Original Research Article

Combination treatment of *Clinacanthus nutans* extract with cisplatin against breast cancer cell line, MCF-7

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ABSTRACT

Chemotherapy is one of the treatments for cancer and among the important agent is cisplatin. However, owing to the adverse effects associated with modern medicine, patients often explore traditional herbal remedies for cancer management. *Clinacanthus nutans* is widely believed to possess healing properties for various ailments, including cancer. The cultural and traditional background of this plant increases its acceptance among patients seeking alternative or complementary treatments. There exists a growing concern regarding the potential impact of *C. nutans* on the efficacy of cisplatin in treating breast cancer. Thus, this study aims to assess the anticancer properties of *C. nutans* water extract on MCF-7 cells and examine the interaction between *C. nutans* water extract and cisplatin on MCF-7 cells. Cells were treated separately with *C. nutans* water extract or cisplatin to determine their respective IC_{50} values. Subsequently, a combination treatment involving *C. nutans* water extract and cisplatin was administered, and the percentage of MCF-7 cell viability was assessed using MTT assay. The findings indicate that *C. nutans* water extract exhibits limited anticancer activity on MCF-7 cells and demonstrates an antagonistic interaction with cisplatin on MCF-7 cells. Further research is warranted to explore potential interactions between *C. nutans* and other anticancer agents, thereby paving the way for more informed approaches in cancer treatment.

Keywords: *Clinacanthus nutans*, Breast cancer, Cisplatin, Drug-drug interaction

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1.0 Introduction

Cisplatin is a potent cytotoxic drug widely employed in the treatment of breast cancer across various stages of the disease. This agent, especially in combination with other agents like paclitaxel or as part of a platinum-based chemotherapy regimen, can be highly effective in certain breast cancer subtypes, especially triple-negative breast cancer (TNBC) and BRCA1-associated breast cancer (1,2).

Classified as an alkylating agent, cisplatin exerts its effects by disrupting transcription and DNA replication processes. This disruption occurs through the formation of cross-links predominantly with guanine and adenine bases within the DNA double-helix strands, thereby impeding their separation and subsequent division. Cisplatin can induce cell apoptosis by triggering nucleotide mispairing, leading to erroneous DNA coding (3,4).

Despite its effectiveness, cisplatin lacks selectivity, impacting both cancerous and normal cells alike. Thus, platinum anti-cancer drugs have serious undesirable effects, including dose-limiting toxicity, especially nephrotoxicity, neurotoxicity, ototoxicity (3). Due to the adverse effects associated with conventional treatments, many cancer patients are turning to alternative therapies in search of a cure with fewer side effects. Herbal remedies have gained popularity as they are believed to offer disease management without the accompanying side effects. Some patients even combine modern medications with herbs, hoping to expedite the healing process (5). While this approach may yield positive outcomes in certain cases, the potential risks of improper combinations can pose greater dangers and impede recovery (5,6).

Clinacanthus nutans, also known as 'Belalai Gajah', is commonly utilized in Thailand for treating various conditions such as inflammation, mild fever, and as an anti-venom for snake or insect bites (7). In Malaysia, this herb has traditionally been employed for addressing ailments like sore throat, kidney problems, gout, and several other health issues (7,8).

Numerous studies have demonstrated the antioxidant properties of *Clinacanthus nutans* along with its antiproliferative effects on human cancer cell lines (7,9–12). *C. nutans* exhibits higher cytotoxicity against cervical cancer cell lines compared to bladder cancer cell lines (7). Phytochemical compounds present in *C. nutans* have been found to produce antiproliferative effects (13), with flavonoids likely contributing to its antioxidative activity (14,15). Additionally, a study on the anticancer activity of *C. nutans* has demonstrated its ability to inhibit the proliferation of breast cancer cell lines, specifically MDA-MB-231 (10). However, the efficacy of this plant against any types of cancer is still unproven.

C. nutans has gained popularity in Malaysia for its purported cancer treatment properties despite its therapeutic potential for breast cancer prevention and treatment remains largely unexplored. Many cancer patients use herbal remedies as a complementary treatment alongside conventional therapies, with local practices claiming its potential to manage and even cure cancer (16–18). The escalating usage of this herb raises concerns, particularly among breast cancer patients who may be concurrently using it alongside chemotherapeutic agents like cisplatin. Therefore, it is imperative to discern the potential interactions between *C. nutans* and cisplatin to ensure safe and effective treatment protocols.

