# *Dicoma anomala* enhances the zinc phthalocyanine tetrasulphonic acid ($ZnPcS_4$) mediated photodynamic therapy in breast cancer cells


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## ABSTRACT

***Background**:* Globally, cancer has been identified as one of the leading causes of death in both men and women. Breast cancer is the common type of cancer that affects women, and it is the leading cause of cancer related death seconding lung cancer. Predisposing risk factors that lead to the development of breast cancer includes alcohol consumption, age, body mass index, hormonal therapy, radiation, tumor causing viruses, inflammation, mutations, smoking cigarettes, environmental and disease history such as chronic obstructive disease.

*Methods: Dicoma anomala* root methanol extract and zinc phthalocyanine tetrasulfonate ($ZnPcS_4$) Photosensitizer (PS) were used to treat MCF-7 breast cancer cell line at different concentrations (25, 50, and 100 µg/mL of *D. anomala* and 5, 10, 20, 40, and 60 µM of zinc phthalocyanine tetrasulfonate ($ZnPcS_4$)) in photodynamic therapy (PDT) using a laser diode 680 nm at 10 J/cm$^2$ fluency. After 24 h of treatment, the cells were analyzed for lactate dehydrogenase (LDH) cytotoxicity, adenosine triphosphate (ATP) proliferation rates to establish $IC_{50}$ concentrations of plant extract and PS. The $IC_{50}$ were used in combination therapy, in which the treated groups were analyzed for possible morphological changes using an inverted microscope. The LDH cytotoxicity and ATP proliferation rates were analyzed to determine the anticancer effects of the combination therapy. Experiments were performed 4 times (n=4) and results obtained were analyzed by using SPSS statistical software version 27.

***Results**:* Biochemical assays were performed after 24 h of treatment. LDH cytotoxicity assay was performed to assess the integrity of the cell membrane. When *D. anomala* and PDT ($ZnPcS_4$) treated, cells were compared to untreated MCF-7 cells, the untreated MCF-7 cells released less LDH. In MCF-7 cells, *D. anomala* and PDT ($ZnPcS_4$) caused a greater release of LDH. Cancer cells that are metabolically active proliferate and synthesize more ATP. However, ATP in *D. anomala* and PDT ($ZnPcS_4$) treated cells were decreased. A reduction in ATP levels observed in the treated cells was linked to a decrease in cell proliferation. In combination therapy, the $IC_{5O}$ concentrations of *D. anomala* and $ZnPcS_4$ at 680 nm and 10 J/cm$^2$ showed a significant decrease in ATP proliferation and increase in LDH cytotoxicity at $p$ value < 0.001. Morphological changes were observed using an inverted microscope. Untreated MCF-7 cells showed no alterations in morphology. The $IC_{5O}$ of *D. anomala* and $ZnPcS_4$ treated cells showed morphological alterations when compared to the untreated cells.

***Conclusion**:* Phytochemicals are essential in the medical research. Traditional herbal medicinal practices have played a very critical role in the development of strong cancer therapeutic agents. Many plants have proven to posses diversified pharmacological properties. Furthermore, the outcome from this research suggests the use of *D. anomala* root methanol extract as a natural anticancer agent for the treatment of breast cancer. In monotherapy, *Dicoma anomala* induced MCF-7 cell death, while in combination with $ZnPcS_4$ mediated PDT, the plant extract significantly enhanced the efficacy of PDT outcome.

