Trop J Nat Prod Res, November 2025; 9(11): 5490 - 5496
ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Tropical Journal of Natural Product Research

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

Original Research Article

Cytotoxic Effects of *Crassocephalum crepidioides* Leaf Extract on T47D Cells: A Network Pharmacology Approach

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ARTICLE INFO

Article history:
Received 13 July 2025
Revised 27 October 2025
Accepted 12 November 2025
Published online 01 December 2025

ABSTRACT

Crassocephalum crepidioides, locally known as Sintrong, is a medicinal plant extensively utilized in traditional Southeast Asian medicine. Despite the potential, its pharmacological properties, particularly anticancer effects, remain insufficiently studied. Therefore, this study aims to evaluate the cytotoxic effects of *Crassocephalum crepidioides* ethyl acetate leaf fraction on T47D luminal breast cancer cells and to elucidate the underlying mechanisms through an integrated experimental and network pharmacology approach. Phytochemical screening and MTT cytotoxicity assays were conducted on the ethyl acetate fraction. Computational analyses included target prediction using SwissTargetPrediction, protein-protein interaction (PPI) mapping with STRING, and functional enrichment analysis through WebGestalt. The results showed that the extract had significant cytotoxicity, with EC_{50} value of 28.07 ± 0.67 µg/mL, comparable to doxorubicin. Phytochemical profiling revealed a high abundance of alkaloids, terpenoids, and phenolics. In addition, network pharmacology analysis identified 3 major compounds predicted to target PTGS1 (COX-1), PPARA (PPAR-α), and CNR2 (CB2). Enrichment analysis implicated pathways related to cancer, including PPAR signalling and the modulation of inflammatory responses. These results suggest that anticancer effects of Sintrong involve a novel multi-target mechanism associated with lipid metabolism and the endocannabinoid system, exhibiting selective toxicity toward cancer cells. This current study provides comprehensive scientific validation of Sintrong's potential as a source of anticancer agents for luminal breast cancer. However, further studies are necessary to isolate active compounds, evaluate *in vivo* efficacy and safety, and explore potential synergistic effects with conventional therapies.

Keywords: *Crassocephalum crepidioides*, Network pharmacology, Cytotoxicity, Breast cancer

Introduction

Breast cancer continues to pose a significant global health challenge, with approximately 2.3 million new cases and 685.000 deaths reported annually, according to the latest GLOBOCAN 2022 data.[1] The disease accounts for approximately 25% of all cancer diagnoses in women worldwide, with incidence rates continuing to increase, specifically in developing countries where late-stage detection is prevalent.[2] In addition, the economic burden exceeds $88 billion annually in treatment costs and lost productivity, with luminal subtypes (ER+/PR+) constituting approximately 60-70% of cases.[3] Although 5-year survival rates in high-income countries approach 90%, values below 40% are recorded in low-resource settings, emphasizing significant disparities in access to effective therapies.[4]

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Citation: Slamet S, Balqis FI, Wirasti W, Oktaviani RK, Muthoharoh A, Cahyanta AN, Adikusuma W, Mugiyanto E. Cytotoxic Effects of *Crassocephalum Crepidioides* Leaf Extract on T47D Cells: A Network Pharmacology Approach. Trop J Nat Prod Res. 2025; 9(11): 5490 – 5496
https://doi.org/10.26538/tjnpr/v9i11.32

