Trop J Nat Prod Res, December 2025; 9(12): 6033 – 6040 **ISSN 2616-0684 (Print)**
ISSN 2616-0692 (Electronic)

Tropical Journal of Natural Product Research

Available online at https://www.tjnpr.org

Original Research Article

Synergistic Antiproliferative Effects of *Garcinia cowa* Ethyl-Acetate Fraction Combined with Paclitaxel in T47D Breast Cancer Cells

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ARTICLE INFO

Article history:
Received 09 October 2025
Revised 06 November 2025
Accepted 24 November 2025
Published online 01 January 2026

ABSTRACT

Breast cancer therapy is often faces drug resistance and chemotherapeutic side effects with agents, -such as paclitaxel. A combination approach with natural agents may enhance therapeutic efficacy and reduce toxicity. This study aimed to assess the cytotoxic activity of the ethyl acetate fraction of *Garcinia cowa* leaves (EAGCL) against T47D breast cancer cells and to quantify the synergistic interaction between EAGCL and paclitaxel. T47D cells were treated with various concentrations of the ethyl acetate fraction of *Garcinia cowa* leaves (EAGCL; 0.1–100 µg/mL) and paclitaxel (0.001–1 µg/mL), either individually or in combination. Viability was assessed using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. The IC_{50} was determined, and interactions were analysed using the Chou–Talalay method with CompuSyn software to obtain the Fraction Affected (Fa), Combination Index (CI), and Concentration Reduction Index. The IC_{50} of EAGCL and paclitaxel against T47D were 14.83 µg/mL and 0.014 µg/mL, respectively. The sub-IC_{50} combination (EAGCL 15–30 µg/mL + paclitaxel 0.00175 µg/mL) produced Fa 0.68–0.82 and CI 0.28–0.59, indicating moderate to strong synergism. Concentration (Dose) Reduction Index showed the potential for a 17-fold reduction in paclitaxel concentration in the optimal combination and a 3–4-fold reduction in EAGCL concentration. The combination of EAGCL with paclitaxel demonstrated significant synergism *in vitro* in T47D cells, suggesting the potential to reduce the paclitaxel concentration. Further studies are needed such as apoptosis assays, cell cycle analysis, and evaluation of apoptosis/survival-related protein expression—to confirm the mechanism of action and safety.

Keywords: *Garcinia cowa*, Paclitaxel, T47D, Cytotoxicity, Combination Index.

Introduction

Breast cancer remains a serious global public health issue and is the leading cause of death in women; its incidence and mortality continue to rise and place a burden on healthcare systems, particularly due to the cost of treatment and the side effects of conventional chemotherapy.[1] The molecular heterogeneity of breast tumours causes varying responses to therapy between subtypes, making resistance to chemotherapeutic agents such as paclitaxel a significant clinical challenge.[2,3] Furthermore, the use of paclitaxel is limited by apparent dose-limiting side effects, particularly cumulative peripheral neuropathy and bone marrow suppression (neutropenia), which reduce patients' quality of life and limit the duration of therapy.[4–6] Therefore, adjuvant strategies are needed that enhance the effectiveness of therapy while reducing toxicity to healthy tissue.[7,8] To overcome these limitations, a combined approach that combines standard chemotherapy with adjuvant agents—especially natural compounds—has become the focus of research, to increase antitumour efficacy while reducing drug dosage and toxicity to normal tissues.[9,10] Tropical plants from the Guttiferae family, particularly *Garcinia cowa* Roxb., are known intraditional medicine and contain various bioactive metabolites (e.g. xanthones, cowanin, garcinisidone-A) which, in several studies, have shown anticancer activity in breast cell lines such as T47D and MCF-7.[11–18] Several extracts and isolated compounds from *G. cowa* have been reported to have proliferation-inhibitory effects, cell cycle modulation, and the potential to interact with molecular targets that regulate proliferation based on reports from *in vitro* and *in silico* studies.[19–27] Although several studies have reported the antiproliferative effects of extracts or compounds from *G. cowa* extracts or isolates, systematic evidence regarding the synergistic potential of the ethyl acetate fraction of *G. cowa* leaves (EAGCL) when combined with paclitaxel is still limited—particularly in ER/PR-positive, HER2-negative models such as T47D cells. Most previous studies have reported the activity of single extracts or specific isolated compounds, but few have evaluated combinations with standard chemotherapeutic agents.[10,28,29] Therefore, a knowledge gap remains regarding whether and how the ethyl acetate fraction of *G. cowa* leaves (EAGCL) can enhance the efficacy of paclitaxel, reduce dosage requirements, and reduce side effects on healthy tissue. Based on this gap, this study aims to evaluate the synergistic effects of EAGCL and paclitaxel on T47D breast cancer cells. The scope of the study includes determining the single and combination cytotoxic potency (MTT assay) and quantitative drug interaction analysis using the Chou–Talalay and CompuSyn method (Fa, CI, DRI). The results are expected to provide comprehensive *in vitro* evidence of EAGCL's ability as an adjuvant agent to enhance the efficacy of paclitaxel and its concentration reduction potential, as a basis for further *in vivo* and mechanistic studies.

