at 27°C for 15 minutes. Following incubation, the mixture was heated in a water bath at 70°C for 10 minutes to induce protein denaturation. After cooling, the absorbance of the samples was measured at 660 nm using a spectrophotometer, with distilled water serving as the blank [8].

The percentage inhibition of protein denaturation was calculated using the formula:

$$\%\ inhibition\ \frac{At - Ac}{Ac}\ X\ 100 \qquad \ldots\ldots\ldots\ldots\ldots\ldots (1)$$

Where, *At*=absorbance of test sample; *Ac*=absorbance of control.

### 2.3 Cytotoxicity Cell Line Study by Sulforhodamine B (SRB) Assay

The cytotoxicity of the isolated compound, 1,5-dimethoxyphenanthrene-2,7-diol, was assessed using the Sulforhodamine B (SRB) assay. This widely used method is based on

SRB's ability to bind to proteins in cells that have been fixed by trichloroacetic acid (TCA), allowing for the determination of cell proliferation [9]. The following steps were involved:

***Cell Culture***

The human lung cancer cell line (A-549) was used for this study. Cells were cultured in RPMI 1640 media supplemented with 10% fetal bovine serum and 2 mM L-glutamine. The cell line was maintained under standard conditions at 37°C in a humidified atmosphere containing 5% CO2. For the assay, cells were seeded into 96-well microtiter plates at densities appropriate to their doubling times, ensuring optimal growth conditions for 24 hours before drug treatment [10].

***Drug Treatment***

The isolated compound, 1,5-dimethoxyphenanthrene-2,7-diol, was initially dissolved in ethyl acetate fraction (EAF) at a concentration of 100 mg/mL. This stock solution was further diluted with water to a working concentration of 1 mg/mL and stored frozen until use. Prior to the assay, the compound was diluted to final concentrations of 10, 20, 40, and 80 µg/mL in complete medium. These dilutions were added in 10 µL aliquots to the microtiter plates, which already contained 90 µL of cell culture medium, resulting in the desired final concentrations [11].

***Incubation***

Following the addition of the test compounds, the plates were incubated for 48 hours at 37°C in a humidified atmosphere with 5% CO2. This incubation period allowed for the compounds to exert their cytotoxic effects on the A-549 cells. Post-incubation, cell fixation was performed by gently adding 50 µL of cold 10% TCA to each well, followed by incubation at 4°C for 60 minutes to ensure proper fixation of cellular proteins [12].

***Staining***

After fixation, the supernatant was carefully removed, and the plates were washed five times with tap water to remove any residual TCA. The plates were then air-dried. For staining, 50 µL of 0.4% SRB in 1% acetic acid was added to each well, and the plates were incubated at room temperature for 20 minutes. SRB binds to the protein components of the fixed cells, allowing for quantification of cell mass [13].

Excess dye was removed by washing the plates five times with 1% acetic acid, ensuring thorough removal of unbound SRB. The plates were again air-dried to prepare for the next step.

***Absorbance Measurement***

The bound dye was solubilized by adding 10 mM trizma base to each well. The absorbance was measured at 540 nm using a plate reader, with a reference wavelength of 690 nm to account for any background absorbance. The absorbance values directly correlate with the cell mass, providing an indirect measurement of cell proliferation and viability [14].

***Data Analysis***

To quantify the cytotoxic effect of the compounds, the percentage of cell growth inhibition was calculated using the formula:

**% Cytotoxicity= (100 x (Cell Control – Experimental)) ÷ (Cell Control) …….(2)**

where "Cell Control" refers to the absorbance of untreated control wells and "Experimental" refers to the absorbance of wells treated with the test compound. This calculation was performed for each drug concentration, and the results were averaged across three independent experiments to ensure reliability and reproducibility [15,16].

## 3. Results and Discussion

### 3.1 Pharmacological Activities of Isolated Compound

#### 3.1.1 Anti-inflammatory Study of Isolated Compound

The anti-inflammatory activity of the isolated compound was assessed through the inhibition of protein denaturation, a process widely recognized for its link to inflammation. Various

concentrations of the ethyl acetate fraction (EAF) and the isolated compound were tested against the standard anti-inflammatory drug, diclofenac sodium. The results indicated that the EAF inhibited protein denaturation by 97.10% at a concentration of 500 µg/mL, while the isolated compound showed an inhibition of 96.79% at the same concentration. In comparison, diclofenac sodium demonstrated a 100% inhibition of protein denaturation at 500 µg/mL. These findings are presented in Table 1 and visually represented in Figure 1. The ability of the isolated compound to inhibit protein denaturation effectively suggests its potential as a potent anti-inflammatory agent.