2.0 Materials and methods

2.1 Plant extract

The authenticated of *Clinacanthus nutans* was obtained from a local seller in Sepang, Malaysia and the plant sample was sent to Forest Research Institute Malaysia

(FRIM) for authentication with voucher number (SBID: 007/21). *C. nutans* leaves were dried and pulverised before subjected to water extraction. A slightly modified method described before was used (19). 100 g of powdered sample was boiled in 200 mL of water for 60 min. The extract then allowed to cool at room temperature for one hour before vacuum filtered using Whatman No. 1 filter paper. The supernatant of aqueous extract and were frozen at −80 °C and lyophilized using a freeze dryer on the next day (20). Dried extract residue kept at −20 °C until further analysis. The extraction yield of each extract was calculated and kept at −20 °C until further analysis.

2.2 Cell Culture

Human breast cancer cell lines MCF-7 was purchased from the American Type Culture Collection (ATCC, Rockville, MD, USA). The MCF7 cells were used in this experiment because it has been reported to have multiple copies of BRCA1 despite of the apparent loss of heterozygosity (LOH) of the corresponding genomic region (4,21). This implicated that this type of cell is prone to become resistant towards cisplatin treatment (22,23). The cells also firstly derived from an advance metastatic breast cancer, which represent the type of breast cancer indicated for cisplatin treatment (24). The cells were passaged a few times before proceeded with the experiment. Briefly, the cells were cultured in RPMI medium supplemented with 10% fetal bovine serum, 100 U/ml of penicillin and 100 g/ml of streptomycin at 37ºC in 5% CO_2 in a humidified atmosphere. The cells were incubated until 90% confluency (2-3days).

2.3 Cell Viability Test Assay

(3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay were used to evaluate the cell viability as described previously (14,15). After cell count, the MCF-7 cells were seeded into 96-well plates at a density of $2.5x10^4$ cells per well and maintained at the temperature of 37ºC for 24 hours. The cells were exposed to various concentration of cisplatin (10, 100, 250, 500, 750, 1000 µmol/ml) to evaluate the IC_{50}. After 24 hours, the media were removed and 50µL of MTT solution (5mg/ml) were added to each well and the plate was incubated for 3 hours at 37ºC. Then, 50µL of stop solution (10% sodium dodecyl sulfate + hydrochloric acid) was added to each well to dissolve the formazan crystals. The plate was shake for two hours. The amount of formazan formed was measured by using a spectrophotometer at a wavelength of 540nm. The graph was plotted by using compound concentration against percentage of cell inhibition. The IC_{50} of cisplatin was then calculated using *Equation 1*.

Equation 1: Formula for cell viability

$$Cell\ viability = \frac{Absorbance\ measured\ for\ each\ concentration}{Absorbance\ of\ negative\ control} \times 100\%$$

The same steps were used for the treatment of the cells with *Clinacanthus nutans* water extract. The concentration of *C. nutans* water extract used were 100, 250, 400, 550, 750 and 1000µg/ml. The IC_{50} of *C. nutans* water extract was calculated. N=3 replicates for each concentration (6 concentrations). All statistical analysis (ANOVA posthoc Tukey) was done using IBM SPSS Statistics 25 software. After the IC_{50} for both of these drugs were obtained, the same steps were repeated again for the combination treatment of cisplatin and *C. nutans* water extract.

2.4 Isobologram Analysis

The isobologram analysis was used to study the interaction between cisplatin and *Clinacanthus nutans*. The combination index (CI) for each combination of treatments were also calculated *Equation*

2. A CI value less than 1 indicates synergy, a value equal to 1 indicates simple additivity, and a value greater than 1 indicates antagonism. A lower CI, when it is less than 1, indicates stronger evidence in favor of synergy. This calculation will give information on the drugs interaction either synergism, antagonism, or additives (27,28).

Equation 2:
CI= (IC_{50} extract combination / IC_{50} extract single) + (IC_{50} drug combination / IC_{50} drug single)

3.0 Results

MCF-7 cells were treated with 5 different concentrations of *C. nutans* extract or cisplatin. As the concentration of *C. nutans* water extract rises, there is a corresponding increase in the percentage of cell viability inhibition. IC_{50} values of single treatment of *C. nutans* water extract and single treatment of cisplatin on MCF-7 cell line were derived and tabulated in Table 1.

Combination treatments were conducted at concentration IC_{50} of cisplatin. The combination indexes (CI) were calculated, and the CI values as listed in Table 2. The combination treatment of *C. nutans* water extract and cisplatin resulted in antagonistic effect as the CI values are above than one. In the isobologram constructed as illustrated in Figure 1, the plots fall above the line of additivity which also indicates antagonism (28–30).