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Citation: Furqan M, Wahyuni FS, Susanti M, Hefni D, Hamidi D. Synergistic Antiproliferative Effects of *Garcinia cowa* Ethyl-Acetate Fraction Combined with Paclitaxel in T47D Breast Cancer Cells. Trop J Nat Prod Res. 2025; 9(12): 6033 – 6040 https://doi.org/10.26538/tjnpr/v9i12.17

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria

Materials and Methods

Materials

The leaves of *G. cowa* were collected on 20 February 2023 from Kudu Gantiang, Padang Pariaman, West Sumatra (coordinates: 0°30′38.5″S, 100°09′50.4″E; –0.5107, 100.1640). The plant material was identified and authenticated by Dr. Nurainas of Department of Biology, Faculty of Mathematics and Natural Sciences, Andalas University. A herbarium specimen was deposited in the Herbarium of Andalas University (ANDA), Padang, Indonesia, under voucher number 137/K-ID/ANDA/II/2023. Materials used in this study included the ethyl acetate fraction of *G. cowa* leaves, paclitaxel (Sanbe Indonesia), and general solvents and chemicals such as ethyl acetate (Merck) and dimethyl sulfoxide (DMSO) (Merck). Reagents for cellular assays included MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] (Sigma-Aldrich) and Trypan Blue (Sigma-Aldrich). Cell culture reagents included Dulbecco's Modified Eagle Medium (DMEM) (Gibco), penicillin–streptomycin solution (Gibco), fetal bovine serum (FBS) (Gibco), trypsin-EDTA (Gibco), and phosphate-buffered saline (PBS) (Invitrogen). All chemicals and reagents were of analytical or cell-culture grade and were used in accordance with the manufacturers' instructions.

Culture Sel

Human breast cancer cells T47D were obtained from the Cell Culture Laboratory, Faculty of Pharmacy, Andalas University. The cells were maintained in DMEM medium supplemented with 10% FBS and 1% penicillin-streptomycin at 37 °C in an incubator humidified with 5% CO_2. For cytotoxicity assays, cells were seeded in a 96-well plate at 1.0×10^4 cells/well in 180 µL of medium and allowed to attach for 18–24 hours before treatment. All procedures were performed in accordance with Good Cell Culture Practice (GCCP) guidelines.[3,30–33]

Single Cytotoxicity

Inhibitory Concentration (IC_{50}) determination and combination testing were performed using the MTT assay. To determine single cytotoxic potency, T47D cells were treated with a range of concentrations of EAGCL (0.1–100 µg/mL) and paclitaxel (0.001–1 µg/mL) as single agents. After the cells were allowed to adhere for 18–24 hours, each well containing 100 µL of medium was supplemented with 100 µL of solution so that the final volume was 200 µL/well; the treatment was incubated for 48 hours at 37 °C with 5% CO_2. After the exposure period, 20 µL of MTT stock (5 mg/mL in PBS) was added to each well to a total volume of approximately 220 µL/well. Incubation was continued for 4 hours until formazan crystals formed; the medium was then carefully aspirated and 100 µL of DMSO was added to each well to dissolve the formazan, with agitation on a plate shaker for 10–15 minutes to ensure complete dissolution. Blank wells containing medium + extract concentration (without cells) were included to correct for colour interference or direct reduction by extract components; blank values were subtracted from all readings before viability calculations. Absorbance was measured at 550 nm, the percentage viability was calculated as (Abs sample/ Abs control) × 100%, and the IC_{50} value was determined using GraphPad Prism.[17,34–37]

Preparation of test solutions.

The paclitaxel stock solution was prepared at a concentration of 6 mg/mL in DMSO, for the ethyl acetate fraction of the leaves of *Garcinia cowa* (EAGCL), the stock was prepared at 100 mg/mL in DMSO. All final dilutions were performed in complete medium so that the final DMSO concentration in all wells (including the vehicle control) was maintained at ≤ 0.5% (more ideally ≤ 0.1%). Before application to cells, all solutions were visually inspected for precipitation.