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

The heterogeneity of the disease and the development of treatment resistance further exacerbate these challenges, necessitating continuous exploration of novel therapeutic approaches. Current breast cancer therapies face significant limitations, including severe side effects associated with chemotherapy, the development of resistance to endocrine therapies in luminal subtypes, and the high cost of targeted biologics.[5] Approximately 30-50% of patients with estrogen receptor-positive (ER+) breast cancer eventually develop resistance to tamoxifen and aromatase inhibitors, while HER2-targeted therapies remain inaccessible to many in resource-limited settings.[6] The triple-negative subtype presents significant therapeutic challenges due to its aggressive nature and the absence of targeted treatment options. These challenges are further exacerbated by the frequent occurrence of chemotherapy-induced toxicities, such as cardiotoxicity (affecting up to 30% of patients receiving anthracyclines) and myelosuppression, which substantially impair patients' quality of life.[7] Therefore, there is an urgent need for safer, more affordable, and effective treatment alternatives, particularly for hormone receptor-positive cancer, which constitutes the majority of cases. Sintrong (*Crassocephalum crepidioides* S. Moore), a medicinal plant belonging to the Asteraceae family, is recognized as a potential candidate for the development of breast cancer therapies. Indigenous communities in the Southeast Asia have traditionally used this plant for various health conditions, and preliminary studies indicate its possible anticancer properties.[8] Phytochemical analysis reveals the presence of abundant bioactive compounds, including jacobine-type alkaloids, β-caryophyllene, and phenolic derivatives-classes known to exhibit anticancer effects through

multiple mechanisms. In addition, these compounds demonstrate potential for multi-target activity, in line with the current paradigm shift toward polypharmacology approaches. Compared to many studied medicinal plants, Sintrong remains pharmacologically underexplored, representing both a novel resource and an opportunity to validate traditional knowledge through rigorous scientific investigation. This is particularly relevant to luminal breast cancer, which has received less attention in ethnopharmacology studies compared to more aggressive subtypes.

The integration of bioinformatics and network pharmacology has revolutionized drug discovery from natural products by facilitating the prediction of molecular targets, pathway interactions, and therapeutic mechanisms before laboratory validation.[9] Tools, such as SwissTargetPrediction, STRING, and WebGestalt, facilitate comprehensive in silico analyses that can accelerate the identification of active constituents and their roles in cancer biology. Therefore, this study aims to (1) identify key bioactive compounds in Sintrong with potential anti-breast cancer activity, (2) predict molecular targets and enriched pathways using network pharmacology, and (3) validate cytotoxic effects of the extract experimentally. The current study represents the first systematic investigation of Sintrong against luminal breast cancer, combining in silico network pharmacology with in vitro cytotoxic evaluation on T47D cell line. The results reveal novel mechanisms of action, including the involvement of CB2 receptors and the modulation of PPAR-*α*/lipid metabolism pathways, which may facilitate new avenues for therapeutic development. This not only advances the search for affordable plant-based treatments but also provides a replicable framework for investigating other understudied medicinal plants.

Materials and Methods

Plant Collection and Identification

On 12 March 2023, 3 kg of fresh leaf material was collected from Kalipaingan forest, Kali Paingan, Pekalongan, at geographic coordinates -7.116309, 109.593031. The specimen was authenticated and identified by Nurul Suwarningsih, a taxonomist affiliated with the Biology Learning Laboratory, Faculty of Applied Science and Technology, Ahmad Dahlan University (UAD), and deposited under voucher number 184/Lab.Bio/B/III/2023.

Extraction and Phytochemical Screening

The leaves were air-dried, ground to powder (60-mesh), and 300 g of the resulting powder was macerated in ethyl acetate (Merck, Darmstadt, Germany) (1:6, 1800 mL) for 5×24 hours with 2×24 hours re-maceration, filtered, and concentrated by rotary evaporation (50°C) to obtain ethyl acetate fraction (designated as EA. F). Phytochemical screening was performed on the extract, alkaloids were detected using Mayer's and Dragendorff's reagents (Merck, Darmstadt, Germany) after chloroform-ammonia extraction, and flavonoids were identified by color change with Mg/HCl (Merck, Darmstadt, Germany). In addition, phenolics showed blue/green with $FeCl_3$ (Merck, Darmstadt, Germany), saponins produced stable foam in water, tannins formed dark green-black with $FeCl_3$, and steroids/triterpenoids were distinguished by color using acetic acid/H_2SO_4 (Merck, Darmstadt, Germany).[10]

Cytotoxicity Assay (MTT Method)