Table 1: Inhibitory concentrations (IC) values of single treatment of *C. nutans* water extract and single treatment of cisplatin on MCF-7 cell line

	IC_{25}	**IC_{50}**	**IC_{75}**
***Clinacanthus nutans* (µg/ml)**	266.30	**374.75**	457.65
Cisplatin (µmol/ml)	6.56	**13.12**	19.68

Table 2: Combination index

Treatment	**IC_{25} of *C. nutans* + IC_{50} of cisplatin**	**IC_{50} of *C. nutans* + IC_{50} of cisplatin**	**IC_{75} of *C. nutans* + IC_{50} of cisplatin**
CI	1.96	2.32	3.82
Interaction	Antagonism	Antagonism	Antagonism

Table 3: Concentration of *Clinacanthus nutans* water extract against IC_{50} of cisplatin for combination treatment on MCF-7

***Clinacanthus nutans* water extract µg/ml**	**374.753 (IC_{50})**	**0**	**266.30 (IC_{25})**	**374.75 (IC_{50})**	**457.65 (IC_{75})**
IC50 Cisplatin µmol/ml	0	43.59	41.69	57.54	123.03

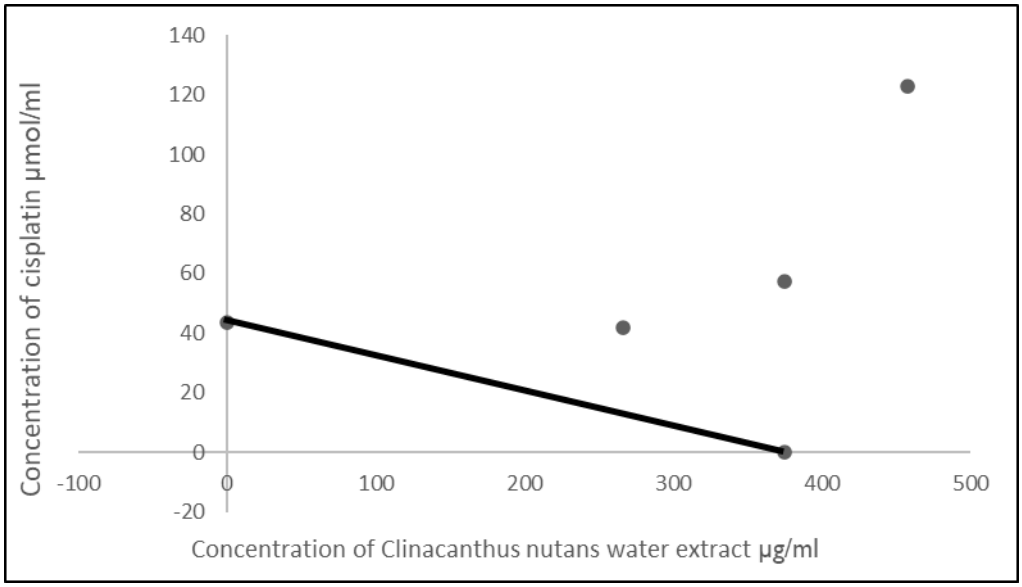

Figure 1: Analysis of combination treatment for MCF-7 treated with *C. nutans* water extract and cisplatin

4.0 Discussion

Cancer patients often use herbal supplements as complementary treatments to alleviate symptoms. A survey conducted in a Malaysian tertiary hospital found that 51.6% of 103 breast cancer patients utilized Malay traditional/folk medicine, and 34.1% used herbs as complementary and alternative medicine (6). These patients, who self-medicated with herbs, were also receiving chemotherapy, leading to common drug-herb interactions that could cause complications (16–18).

While preclinical studies have demonstrated that *C. nutans* extracts can induce apoptosis and inhibit the proliferation of various cancer cell lines (7,11,31,32), including breast cancer cells (10), it still lacks comprehensive clinical evidence to substantiate these findings. However, the concurrent use of *C. nutans* with chemotherapy drugs raises concerns about potential drug-herb interactions. Such interactions can lead to reduced efficacy of conventional treatments or increased toxicity, posing significant risks to patients. It is crucial for healthcare providers to be aware of their patients' use of herbal supplements and to monitor for any adverse effects or interactions. Thus, this study aimed to provide a preliminary insight into possible interactions between *C. nutans* extracts and cisplatin, a commonly used chemotherapeutic agent for advanced breast cancer.

The decoction method was chosen to mimic traditional herbal tea preparation in herbal medicine. We have shown previously that this method will provide phenolics compounds important for *C. nutans* antiproliferative activities on WRL68 cell line (19). Numerous studies have also reported the antioxidant and antiproliferative properties of *C. nutans* (7,11,32). The results on MCF7 cell line in this study also demonstrate that as the concentration of *C. nutans* water extract increases, there is a corresponding decrease in cell growth. The determined IC50 value for *C. nutans* is 374.753µg/ml, significantly higher than that of cisplatin. This value surpasses the recommended IC_{50} value for crude extracts by the National Cancer Institute, which is <20µg/ml (33).