Combination design and synergy analysis.

Combination testing was performed using a checkerboard design with a 5 × 5 concentration matrix created in a serial two-fold manner; the concentration series used for EAGCL were 30, 15, 7.5, 3.75 and 1.875 µg/mL, while for paclitaxel they were 0.028, 0.014, 0.007, 0.0035 and 0.00175 µg/mL. For each condition, a 2× solution was prepared in complete medium and the plate was arranged so that one axis (column) contained the paclitaxel series and the other axis (row) contained the EAGCL series; then 100 µL of each 2× solution was added to wells containing 100 µL of medium with cells so that each combination had a final volume of 200 µL per well. Each condition was run in technical triplicate. The interaction between EAGCL and paclitaxel was analysed using the Chou–Talalay method with CompuSyn software to obtain the Fraction Affected (Fa), Combination Index (CI) at various effect levels, and Dose Reduction Index (DRI). CI values were reported at Fa = 0.25, 0.5 and 0.75, and interpretation was based on the criteria CI 1 (synergism), CI = 1 (additive) and CI > 1 (antagonism).[38–41]

Data Analysis

Cytotoxic activity was evaluated based on IC_{50} values using GraphPad Prism 10 (GraphPad Software, 2023, San Diego, CA, USA). The interaction between EAGCL and paclitaxel was analysed using the Chou–Talalay method with CompuSyn software to obtain the Fraction Affected (Fa), Combination Index (CI) at various effect levels, and Dose Reduction Index (DRI); CI values were reported at Fa = 0.25, 0.5 and 0.75, and interpretation was performed based on the criteria CI 1 (synergism), CI = 1 (additive) and CI > 1 (antagonism).

Results and Discussion

Cytotoxicity testing using the MTT method showed that the ethyl acetate fraction of *Garcinia cowa* leaves (EAGCL) and paclitaxel reduced the viability of T47D cells in a concentration-dependent manner. Cytotoxic potential was assessed based on the IC_{50} value, which is the concentration required to inhibit cell growth by 50%; according to the National Cancer Institute (NCI) criteria, a lower IC_{50} value reflects a stronger potential for inhibiting cancer cell proliferation.[3,10] The interpolated IC_{50} values for the two agents were, IC_{50} EAGCL = 14.83 µg/mL and IC_{50} paclitaxel = 0.014 µg/mL (Table 2), indicating a significantly higher antitumour potential of paclitaxel in this cellular model.

The cytotoxicity of EAGCL is likely related to the phytochemical diversity of the fraction — including xanthones, flavonoids, and benzophenones — which have been reported to modulate proliferation, apoptosis, and cell survival pathways.[17,42–45] The difference in sensitivity between EAGCL and paclitaxel may be attributed to the complex nature of the EAGCL mixture. Although fractionation aims to enrich active compounds in the ethyl acetate fraction, each bioactive component is still present at relatively low concentrations compared to a pure compound such as paclitaxel. Furthermore, the complex phytochemical composition and potential intrinsic resistance mechanisms in T47D cells may also contribute to the observed cytotoxic response.

The ethyl acetate fraction of *Garcinia cowa* leaves (EAGCL) reduced the viability of T47D cells in a concentration-dependent manner. At concentrations of 0.1, 1, 10, and 100 µg/mL, viability was 86.47 ± 9.66%, 79.86 ± 7.70%, 64.00 ± 4.93%, and 5.20 ± 0.34%, respectively (Table 1, Figure 1a). The decrease in viability became apparent starting at 10 µg/mL and reached almost complete elimination of the population at 100 µg/mL. The error bars indicate the greatest inter-replication variability at low concentrations and at 100 µg/mL, the values were very consistent. The data show the cytotoxic effect of EAGCL on T47D with a relatively flat concentration-response curve between 0.1–10 µg/mL and a sharp decrease between 10–100 µg/mL.