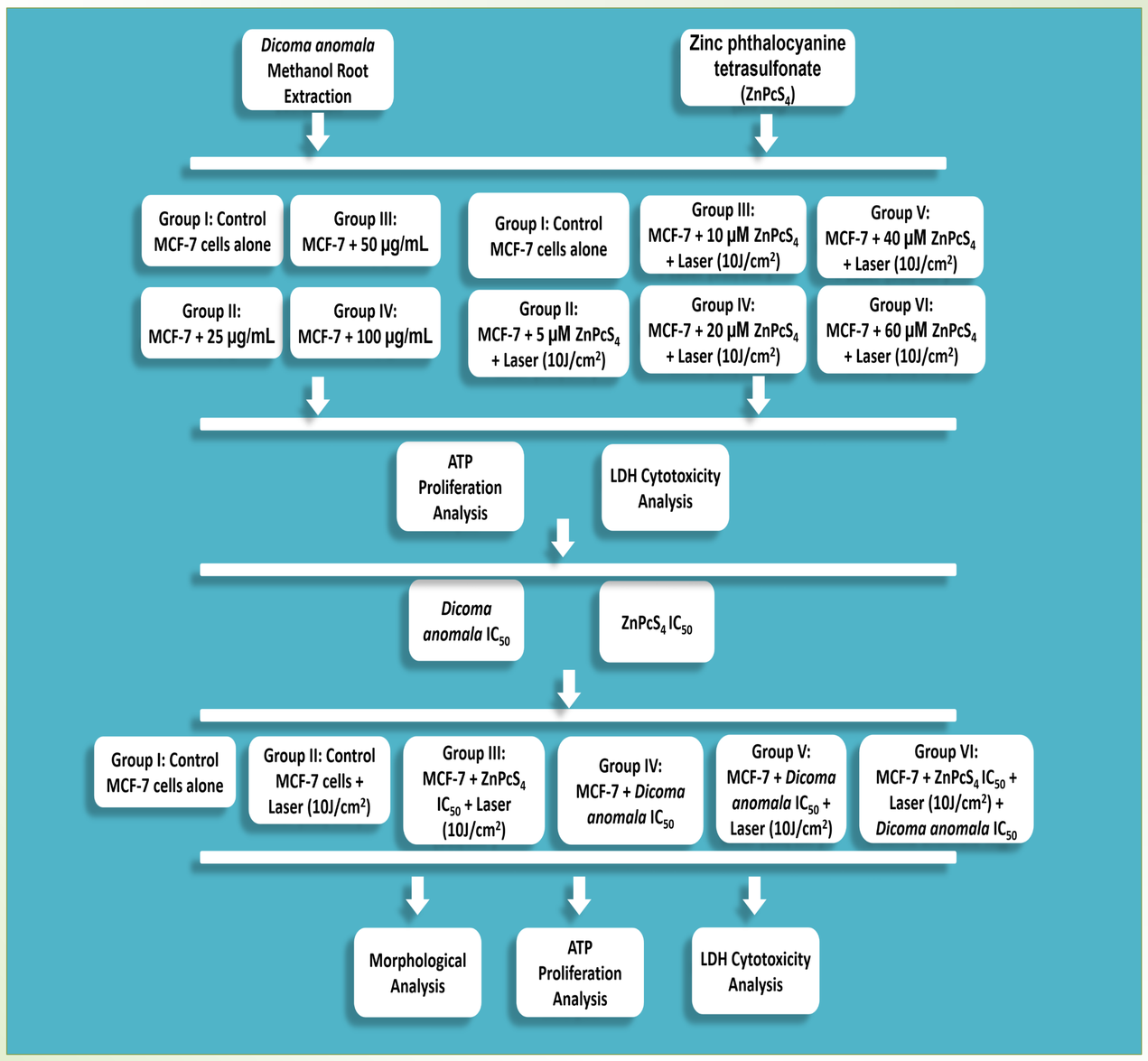

## INTRODUCTION

Cancer is a public health problem that affects both men and women. It is the second leading cause of death after cardiovascular conditions. Breast cancer (BC) is a type of cancer that originates in the breast, and it is the most common type of cancer in female. According to the GLOBOCAN report of 2020, BC incidence rate is expected to increase from 2.63 million cases of 2020 to 3.19 million new cases by 2040. In South Africa, the incidence rate was estimated to be 15.5 thousand cases and is expected to rise to 24.4 thousand new cases by 2040 [1]. There are several factors that attribute to the development of BC. These risk factors include alcohol consumption, aging, body mass index, hormonal therapy, radiation, tumor causing viruses, inflammation and mutations [2]. Conventional treatment modalities employed in the treatment of BC include surgery, radiation therapy, chemotherapy, hormonal therapy, and immunotherapy. It is worth to note that the preference of these modalities is dependent on the stage and progression of the tumor. However, conventional treatment modalities are known to elicit side effects. In order to reduce the therapy related adverse side effects, many researchers are focusing on designing plant derived anticancer drugs with improved efficacy and use in combination with photodynamic therapy [3-5].

## AIM

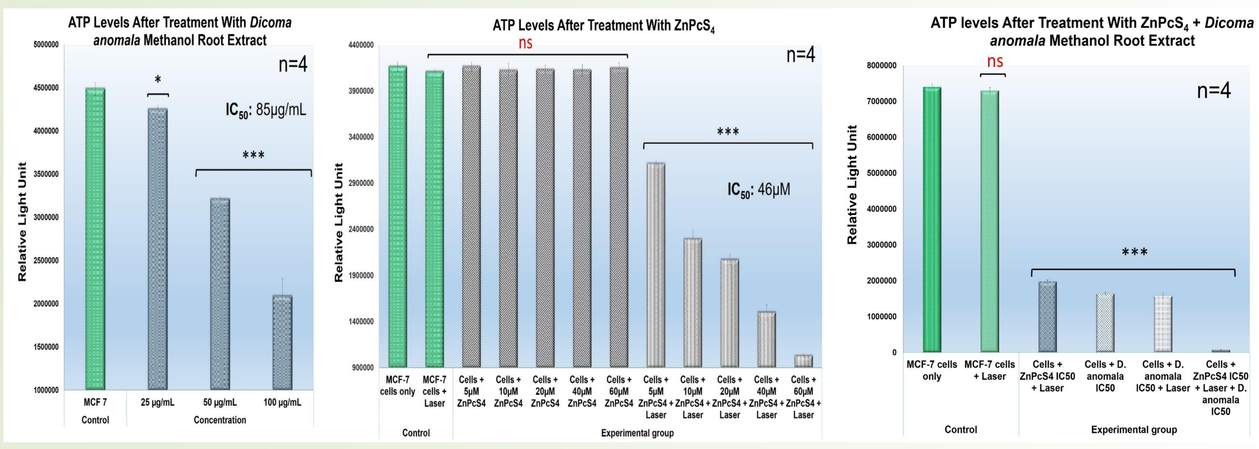
The aim of this study is to explore *in vitro* anticancer effects of *Dicoma anomala* in combination with Photodynamic therapy using zinc phthalocyanine tetrasulfonate ($ZnPcS_4$) photosensitizer on MCF-7 breast cancer cell line using 680 nm diode laser at 10 J/cm$^2$ fluency.