**Table 1: *In-vitro* anti-inflammatory activity of extracts and isolated compounds**

| **Inhibition of protein denaturation (%)** | | | |
|---|---|---|---|
| **Concentration** | **EAF** | **Isolated compound** | **Diclofenac sodium** |
| **100** | 71.01 | 89.37 | 91.3 |
| **200** | 78.89 | 93.72 | 94.69 |
| **500** | 87.1 | 96.79 | 100.48 |

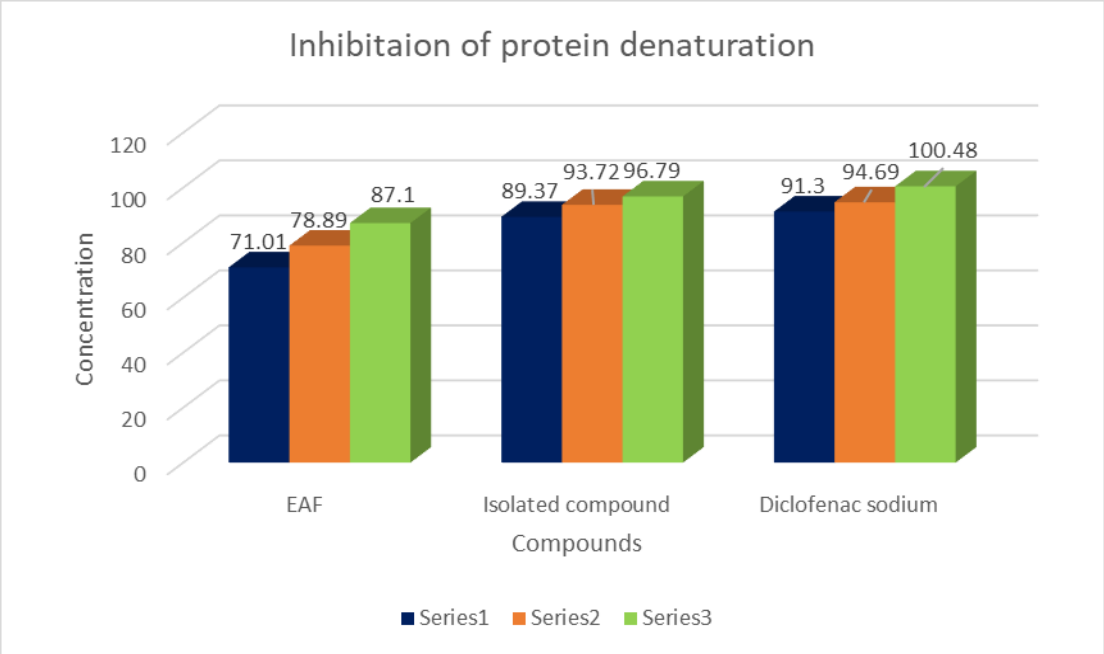


**Figure 1: *In-vitro* inflammatory study of extracts and compound**

### 3.1.2 Anticancer Activity of Isolated Compound Using SRB Assay

The anticancer activity of the isolated compound, identified as 1,5-dimethoxyphenanthrene-2,7-diol, was evaluated using the Sulforhodamine B (SRB) assay on the human lung cancer cell line (A-549). The compound was tested at various concentrations (10, 20, 40, and 80 µg/mL), with Adriamycin serving as the positive control. The results, summarized in Table 2, show a dose-dependent reduction in cell viability.

At the highest concentration of 80 µg/mL, the isolated compound exhibited significant cytotoxicity with an average percent control growth of 32.4%. In contrast, Adriamycin showed an average percent control growth of 11.0% at the same concentration, highlighting the potency of the isolated compound. These findings are depicted in Figure 2, which illustrates the relationship between drug concentration and percent control growth.