Table 1: Percentage viability of the ethyl acetate fraction of *G. cowa* leaves

Cell lines	Concentration (µg/mL)			
	0.1	**1**	**10**	**100**
T47D	86.47 % ± 9.66 %	79.86 % ±7.70 %	64.00 % ± 4.93 %	5.20 % ± 0.34 %

This pattern indicates the presence of a dose threshold at which the bioactive components of the fraction begin to cause massive cell death; possible causes include the effective concentration of the active

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

components, as well as pharmacodynamic factors (e.g. activation of the apoptosis pathway) or the solubility and absorption limits of the fraction.[46–48]

Table 2: IC_{50} of EAGCL and paclitaxel

Cell lines	Concentration (µg/mL)	
	EAGCL	Paclitaxel
T47D	14.83 ± 0.730 µg/mL	0.014 ±0.007 µg/mL

Paclitaxel reduced the viability of T47D cells in a concentration-dependent manner. At concentrations of 0.001, 0.01, 0.1, and 1 µg/mL, viability was 81.7 ± 20.92%, 54.31 ± 7.50%, 32.34 ± 1.20%, and 27.08 ± 3.40%, respectively. The decrease in viability was sharp between 0.01 and 0.1 µg/mL, while the decrease was smaller between 0.1 and 1 µg/mL, indicating that the range of 0.01–0.1 µg/mL covers the area around IC_{50} (Figure 1b). These results suggest that paclitaxel is highly potent against T47D. The concentration –response pattern shows a transition (threshold) region between 0.01–0.1 µg/mL where most cells shift from survival to a significantly affected state. The relatively high variability at a concentration of 0.001 µg/mL indicates that the effect at very low concentrations is less consistent between replicates—this is reasonable because it is far below the active range of paclitaxel.

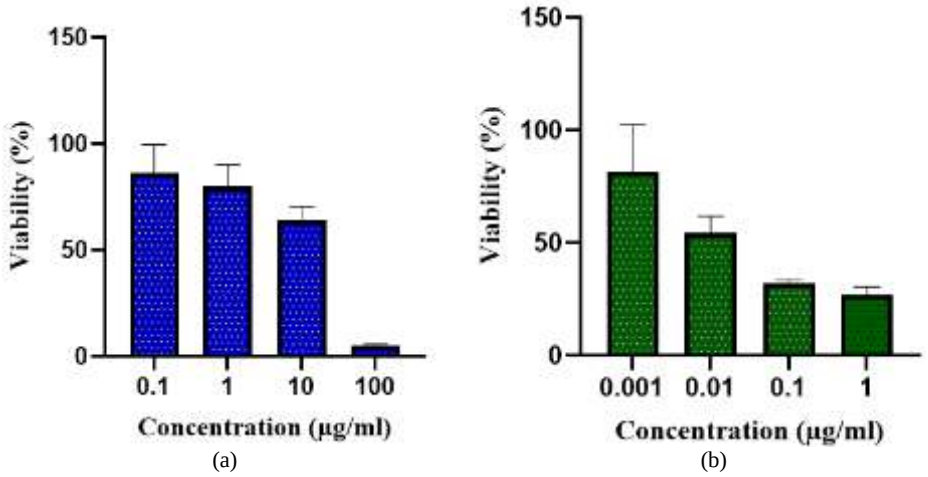

Figure 1: Concentration–response effect of EAGCL (a) and paclitaxel (b) on T47D cell viability. T47D cells were treated with indicated concentrations (µg/mL) for 48 h and viability was measured by MTT assay. Data are presented as mean ± SEM (n = 3 biological replicates

Combination analysis using CompuSyn software shows that the interaction between paclitaxel and EAGCL is concentration-dependent, as shown in Table 3. This evaluation is based on two main parameters, namely the Affected Fraction (Fa), which describes the degree of inhibition of cancer cell growth, and the Combination Index (CI), which determines the nature of the interaction between the two agents. Based on the Chou–Talalay principle, a CI value of 1 indicates synergism, CI = 1 indicates an additive effect, and CI > 1 indicates antagonism.[49–51] In addition, the Dose Reduction Index (DRI) provides information on how much the dose of each agent can be reduced when used in combination to achieve the same effect compared to single use. [38,40,52–54]

The EAGCL (µg/mL) column denotes the concentrations of the ethyl acetate fraction of *Garcinia cowa* leaves used in the combinations; values are expressed as multiples (or fractions) of the previously determined IC_{50}**:** 30 µg/mL = 2× IC_{50}, 15 µg/mL = 1× IC_{50}, 7.5 µg/mL = 1/2× IC_{50}, 3.75 µg/mL = 1/4× IC_{50}, and 1.875 µg/mL = 1/8× IC_{50}. The Paclitaxel (µg/mL) column is reported similarly relative to the paclitaxel IC_{50} used in this study: 0.028 µg/mL = 2× IC_{50}, 0.014 µg/mL = 1× IC_{50}, 0.007 µg/mL = 1/2×IC_{50}, 0.0035 µg/mL = 1/4×IC_{50}, and 0.00175 µg/mL = 1/8×IC_{50}**.** Arranging multiple relative IC_{50} levels for both agents into a concentration–response matrix provides several methodological advantages.