T47D breast cancer cells were cultured in RPMI 1640 medium (Sigma, MA, USA) with 10% FBS (Gibco, CA, USA) at 37°C under 5% CO_2. At 80% confluency, cells were harvested using 0.25% trypsin-EDTA (Gibco, CA, USA), neutralized with complete medium, and counted using a haemocytometer. Cells were seeded (1×10^4 cells/well) in a 96-well plate (Thermo Scientific, CA, USA) and incubated for 4 hours. Meanwhile, Sintrong leaf extract (EA. F) was dissolved in DMSO (Merck, Darmstadt, Germany) and diluted in medium to concentrations of 7.8-1000µg/mL. Cells were treated in triplicate for 24 hours. After incubation, MTT (Bio Basic, NY, USA) (0.5 mg/mL) was added and incubated for 4 hours. Formazan crystals were dissolved in DMSO, and absorbance was measured at 550–600 nm using a Tecan Spark® plate reader (Tecan, Zurich, Switzerland). EC_{50} values were calculated using dose-response analysis. Doxorubicin (Merck, Darmstadt, Germany) functioned as the positive control.[11] Moreover, before solubilization, microscopic imaging at 10x magnification was performed using an inverted microscope to document cell morphology and formazan distribution, with photos taken of untreated control wells (healthy cells with dense purple crystals), treated wells (for comparison), and blank wells (background control).

Database Mining for Phytochemical Analysis of Sintrong

To identify phytoconstituents of Sintrong, compound screening was conducted through the Indian Medicinal Plants, Phytochemistry and Therapeutics (IMPPAT) (https://cb.imsc.res.in/imppat).[12] Additional compound data and molecular identifiers were obtained from EMBL-EBI (https://www.ebi.ac.uk/),[13] PubChem, and KEGG, all accessed on March 25, 2025. IMPPAT provided phytochemical profiles of medicinal plants, while EMBL-EBI offered bioactivity data and molecular interactions. Key compounds were cross-referenced with PubChem and KEGG Pathway databases to assess their pharmacological potential, particularly focusing on anticancer mechanisms relevant to T47D breast cancer cells.

Structure-Based Target Prediction of Sintrong Leaf Constituents

Following the extraction of bioactive compounds from Sintrong leaf, SwissTargetPrediction (http://www.swisstargetprediction.ch) was used to determine potential protein targets in Homo sapiens. This platform used a combination of 2D/3D molecular similarity metrics and machine learning to identify probable targets based on known ligand-protein interactions. The prediction was specifically set for Homo sapiens with a structural similarity threshold >0.5 to ensure high confidence matches, as values above this cutoff showed significant pharmacophore overlap with validated ligands. Only targets with probability scores >30% were considered to minimize false positives.[14]

Network-Based Validation of Sintrong Targets in Breast Cancer Pathways

The predicted protein targets obtained from SwissTargetPrediction were converted to their corresponding Gene IDs for further validation. Protein-protein interaction (PPI) network was constructed using STRING database (https://string-db.org) to investigate its potential role in T47D breast cancer development. Initially, 3 core Gene IDs from the target prediction were input, which were then supplemented with established breast cancer-associated genes (ESR1, PI3K, AKT, and mTOR) to provide biological context. The network was generated with high confidence (interaction score >0.7) to ensure reliable interactions. Subsequently, the network was expanded to incorporate the 3 Sintrong-derived target proteins, analyzing their topological features (degree centrality, betweenness) to identify hub proteins. The resulting network visualization (nodes representing proteins, edges indicating interactions) revealed both direct and indirect connections between Sintrong's targets and key breast cancer pathways, particularly those related to hormone signalling and cell proliferation.[15]

Validation of Breast Cancer-Associated Proteins Using cBioPortal

Comprehensive genomic profiling was performed using cBioPortal (https://www.cbioportal.org) to confirm the relevance of all identified proteins from the previous network analysis in breast cancer pathogenesis. This platform was specifically selected as it aggregated multi-omics data (mutations, copy number variations, mRNA expression) from >300 cancer studies, including TCGA breast cancer datasets. All target proteins were queried across 1) TCGA Breast Invasive Carcinoma (TCGA-BRCA), 2) METABRIC (Nature 2012 & 2016), and 3) MSKCC (Cell 2019) cohorts.[16]

Functional Enrichment Analysis Using ORA in Web Gestalt 2024

Functional enrichment analysis was conducted using Web Gestalt (https://www.webgestalt.org) through over-representation analysis (ORA), with the reference set derived from KEGG 2024 pathways.[17,18] Criteria for significance included a minimum of 5 genes per pathway, a false discovery rate (FDR) below 0.05 using the Benjamini-Hochberg method, and a Bonferroni-corrected *p*-value below 0.01.[19] This approach enabled the identification of key biological pathways that could be modulated by Sintrong-derived compounds, particularly those related to oncogenic signalling and cellular homeostasis.