## METHODOLOGY FLOW DIAGRAM

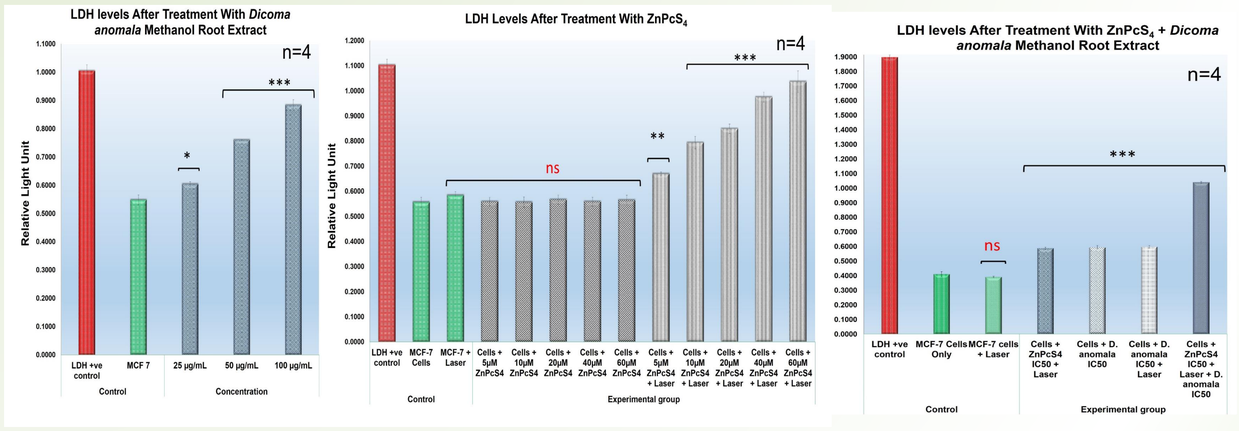



## RESULTS

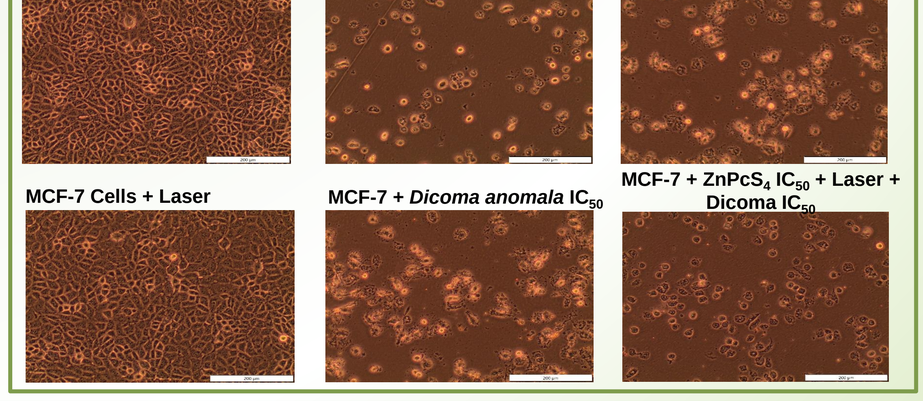
### ATP Proliferation Assay




### LDH Cytotoxicity Assay




### Morphological Changes




## CONCLUSION

In conclusion, *Dicoma anomala* methanol root extract has demonstrated the anticancer effects when administered in monotherapy as well as in combination with photodynamic therapy using zinc phthalocyanine tetrasulfonate ($ZnPcS_4$) photosensitizer on MCF-7 breast cancer cells. Furthermore, the cell death mechanism analysis will be warranted to explore exact mechanisms of *D. anomala* in monotherapy as well as the combination with a photosensitizer. In drug development research, many natural chemicals serve as lead anticancer compounds. Scientific confirmation is required for the finding of anticancer properties in traditional medicinal plants and natural products. In addition, *D. anomala* plant extracts could be considered as an effective adjuvant therapeutic drug after the clinical trails.

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