Table 3: CI values of the combination Ethyl acetate fraction of *Garcinia cowa* leaves (EAGCL) and paclitaxel on T47D cells

EAGCL (µg/mL)	Paclitaxel (µg/mL)	Total concentration (µg/mL)	Fraction affected (Fa)	Combination Index (CI) Value	DRI EAGCL	DRI Paclitaxel
30	0.028	30.028	0.668	6.328	1.459	0.177
15	0.028	15.028	0.447	45.998	0.805	0.022
7.5	0.028	7.528	0.485	32.115	1.998	0.031
3.75	0.028	3.778	0.537	19.906	5.362	0.050
1.875	0.028	1.903	0.314	163.754	2.877	0.006
30	0.014	30.014	0.667	3.520	1.455	0.352

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

15	0.014	15.014	0.543	10.029	1.389	0.107
7.5	0.014	7.514	0.428	27.420	1.441	0.037
3.75	0.014	3.764	0.307	89.222	1.368	0.011
1.875	0.014	1.889	0.198	337.028	1.193	0.002
30	0.007	30.007	0.666	2.119	1.448	0.699
15	0.007	15.007	0.545	5.297	1.402	0.218
7.5	0.007	7.507	0.482	8.625	1.965	0.123
3.75	0.007	3.757	0.610	2.615	8.193	0.401
1.875	0.007	1.882	0.463	9.814	7.050	0.103
30	0.0035	30.0035	0.647	1.652	1.280	1.147
15	0.0035	15.0035	0.427	8.127	0.717	0.148
7.5	0.0035	7.5035	0.449	6.124	1.625	0.181
3.75	0.0035	3.7535	0.429	6.948	2.904	0.151
1.875	0.0035	1.8785	0.475	4.462	7.553	0.231
30	0.00175	30.00175	0.815	0.284	4.443	16.966
15	0.00175	15.00175	0.685	0.599	3.271	3.404
7.5	0.00175	7.50175	0.535	1.638	2.643	0.793
3.75	0.00175	3.75175	0.253	21.367	0.938	0.049
1.875	0.00175	1.87675	0.142	105.534	0.678	0.009

First, it yields multiple fractional-effect data points required for quantitative interaction analyses, such as Combination Index (CI) and Dose Reduction Index (DRI) calculations based on the Chou–Talalay method. Second, this matrix format facilitates evaluation of ratio-dependent interactions, allowing identification of concentration ratios at which combinations exhibit synergistic, additive, or antagonistic effects.

From both biological and clinical perspectives, this strategy is also practical. If sub-IC_{50} combinations elicit substantial cytotoxicity, this suggests the potential to reduce the concentration of a toxic agent (e.g., paclitaxel) without compromising efficacy, thereby lowering possible clinical side effects. Conversely, if only supra-IC_{50} combinations are effective, this may indicate the need for higher concentrations or further isolation and evaluation of active constituents within the plant fraction.[49,55–57]

Figure 2 shows the Combination Index (CI) values against total concentration (µg/mL) for 25 combination pairs between the ethyl acetate fraction of *Garcinia cowa* leaves (EAGCL) and paclitaxel in T47D cells. Each point represents one concentration combination (EAGCL + paclitaxel); the horizontal line marks the additivity limit (CI = 1), the area below it indicates synergism (CI 1) and the area above it indicates antagonism (CI > 1).

Plot colours are coded: red = CI < 1 (synergistic), blue = CI > 1 (antagonistic). This normalization approach minimizes the disparity in absolute potency between a plant-derived fraction and a pure compound, allowing the "1× IC_{50}" level to serve as an equivalent relative reference point for both agents despite significant differences in their absolute IC_{50} values. Using a two-fold serial dilution scheme produces a logarithmically spaced concentration series, which enhances the stability of concentration –response curve fitting and facilitates interpolation across a wide effect range. By encompassing concentrations both above (supra-IC_{50}) and below (sub-IC_{50}) the median inhibitory level, this design enables comprehensive mapping of the concentration–response profiles for each agent and their combinations