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

Results and Discussion

Despite the widespread traditional use of Sintrong in several ethnomedicinal practices, scientific studies investigating its pharmacological potential, particularly in cancer, remained remarkably limited. This study provided the first comprehensive network pharmacology and experimental validation of Sintrong's anticancer effects against T47D breast cancer cells, addressing a critical gap in the scientific literature. The phytochemical profile of ethyl acetate leaf extract was initially characterized. Phytochemical screening revealed several bioactive compounds with varying intensities that contributed to the observed cytotoxic effects (**Supplementary Table 1**). The extract showed strong positive results for alkaloids, phenolics, steroids, and tannins, which are compound classes known to exhibit anticancer properties through multiple mechanisms.

Dose-Dependent Cytotoxicity of EAF Against T47D

The cytotoxic potential of Sintrong against T47D luminal breast cancer cells was evaluated through a comprehensive MTT assay, with results presented in **Figure 1**. Furthermore, the dose-response analysis revealed a significant, concentration-dependent reduction in cell viability across a concentration range of 7.8 to 1000 µg/mL, achieving an EC50 value of 28.07 ± 0.675 µg/mL after 24 hours of treatment. Cytotoxic activity was comparable to the reference chemotherapeutic agent doxorubicin (EC50 19.93 ± 0.025 µg/mL), demonstrating only a 1.4-fold difference in potency (right panel). The steep slope (1.8) of the dose-response curve suggested potential cooperative binding or multi-target mechanisms, possibly attributable to the extract's complex phytochemical composition (left panel). Notably, EA.F induced 80% maximal inhibition at 100 µg/mL, approaching the 85% inhibition observed with doxorubicin. Furthermore, the demonstrated bioactivity at concentrations below 30 µg/mL supported the potential for further fractionation and purification to identify the most active constituents, while the extract's favourable cytotoxicity profile warranted exploration of potential synergistic effects with conventional chemotherapeutic agents.

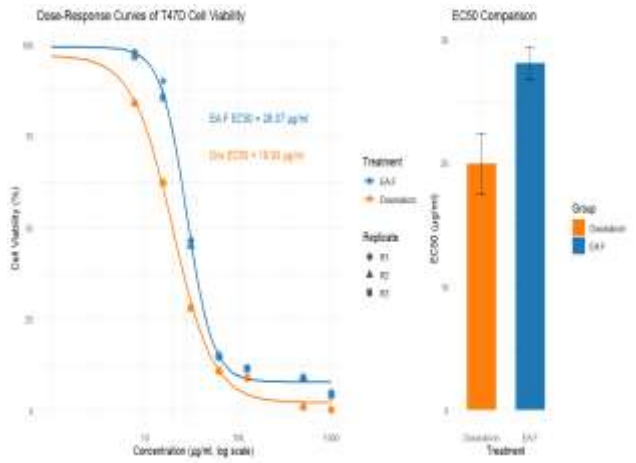


Figure 1: Dose-dependent cytotoxicity of EA.F against T47D breast cancer cells. (Left panel) Concentration-response curve and comparative cytotoxicity with doxorubicin (Right panel).

Morphological Assessment of Cytotoxic Effects Through Microscopic Analysis

Figure 2 presented compelling visual evidence of cytotoxic effects exerted by EA.F on T47D breast cancer cells, as demonstrated through microscopic evaluation of formazan crystal formation in MTT assay. The control group **(A)** displayed a dense population of viable cancer cells, evidenced by abundant purple formazan crystals. **Figure 2(B)**, representing media control, shows no cytotoxic activity. Meanwhile, the extract-treated cells **(D)** exhibited significant cytotoxic activity, with markedly reduced formazan crystal formation compared to the control, showing substantial inhibition of cellular metabolic activity. This effect closely paralleled the response observed with doxorubicin treatment **(C)**, where formazan crystals were nearly absent, confirming the potent cytotoxic capability of this standard chemotherapeutic agent.

