Membrane Permeabilization and Cytotoxicity of Natural and Synthetic Compounds as Potential Therapeutic Drugs

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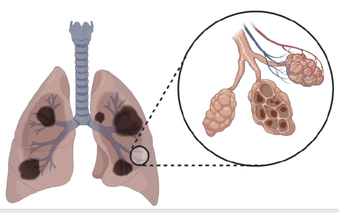
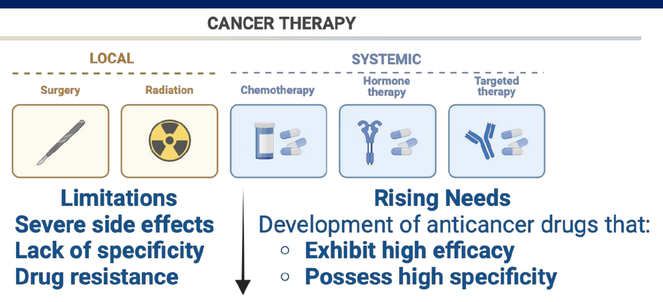
Abstract

Membrane disruption is a key mechanism used by several natural and synthetic compounds to exert biological effects or induce cytotoxicity. This mechanism is often explored to identify potential drug candidates by focusing on analyzing the interaction between compounds and the cell membrane, as well as their ability to alter membrane structures, leading to cell death. This study investigates the membrane permeabilization properties of selected natural and synthetic compounds, including curcumin, extracts from *Curcuma longa*, and the synthetic 78-molecule (benzo[*a*]phenazine), which were previously reported to exhibit some biological activities. The study employed a fluorescence leakage assay using liposomes as a model for cell membranes. The liposomes were composed of POPE:POPC (1:1), which are common phospholipids found in cell membranes, and encapsulated with 5,6-carboxyfluorescein (CF) fluorescent dye at 80 mM. The results indicated that CF leakage from liposomes increased significantly upon the addition of curcumin, while no substantial leakage was observed with the 78-molecule. Furthermore, the cytotoxicity of the compounds was tested on A549 lung cancer cells using the MTT assay, with gefitinib as a drug reference. Additionally, the cytotoxicity of the new strain of curcuma has been tested. The IC50 values of *Curcuma globulifera* extracted with various solvents, including Hexane, MeOH, and EtOAc, were found to be 79.25 ± 35.0 µg/mL, 75.98 ± 19.9 µg/mL, and 85.18 ± 20.5 µg/mL, respectively. The IC50 values for gefitinib, 78-molecule and curcumin were determined to be 5.48 ± 0.7 µM, 9.54 ± 0.5 µM and 20.89 ± 1.4 µM, respectively, suggesting that gefitinib and 78-molecule was more effective in inhibiting A549 cell growth than curcumin and *C. globulifera* extracts. However, the membrane permeabilization did not correlate with cytotoxicity, possibly due to other complex mechanisms in cells. Overall, *Curcuma* plants, curcumin and 78-molecule could still be beneficial for pharmaceutical research, offering potential for drug development.

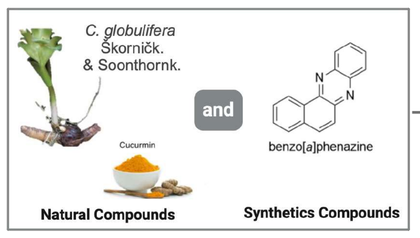
Introduction

Lung cancer is the leading cause of cancer deaths worldwide





Seeking alternative anticancer agents: Natural products and synthetic compounds



Membrane permeabilization

- Assesses cell membrane disruption, potentially leading to cell death.

Cytotoxicity

- Measures a compound's ability to kill or inhibit cells, essential for drug screening.