The water-soluble portion of *C. nutans* stems and leaves extracts is rich in C-glycosyl flavones, which were successfully isolated in previous studies (19,26). These flavones include schaftoside, vitexin, isovitexin, orientin, isoorientin, and isomollupentin 7-O-β-glucopyranoside. These compounds are believed to contribute significantly to the antiproliferative activity of *C. nutans* (15,34).

These findings suggest that while *C. nutans* water extract may exhibit some antiproliferative activity, it is not as potent as cisplatin in breast cancer anticancer regimens. Consequently, research on *C. nutans* water extract has primarily focused on its antioxidant properties due to its poor antiproliferative activity (11,14,15). One study discovered that the aqueous extract of *C. nutans* exhibits the highest nitric oxide (NO) radical scavenging activity compared to other extracts (11,14). However, there is limited information available regarding the specific compounds within the water extract responsible for this activity.

Based on the findings, treating MCF-7 cells with IC_{25}, IC_{50}, and IC_{75} concentrations of *C. nutans* water extract in combination with cisplatin led to an increase in the IC_{50} of cisplatin compared to when cisplatin was used alone. The IC_{50} of cisplatin increased from 41.69 µmol/ml to 57.54 µmol/ml and 123.03 µmol/ml, respectively. This suggests that as the concentration of *C. nutans* water extract increases, a higher concentration of cisplatin is needed to inhibit 50% of cell growth. The combination treatment of *C. nutans* and cisplatin resulted in an antagonistic effect, as indicated by combination index (CI) values greater than one. The isobologram (Figure 1) also demonstrated antagonism, with plots falling above the line of additivity.

Cisplatin's mechanisms of action include inducing oxidative stress within the cell, which leads to cell death. It also disrupts normal calcium homeostasis, thereby interfering with essential cellular functions. Additionally, cisplatin can induce apoptosis through both intrinsic and extrinsic pathways.

The antagonistic interaction caused by *C. nutans* extract may directly disrupt the mechanism of action of cisplatin. A previous report elucidated that cisplatin exerts its cytotoxic effects by forming DNA adducts and increasing reactive oxygen species (ROS), resulting in mitochondrial DNA damage, ultimately leading to cell death (3). The generation of ROS is dependent on the concentration of cis-diamminedichloroplatinum (II) and the duration of exposure, which induces apoptosis through intrinsic or extrinsic pathways (35). However, compounds that hinder ROS generation could potentially prevent cisplatin-induced apoptosis. Data from a previous study indicated that the ethanol extract of *C. nutans* leaf contains flavonoids, terpenoids, phenols, saponins, cardiac glycosides, and alkaloids (9,36,37).

It has been suggested that the antioxidant properties of phenolic compounds possess the ability to scavenge and inhibit the generation of reactive oxygen species/nitrogen species (ROS/RNS) by inhibiting certain enzymes or chelating metals involved in free radical synthesis, or by enhancing antioxidant defense mechanisms (38,39). Consequently, this mechanism could potentially interfere with cisplatin's ability to induce cancer cell death through ROS (40–42). Therefore, further investigation is warranted to determine whether *C. nutans* extract significantly suppresses ROS production or if there are other mechanisms at play contributing to its antagonistic interaction with cisplatin.

Despite the documented low antiproliferative activity of polar extracts of *C. nutans*, patients undergoing chemotherapy often incorporate plant products like *C. nutans* tea into their regimen as

complementary treatment, unaware of its potential effects on chemotherapy. Drug-herb interactions have the potential to impact drug efficacy in various manners, potentially leading to antagonistic, additive, or synergistic effects.

5.0 Conclusion

This study indicates that *C. nutans* water extract exhibits poor antiproliferative activity on MCF-7 cells. Additionally, combining *C. nutans* water extract with cisplatin at specific concentrations increases the IC_{50} of cisplatin, suggesting an antagonistic interaction. This antagonism may result from the oxidative stress induced by cisplatin and the antioxidant activity of *C. nutans* water extract.

However, these findings are not conclusive, and further research is necessary to explore how specific *C. nutans* affect cisplatin treatment. Future studies could also investigate the interactions of *C. nutans* with other solvent extracts and chemotherapeutic agents, potentially providing significant insights into its use in cancer treatment.

Authorship contribution statement

NAO: Data analysis, Methodology, Formal analysis, Writing–original draft. **AAD:** Visualization, Methodology, Writing – review & editing. **NAL:** Supervision, Funding acquisition, Writing – review & editing.

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Conflict of Interest

The authors declared that they have no conflicts of interest to disclose.

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