Bioactive Compound Identification through Database Mining

Our bioinformatics analysis, using 2 authoritative databases and EMBL-EBI, successfully identified 38 distinct bioactive compounds present in Sintrong. These phytochemical constituents, systematically compiled from 3 peer-reviewed publications, represented diverse chemical classes with significant pharmacological potential.[20-22] The identified compounds included characteristic pyrrolizidine alkaloids such as jacobine, along with several terpenoid derivatives, reflecting the complex secondary metabolite profile typical of the Asteraceae family. As detailed in **Table 1**, the phytochemical inventory comprised (1) nitrogen-containing compounds (particularly pyrrolizidine-type alkaloids), (2) various terpenoid skeletons, and (3) associated phenol derivatives. Furthermore, these 38 curated compounds functioned as the essential chemical basis for subsequent target prediction.

Computational Target Prediction of Key Bioactive Compounds from Sintrong

SwissTargetPrediction analysis revealed 3 major bioactive compounds from Sintrong with significant pharmacological potential, namely carvacrol and *β-caryophyllene* (appearing twice in this analysis). These compounds demonstrated specific and potentially therapeutic interactions with key human protein targets, as detailed in **Table 2**.

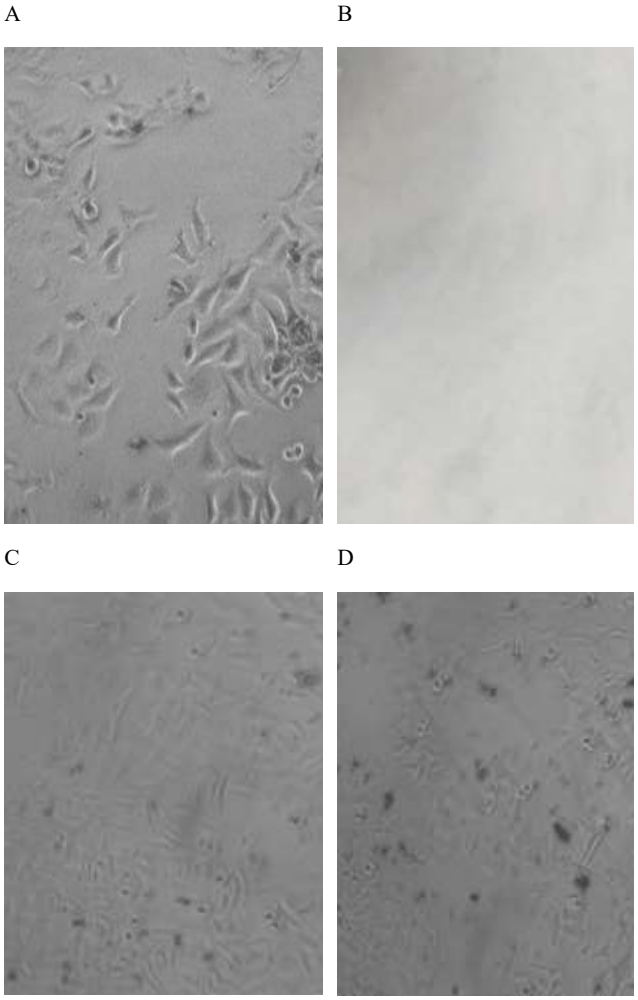
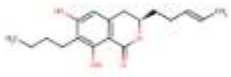
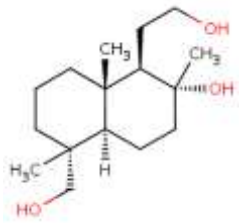
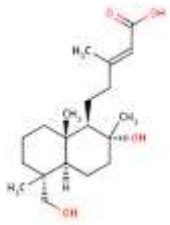


Figure 2: Morphology of T47D cells after treatment at 10x magnification: (A) control cells; (B) media control; (C) T47D cells treated with doxorubicin; (D) extract-treated T47D cells

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

Table 1: Representative identified bioactive compounds from Sintrong through database mining.