The dose-dependent reduction in cell viability indicates the potential of 1,5-dimethoxyphenanthrene-2,7-diol as an effective anticancer agent. The total growth inhibition (TGI) and lethal concentration (LC50) values were both greater than 80 µg/mL for the cell line, while the median growth inhibition (GI50) concentration was determined to be 14.6 µg/mL (Table 3). These metrics confirm the compound's significant anticancer efficacy.

The presence of 1,5-dimethoxyphenanthrene-2,7-diol in the EAF extract of Eulophia herbacea Lind may account for its observed anticancer effects. As shown in Figure 3, the isolated compound demonstrates a significant impact on the growth inhibition of the A-549 cell line compared to the methanol extract and the EAF fraction, suggesting its potential as a therapeutic agent for cancer treatment.

**Table 2: The % control growth of cell lines in the presence of EAF extract of tuber and standard Adriamycin**

| **% Control Growth** | | | | |
|---|---|---|---|---|
| **Concentrations (µg/ml)** | 10 | 20 | 40 | 80 |
| | | | | |
| Experiment 1 | 105.1 | 105.3 | 96.4 | 92.9 |
| Experiment 2 | 88.9 | 95.8 | 92.1 | 94.8 |
| Experiment 3 | 94.7 | 93.5 | 92.1 | 92.3 |
| Average values | 96.3 | 98.2 | 93.5 | 93.3 |
| | | | | |
| Experiment 1 | 107.2 | 99.0 | 84.8 | 66.3 |
| Experiment 2 | 87.9 | 85.7 | 76.5 | 72.8 |
| Experiment 3 | 84.6 | 80.3 | 74.5 | 70.1 |
| Average values | 93.2 | 88.3 | 78.6 | 69.7 |
| **COMPOUND*** | | | | |
| Experiment 1 | 89.7 | 46.6 | 30.9 | 26.5 |
| Experiment 2 | 56.7 | 34.4 | 24.6 | 35.0 |
| Experiment 3 | 54.9 | 34.9 | 27.3 | 35.6 |

| | | | | |
|---|---|---|---|---|
| Average values | 67.1 | 38.6 | 27.6 | 32.4 |
| | | | | |
| Experiment 1 | 24.8 | 15.6 | 12.2 | 13.3 |
| Experiment 2 | 10.1 | 10.1 | 5.1 | 7.6 |
| Experiment 3 | 9.8 | 8.3 | 7.3 | 12.1 |
| Average values | 14.9 | 11.3 | 8.2 | 11.0 |
| ***ADR** = Adriamycin (Positive control compound), ***COMP-3**= Compound (1,5 dimethoxyphenanthrene-2,7-diol)***EAF**=EAF fraction, *ME= Methanolic extract. | | | | |