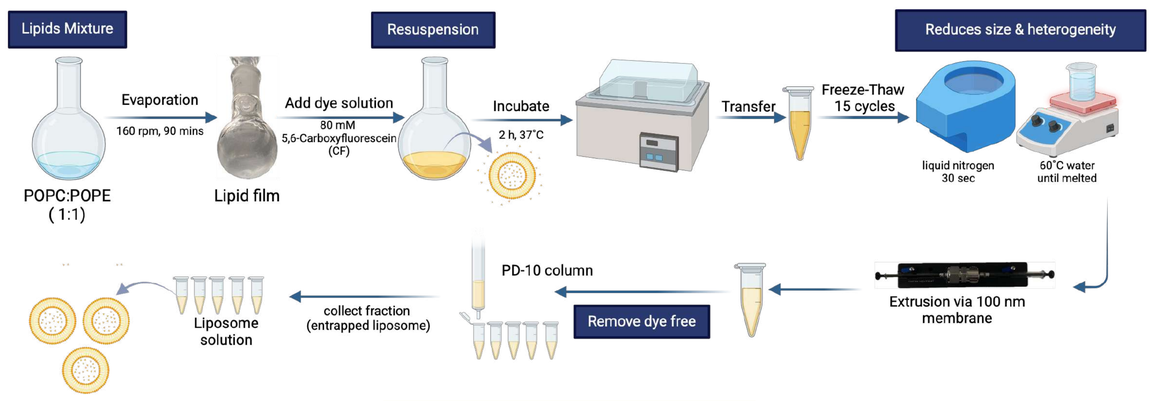
Objectives

- To investigate the membrane permeabilization properties of selected natural and synthetic compounds.
- To investigate the cytotoxic effects of natural and synthetic compounds.

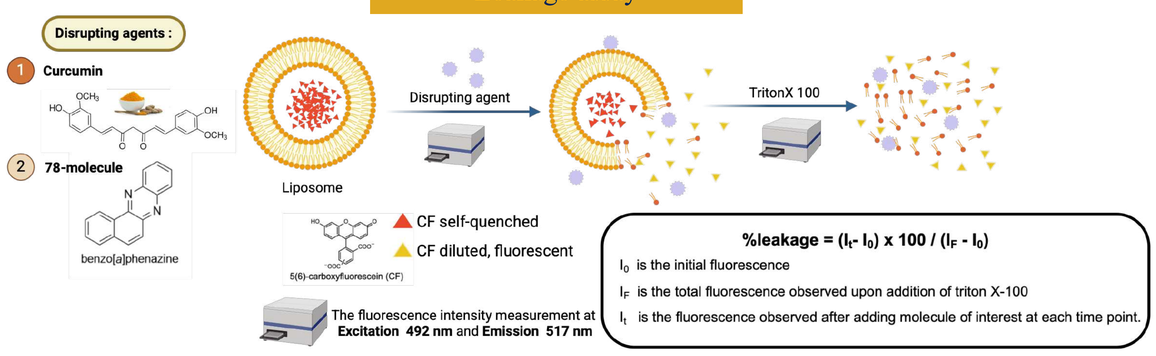
Methodology

Membrane Permeabilization

Liposome Preparation



Leakage assay



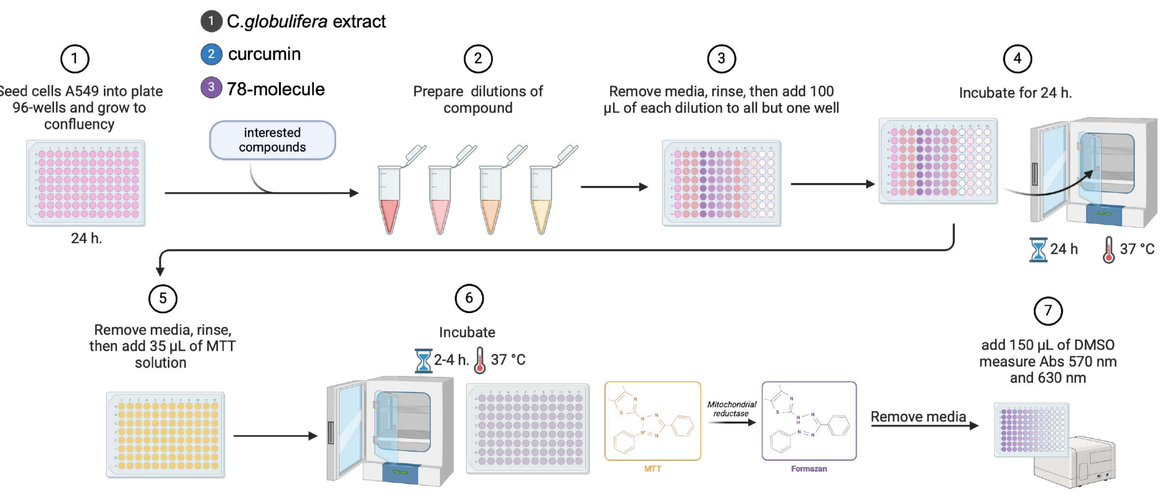
$$\%leakage = (I_t - I_0) \times 100 / (I_F - I_0)$$

I_0 is the initial fluorescence

I_F is the total fluorescence observed upon addition of triton X-100

I_t is the fluorescence observed after adding molecule of interest at each time point.