No.	Name	M.W. (g/mol)	SMILES	Chemical Structure	Ref
1	(R)-7-butyl-6,8-dihydroxy-3-[(3E)-pent-3-en-1-yl]-3,4-dihydroisochromen-1-one	304.3	CCCCc1c(O)cc2C[C@@H](CC\C=C\C)OC(=O)c2c1O		20
2	13,14,15,16-tetranorlabdane-8alpha,12,18-triol	270.4	C[C@@]1(O)CC[C@H]2[C@](C)(CO)CCC[C@]2(C)[C@H]1CCO		21
3	8alpha,19-dihydroxylabd-13E-ene-15-oic acid	338.4	[H][C@@]12CC[C@@](C)(O)[C@H](CC\C(C)=C\C(O)=O)[C@@]1(C)CCC[C@@]2(C)CO		21
4	Jacobine	351.4	O=C1OCC2=CCN3[C@H]2[C@@H](CC3)OC(=O)[C@@]2(C[C@H]([C@@]1(C)O)C)O[C@H]2C		22
5	(15alpha,20R)-12,15,20-Trihydroxy-15,20-dihydrosenecionan-11,16-dione	369.41	C[C@H]([C@@]1(O)C[C@@H](C)[C@@](C)(O)C(=O)OCC2=CCN3[C@H]2[C@H](OC1=O)CC3)O		22

Carvacrol, a monoterpenoid phenol, showed strong predicted binding affinity for cyclooxygenase-1 (COX-1), suggesting potential anti-inflammatory effects through prostaglandin biosynthesis modulation. The first *β*-Caryophyllene entry exhibited significant interaction potential with peroxisome proliferator-activated receptor alpha (PPAR-*α*), indicating possible metabolic regulation and lipid homeostasis effects. Interestingly, the second *β*-Caryophyllene prediction targeted cannabinoid receptor 2 (CNR2), implicating potential immunomodulatory and analgesic pathways.

The 3 key bioactive compounds (carvacrol, *β*-caryophyllene) target PTGS1 (COX-1), PPARA (PPAR-*α*), and CNR2 (CB2) - proteins intimately involved in cancer-related inflammation and metabolism. These results were consistent with studies reporting *β*-caryophyllene's PPAR-*α*-mediated antitumor effects in various cancer cell lines, including HCT116, PANC-1, PC3, and MCF-7.[23] Furthermore, this study was the first to implicate such mechanisms in luminal breast cancer cells (T47D). Further support was provided by protein–protein interaction (PPI) network analysis, which revealed novel connections between Sintrong's predicted targets and established breast cancer-related proteins, specifically those involved in lipid metabolism pathways. This observation reinforced earlier results by Blücher and Stadler (2017), who emphasized altered lipid metabolism as a vulnerability in luminal breast cancer.[24]

Network Analysis and Functional Validation of Sintrong Putative Protein Targets in Breast Cancer

This comprehensive network pharmacology approach yielded significant insights into the potential anticancer mechanisms of Sintrong compounds. The PPI network constructed using the STRING database (**Figure 3A**) showed that the 3 primary targets (PTGS1/COX-1, PPARA/PPAR-*α*, and CNR2/CB2) formed extensive interactions

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

with established breast cancer-associated proteins, suggesting their functional relevance in oncogenic pathways. Furthermore, the network exhibited high connectivity scores (>0.8), with particularly strong interactions between PPARA and lipid metabolism regulators and between CNR2 and immune response modulators. Both processes were critically dysregulated in breast cancer progression. Validation through cBioPortal analysis (**Figure 3B**) confirmed these targets' clinical relevance, showing significant genomic alterations (amplifications/mutations) in >15% of TCGA breast cancer cases.