The Combination Index (CI) provides quantitative evidence of the pharmacodynamic interaction between the ethyl acetate fraction of *Garcinia cowa* leaves (EAGCL) and paclitaxel in T47D cell lines, thus serving as a primary tool for determining whether the combination is synergistic (CI < 1), additive (CI = 1), or antagonistic (CI > 1) according to the Chou–Talalay approach. In the analysis of 25 non-constant-ratio combination pairs (5×5) using CompuSyn software, the majority of pairs (23/25) showed CI > 1, indicating the dominance of antagonism in the test conditions used, while only two pairs showed CI 1: EAGCL 30 µg/mL + paclitaxel 0.00175 µg/mL (Fa = 0.815; CI = 0.284; DRI EAGCL = 4.44; DRI paclitaxel = 16.97) and EAGCL 15 µg/mL + paclitaxel 0.00175 µg/mL (Fa = 0.686; CI = 0.599; DRI EAGCL = 3.27; DRI paclitaxel = 3.40). The difference in potency between the two agents—paclitaxel (IC_{50} = 0.014 µg/mL) and EAGCL (IC_{50} = 14.83 µg/mL)—renders their interaction highly sensitive to the selected concentration ratio. Synergistic effects were predominantly observed when paclitaxel was administered at sub-IC_{50} concentrations in combination with EAGCL at moderate to high levels, indicating that the detected synergy is ratio-dependent. This observation aligns with the conceptual definition of synergism (combined effect greater than the predicted additive effect). However, the significant disparity in absolute potencies makes the estimation of the combination index (CI) particularly susceptible to fluctuations, especially at very low or very high fractional effect (Fa) regions. The occurrence of extreme CI values in some concentration pairs likely reflects statistical or extrapolative instability and should therefore be interpreted with caution. To confirm and validate this interpretation, further replication, CI analysis with confidence intervals (e.g., bootstrap method), visualization of

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

isobologram or ratio–response heatmaps, as well as fixed-ratio and mechanistic studies at synergistic combination points are recommended. Without such supporting analyses, single CI values—particularly those that are extreme—are insufficient to draw robust biological implications.[58,59]

Figure 3a shows the concentration–effect relationship (x-axis = concentration; y-axis = fraction affected, Fa) for EAGCL, paclitaxel, and the tested combination. Paclitaxel achieved a high Fa at a very low concentration, while EAGCL showed a more gradual increase in Fa, requiring a much higher concentration to achieve the same effect. From the median-effect fitting (CompuSyn), the following parameters were obtained: EAGCL — IC_{50}= 14.83 µg/mL, *m* = 0.705 (r = 0.914); paclitaxel — IC_{50}= 0.014 µg/mL, *m* = 0.439 (r = 0.956). The difference in IC_{50} explains the difference in potency seen in the curves and shows that CI calculations are greatly influenced by the selected concentration ratio. The *m* value < 1 for both agents indicates a relatively flat concentration -response curve, such that small changes in concentration result in gradual changes in Fa. Figure 3b shows the normalised isobologram at ED_{50} and ED_{75}; points below the additivity line indicate potential synergy, while points above the line indicate antagonism