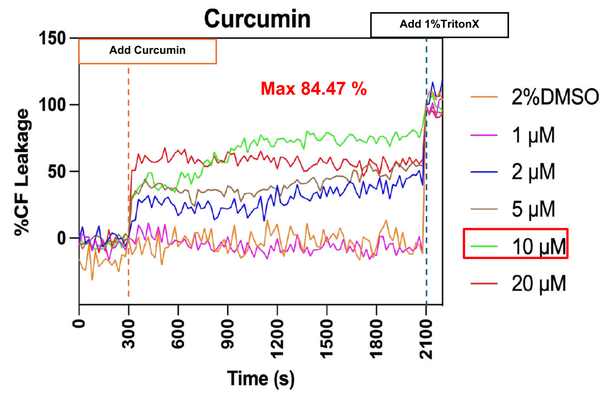
Cytotoxicity: MTT assay

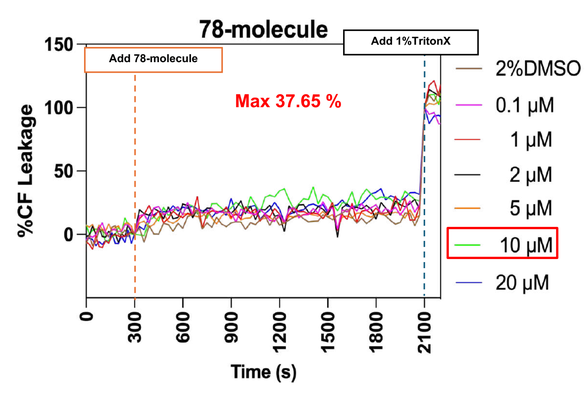


Results & Discussion

Membrane Permeabilization

- Liposome leakage test with **Curcumin** and **78-molecule (benzo[*a*]phenazine)**



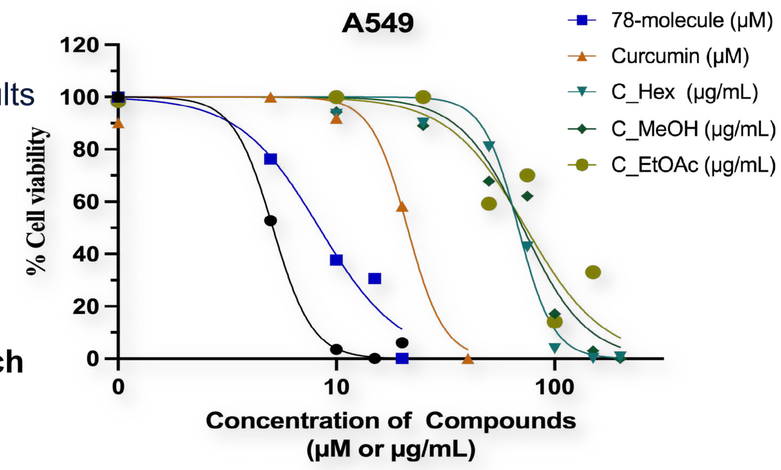


- At a concentration of **10 µM** of curcumin and the 78-molecule, the maximum CF leakage was **84.47%** and **37.65%**, respectively.
- **Curcumin caused membrane permeabilization more effectively than 78-molecule.**

MTT assay

Cytotoxicity test

- **After 24 hours** of incubation with the compounds, The results showed that **gefitinib, compound drug, exhibited significantly higher efficacy in inhibiting cell growth compared to curcumin and *C. globulifera* extracts, which are natural compounds.**



NSCLC cell line	IC50 (µM)			IC50 (µg/mL)		
	Gefitinib	78-molecule	Curcumin	C_ Hexane	C_ MeOH	C_ EtOAc
A549	5.47±0.7	9.54±0.5	20.89±1.4	79.25 ± 35.0	75.98 ± 19.9	85.18 ± 20.5

± standard deviation (SD), n=2

- The **IC50 values** show that **gefitinib** and **78-molecule** achieved 50% inhibition at **lower concentrations** than **curcumin** and ***C.globulifera extracts*, indicating greater efficacy in inhibiting A549 cell growth.**
- The extracts from ***C. globulifera*** **showed no significant differences among the three types** and exhibited lower efficacy compared to other compounds.

Conclusions

- Curcumin exhibited membrane permeabilization effects, whereas the 78-molecule showed minimal effect.
- In cytotoxicity tests, gefitinib and 78-molecule are more effective in inhibiting A549 cell growth than curcumin and *C. globulifera*.
- The membrane permeabilization did not show strong correlation with, possibly due to other complex mechanisms in cells.
- Overall, *Curcuma* plants, curcumin and 78-molecule could still be beneficial for pharmaceutical research, offering potential for drug development.

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