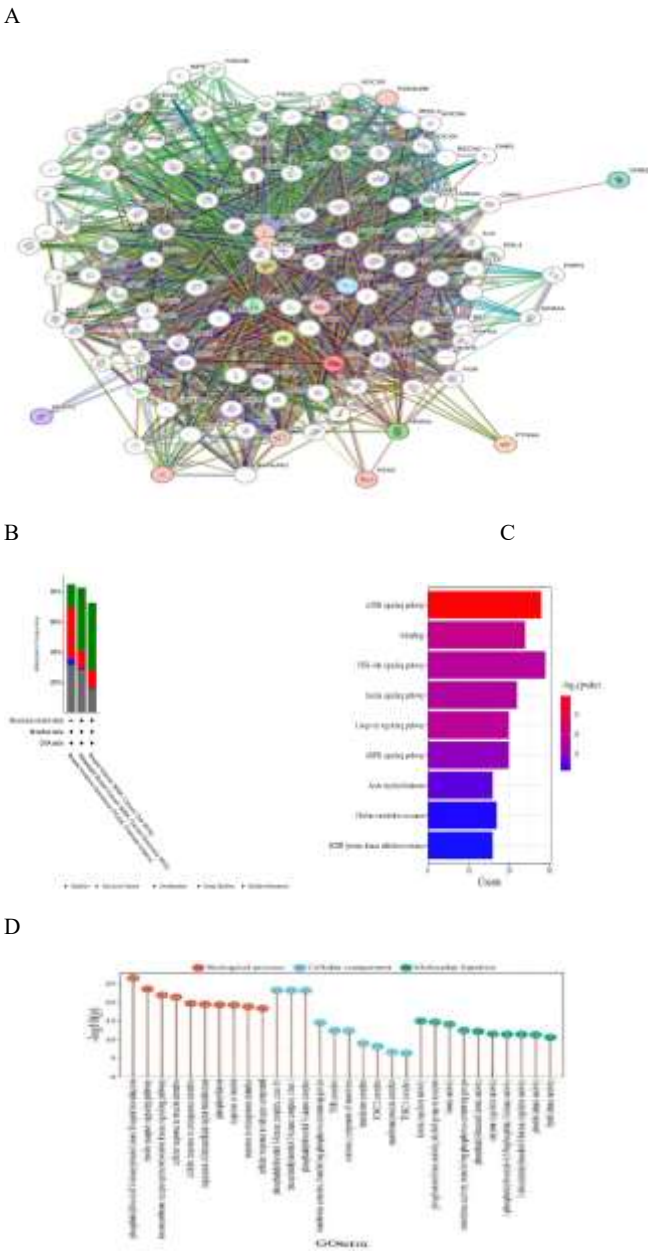


Figure 3: Integrated network pharmacology analysis of Sintrong protein targets in breast cancer. **(A)** Protein-protein interaction (PPI) network from STRING database. **(B)** cBioPortal genomic validation showing alteration frequencies of targets in TCGA breast cancer cohorts. **(C)** WebGestalt pathway enrichment analysis revealing significant cancer-related pathways. **(D)** Functional annotation of targets across biological processes (red), cellular components (blue), and molecular functions (green).

Web Gestalt functional enrichment analysis (**Figure 3C**) further substantiated these results, identifying significant involvement in cancer hallmark pathways including "PPAR signalling pathway," "Eicosanoid inflammation," and "Endocannabinoid signalling." Furthermore, the multi-level functional characterization (Figure 4D) demonstrated the Biological Process, Cellular Component, and Molecular Function of those proteins.Enrichment analysis also showed significant involvement of PPAR signalling and inflammatory pathways, consistent with previous reports on plant-derived terpenoids.[25] The predicted CB2 targeting contrasted with most anticancer phytochemical studies focusing on CB1, suggesting that Sintrong used distinct endocannabinoid modulation. This result warranted further investigation given emerging evidence of CB2's role in tumour microenvironment regulation.[26,27] Moreover, the extract's cytotoxicity at relatively low concentrations (<30 µg/mL) was notable when compared with other Asteraceae species such as Vernonia amygdalina (EC_{50} ~50 µg/mL under similar assay conditions), suggesting that Sintrong could possess more potent or synergistic bioactive constituents.[28] This was consistent with our phytochemical detection of jacobine-type alkaloids, which are compounds shown in Senecio species to induce DNA damage in cancer cells.[29] The lack of cytotoxicity in normal cell controls suggested a degree of selectivity that merited further investigation.