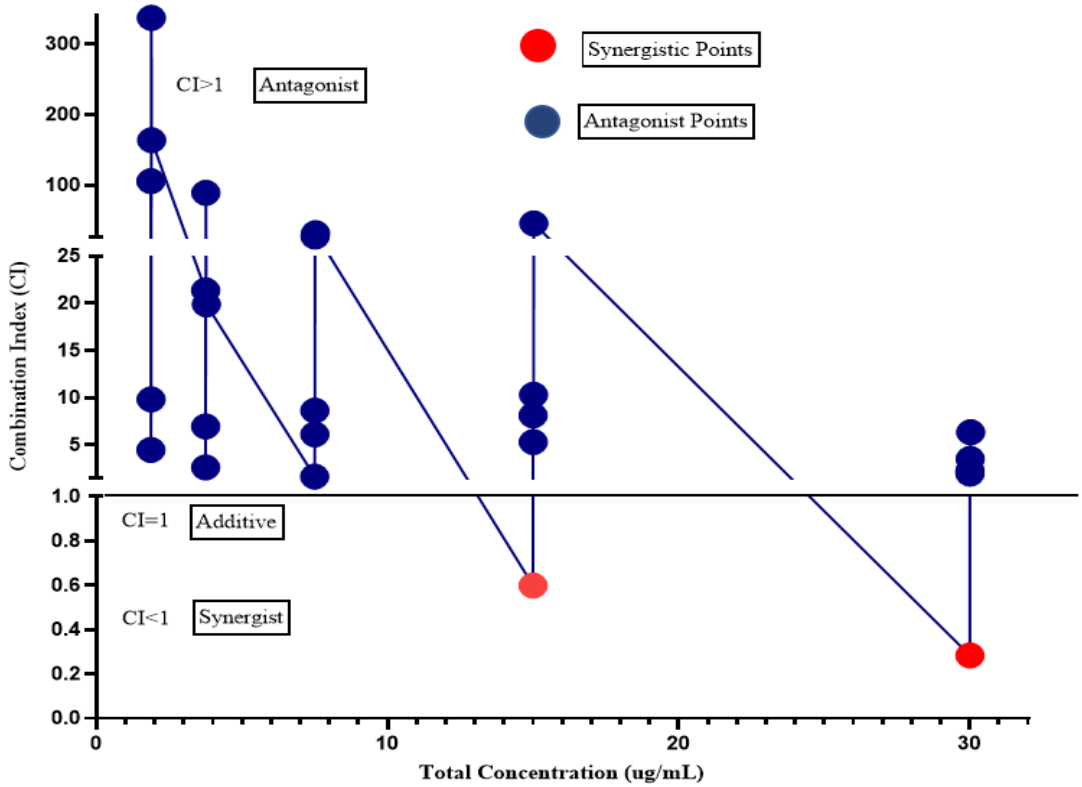

Figure 2: Plot of Combination Index (CI) vs Total Combination Concentration of EAGCL–paclitaxel

Consistent with the CI analysis, the majority of points lie above the line of additivity, while only two points (corresponding to the pair with CI 1 in the CI analysis) occupy positions below the line. Because the non-constant-ratio design produces points that are scattered from the standard ratio, the position of synergistic points represents potential synergy at the specific ratio tested—not evidence of general synergy at all ratios. Therefore, interpretation of the isobologram requires caution and verification through constant-ratio testing.[38,59,60] Figure 3c shows the median-effect plot that forms the mathematical basis for calculating ED_x, CI and DRI. The linearity of the fit in this plot supports the use of the median-effect method in this dataset; however, less stable fitting in the extreme Fa region may result in unstable CI/DRI values. For transparency and reproducibility, the equation of the line, IC_{50}/*m*/r values, number of replicates per point, and error bars or confidence intervals should be reported. Because the EAGCL curve has not reached a plateau approaching Fa = 1 at the maximum test concentration, extending the concentration range and independent replication are recommended when the research objectives include ED_{90}–ED_{95} estimation or synergy validation for translational purposes.[49,61]

Mechanistically, the observed synergism makes the most sense when viewed as a combination of paclitaxel's specific action on microtubules and EAGCL's multi-target action, which lowers the threshold for apoptosis and suppresses intracellular survival pathways.[62] This combination can increase intracellular paclitaxel accumulation (e.g. through efflux pump inhibition) and increase the susceptibility of mitotically arrested cells to enter the apoptosis pathway, thereby explaining the improvement in cytotoxic effects at specific concentration windows. On the other hand, the presence of antagonism in most ratios confirms that pharmacodynamic/compositional interactions can also be inhibitory—e.g. through changes in phase distribution or physicochemical interactions that reduce paclitaxel availability—therefore claims of therapeutic benefit must be weighed against the possibility of reduced efficacy in other ratios.[63] From a resistance perspective, such multi-target combinations have the potential to delay or minimise the emergence of resistant clones by cutting off compensatory pathways (e.g., PI3K/Akt, NF-κB) and suppressing efflux transporter expression; however, this hypothesis requires confirmation through resistance biomarker measurements (e.g., Bcl-2, survivin, phospho-Akt) and longitudinal experiments.[48,64,65]

Unresolved methodological limitations include the use of a single cell line (T47D), the complexity of the fraction composition without identification of active compounds, and indications of unstable CompuSyn fitting in some concentration pairs—limiting current generalisation and clinical significance. Therefore, clear recommendations for further work are: larger biological replication of key combinations; mechanistic testing (cell cycle analysis by flow cytometry, measurement of protein expression activity); bioassay-guided fractionation and isolation to identify active components of EAGCL; testing on a panel of different breast cell lines, as well as pharmacokinetic studies and *in vivo* models to assess translation and safety