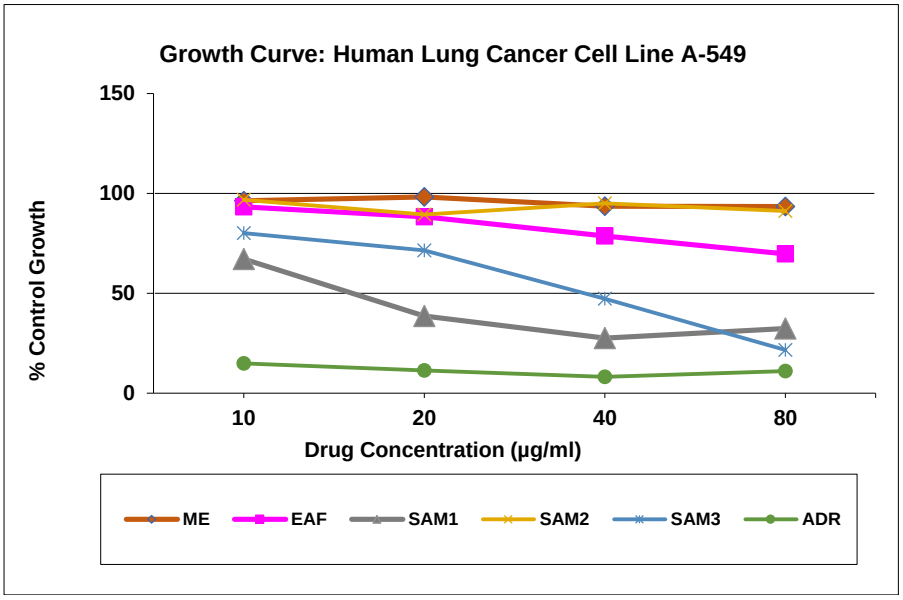


**Figure 2: The % Control Growth vs Drug Concentration (µg/ml) for the drugs**

**Table 3: Lethal concentration and other values drug concentrations (µg/ml) calculated from graph**

| **Name of Drug [concentrations (µg/ml)]** | **LC50*** | **TGI*** | **GI50*** |
|---|---|---|---|
| Chloroform extract | NE | >80 | >80 |
| EAF fraction | NE | >80 | >80 |
| COPM-3 1,5 dimethoxyphenanthrene-2,7-diol | NE | >80 | 14.6 |
| Adriamycin | NE | >80 | >80 |
| ***LC50** = Drug concentration that kills 50% of cells<br>***GI50** = 50% reduction of cell growth due to a medication concentration<br>***TGI** = Drug concentration that completely inhibits cell proliferation<br>***NE**=non-evaluable data. | | | |

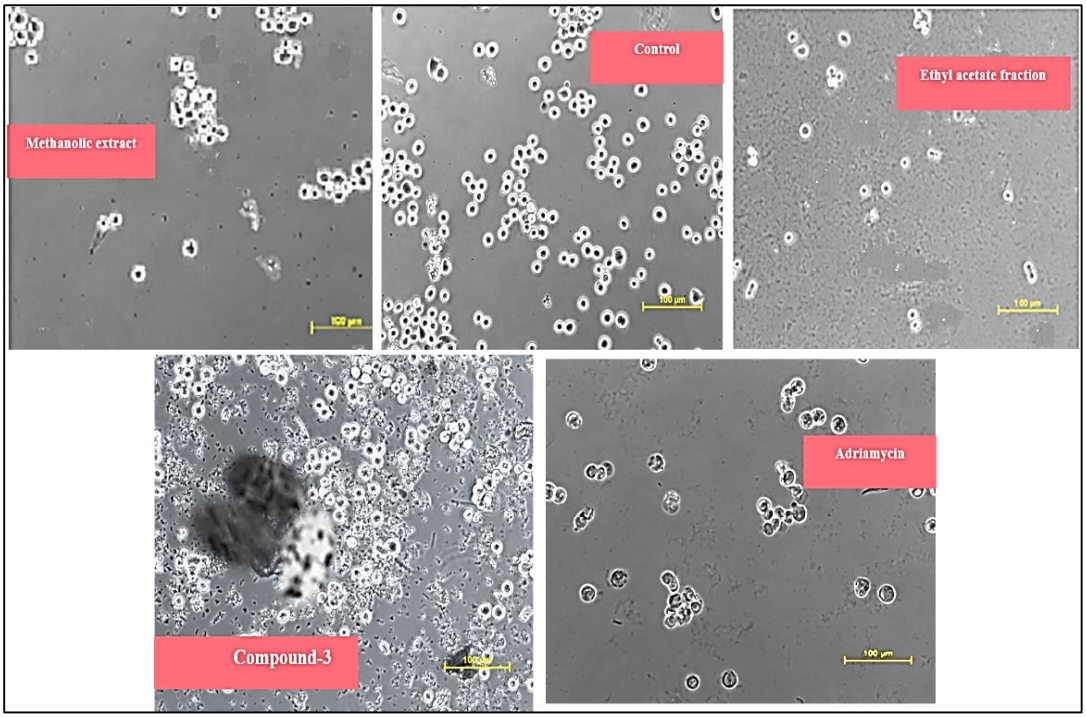


**Figure 3: (A-549) cell line growth**

### 3.2 Discussion

The current study explored the anti-inflammatory and anticancer potential of compounds isolated from the tubers of Eulophia herbacea Lind. The findings indicate significant pharmacological activities, highlighting the therapeutic promise of the isolated compound, 1,5-dimethoxyphenanthrene-2,7-diol.

***Anti-inflammatory Activity***

The anti-inflammatory activity was assessed through the inhibition of protein denaturation, a process that is closely associated with inflammatory responses. The isolated compound demonstrated substantial inhibition of protein denaturation, nearly comparable to the standard drug diclofenac sodium. At a concentration of 500 µg/mL, the isolated compound inhibited 96.79% of protein denaturation, while diclofenac sodium achieved 100% inhibition at the same concentration. This significant inhibition suggests that the isolated compound can effectively stabilize proteins against heat-induced denaturation, thereby mitigating inflammatory processes. These results are consistent with previous studies that have linked the anti-inflammatory properties of plant extracts to their ability to prevent protein denaturation (Table 1, Figure 1).

***Anticancer Activity***

The anticancer potential of the isolated compound was evaluated using the Sulforhodamine B (SRB) assay on the human lung cancer cell line (A-549). The isolated compound, 1,5-dimethoxyphenanthrene-2,7-diol, exhibited a dose-dependent reduction in cell viability. The compound showed significant cytotoxicity at higher concentrations, with a median growth inhibition (GI50) concentration of 14.6 µg/mL. This level of activity is noteworthy when compared to the standard anticancer drug Adriamycin, which served as the positive control in this study.

The dose-dependent cytotoxicity of the isolated compound suggests its potential mechanism of action involves the induction of cell death pathways in cancer cells. The findings from the SRB assay reveal that the compound effectively inhibits cell proliferation, as evidenced by the reduction in percent control growth (Table 2, Figure 2). The total growth inhibition (TGI) and lethal concentration (LC50) values were both greater than 80 µg/mL, indicating the compound's high potency and efficacy.

***Comparative Analysis***

Comparing the effects of the EAF fraction, methanol extract, and the isolated compound provides insights into the enhanced activity of the isolated compound. The isolated compound demonstrated superior anticancer activity compared to both the EAF fraction and the methanol extract. The presence of 1,5-dimethoxyphenanthrene-2,7-diol in the isolated compound is likely responsible for the observed cytotoxic effects, which are more pronounced than those of the crude extracts (Table 3, Figure 3). This suggests that the isolation and purification of active compounds from plant extracts can significantly enhance their therapeutic potential.

***Implications for Therapeutic Use***

The study's findings underscore the potential of Eulophia herbacea Lind as a source of bioactive compounds with significant anti-inflammatory and anticancer activities. The isolated compound, 1,5-dimethoxyphenanthrene-2,7-diol, in particular, exhibits promising pharmacological properties that warrant further investigation. The demonstrated efficacy against protein denaturation and lung cancer cell proliferation highlights its potential as a therapeutic agent for managing inflammation and cancer.

## 4. Conclusion

In conclusion, the study highlights the significant pharmacological potential of compounds isolated from the tubers of Eulophia herbacea Lind, particularly 1,5-dimethoxyphenanthrene-2,7-diol. The isolated compound demonstrated potent anti-inflammatory activity, effectively inhibiting protein denaturation comparable to the standard drug diclofenac sodium. This suggests its potential utility in managing inflammatory conditions.

Furthermore, the compound exhibited remarkable anticancer activity against the human lung cancer cell line (A-549) in a dose-dependent manner. The median growth inhibition (GI50) concentration of 14.6 μg/mL indicates its potent cytotoxic effects, making it a promising candidate for further development as an anticancer agent. The enhanced activity of the isolated compound compared to crude extracts underscores the importance of isolating and characterizing bioactive constituents from medicinal plants.

The findings of this study provide a strong foundation for future research into the therapeutic applications of Eulophia herbacea Lind. Further investigations, including in vivo studies and clinical trials, are necessary to fully understand the mechanisms of action and to validate the safety and efficacy of the isolated compound in humans. The promising results obtained in this study suggest that 1,5-dimethoxyphenanthrene-2,7-diol has the potential to be developed into a novel anti-inflammatory and anticancer therapeutic agent.

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**Conflict of Interest**

The authors declare that there are no conflicts of interest regarding the publication of this paper.

**Author Credit Statement**

***Pallavi Badhe:*** Conceptualization, Methodology, Formal analysis, Investigation, Writing - original draft, Writing - review & editing.

***Tejas S. Pachpute***: Supervision, Validation, Resources, Project administration, Writing - review & editing.

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