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

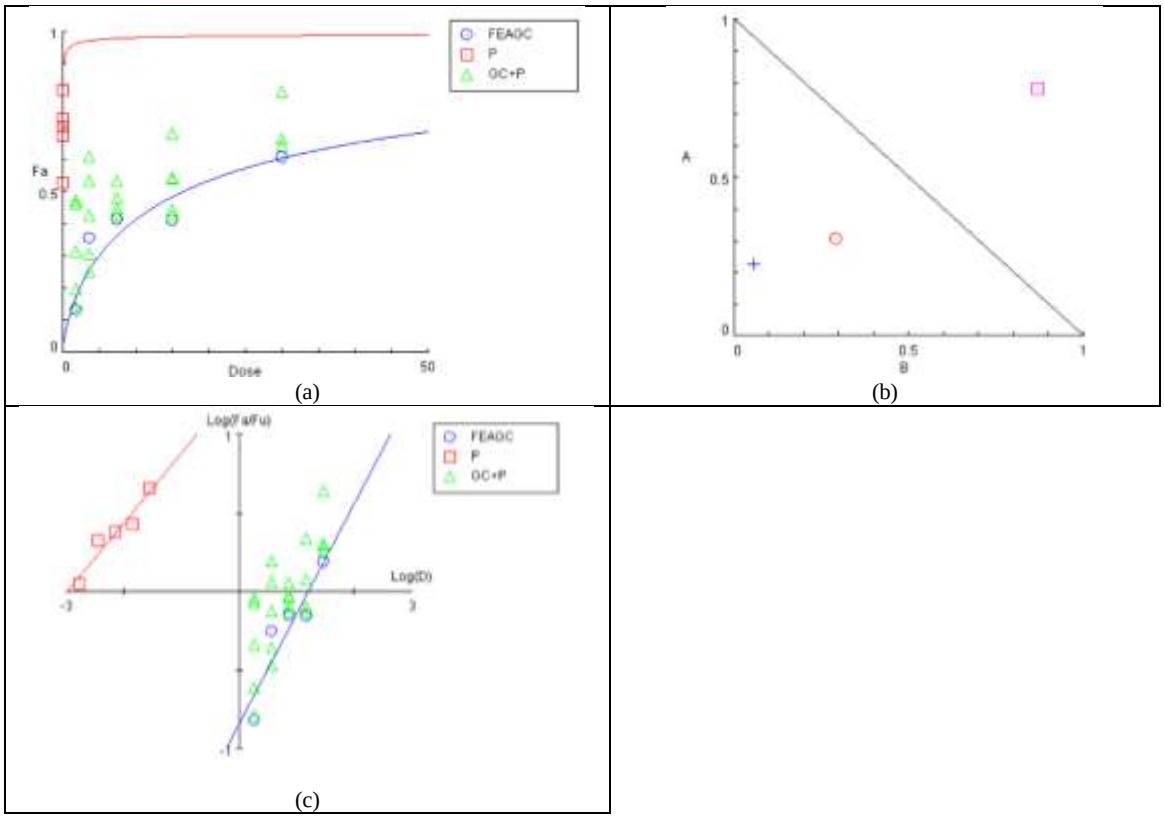

Figure 3: Concentration-effect curve (a), isobologram (b) and median effect plot (c).

Conclusion

This study shows that the ethyl acetate fraction of *Garcinia cowa* leaves (EAGCL) exhibits cytotoxic activity against T47D breast cancer cells, with an IC_{50} value of 14.83 µg/mL, whereas paclitaxel shows markedly stronger potency with an IC_{50} of 0.014 µg/mL. The much lower potency of EAGCL may be attributed to its crude and complex nature, containing multiple bioactive constituents that act through multitarget mechanisms and generally require higher concentrations to exert cytotoxic effects. Nevertheless, this multitarget profile may contribute to the ratio-dependent synergism observed when EAGCL is combined with paclitaxel. Combination analysis using CompuSyn revealed that EAGCL 30 µg/mL + paclitaxel 0.00175 µg/mL (Fa = 0.815; CI = 0.284; DRI paclitaxel = 16.97) and EAGCL 15 µg/mL + paclitaxel 0.00175 µg/mL (Fa = 0.686; CI = 0.599) the interaction between the two agents was synergistic. This condition not only increases cytotoxic efficacy, but also opens up the possibility of substantially reducing the paclitaxel concentration, thereby potentially reducing the risk of toxicity. Thus, the combination of EAGCL and paclitaxel can be considered a more effective therapeutic strategy than monotherapy. However, further research involving in-depth mechanistic analysis is needed to validate these results and explain the molecular interactions underlying this synergy, as well as in vivo studies to support its potential application in a clinical context.

Conflict of Interest

The author declares no conflicts of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

Acknowledgements

The author appreciates the support, guidance, and financial assistance the Faculty of Pharmacy Universitas Andalas provides under contract number 79/UN16.19/PT.01.03/PDD/2025 and expresses gratitude to Universitas Andalas' LPPM for helping to complete this task.

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