The presence of pyrrolizidine alkaloids (PAs), including jacobine derivatives, raised important safety considerations for therapeutic development.[30] Several studies documented the hepatotoxic and genotoxic potential of unsaturated PAs through metabolic activation into reactive pyrrolic derivatives that formed DNA adducts.[31,32] Chronic exposure to these compounds was associated with hepatic sinusoidal obstruction syndrome (HSOS) in humans, as evidenced by outbreaks of PA-induced liver toxicity from contaminated herbal products.[33,34] Particularly relevant to breast cancer applications, certain PAs had demonstrated dose-dependent carcinogenicity in rodent models through activation of pro-inflammatory cytokines (IL-6, TNF-α) and oxidative stress pathways.[35] However, it must be noted that toxicity profiles varied significantly among PA subclasses, with some exhibiting minimal toxicity at therapeutic doses.[36] These safety concerns necessitated (1) rigorous quantification of PAs content in Sintrong preparations, (2) evaluation of hepatic and renal toxicity in preclinical models, and (3) consideration of detoxification strategies such as fractional extraction or combinatorial formulations with hepatoprotective agents. The concentration-dependent duality of PAs, exhibiting both anticancer bioactivity and potential toxicity, emphasized the importance of establishing precise therapeutic windows for any clinical development of Sintrong-based therapies.

While this study provided compelling evidence for the anticancer potential of Sintrong against T47D breast cancer cells, several limitations must be considered when interpreting the results. The use of a crude ethyl acetate extract, while pharmacologically relevant for traditional medicine applications, made it difficult to attribute the observed cytotoxic effects to specific bioactive compounds.[37] Computational target predictions, though validated through multiple bioinformatics approaches (SwissTargetPrediction, STRING, Web Gestalt), required experimental confirmation using techniques such as molecular docking or competitive binding assays. Furthermore, the exclusive focus on T47D cells (ER+/PR+, luminal A subtype) limited the understanding of Sintrong's potential effects on other molecular subtypes of breast cancer (e.g., HER2-enriched or triple-negative) or normal mammary epithelial cells. The 24-hour treatment period in cytotoxicity assays, while standard for initial screening, could not capture longer-term adaptive responses or potential development of resistance. In silico analyses could not account for critical pharmacokinetic factors such as oral bioavailability, metabolic stability, or tissue distribution that influenced therapeutic potential *in vivo*.[38]

Table 2: Predicted protein targets of bioactive compounds from *C. crepidioides* identified through Swiss Target Prediction analysis.

Compound name	Protein	Function	Gen ID
Carvacrol	Cyclooxygenase-1	Oxidoreductase	PTGS1
beta-Caryophyllene	Peroxisome proliferator-activated receptor alpha	Nuclear receptor	PPARA
beta-Caryophyllene	Cannabinoid receptor 2	Family A G protein-coupled receptor	CNR2

Future studies must prioritize bioactivity-guided fractionation to isolate and characterize the specific compounds responsible for Sintrong's cytotoxic effects. This was followed by comprehensive mechanistic studies to validate the predicted protein targets (PTGS1, PPARA, CNR2) using genetic and pharmacological approaches. Expanded cytotoxicity profiling across a panel of breast cancer cell lines representing different molecular subtypes provided essential information about the spectrum of activity and potential therapeutic windows. *In vivo* efficacy studies using appropriate animal models of luminal breast cancer were important to evaluate tumour suppression potential and systemic toxicity. Due to the multi-target nature of the predicted activities, systems biology approaches (e.g., transcriptomics, proteomics) showed novel pathway interactions and potentially synergistic combinations with existing therapies. Pharmaceutical development efforts must focus on optimizing the bioavailability and stability of active constituents through formulation strategies. These investigations validated and expanded on the current results but also facilitated the translation of Sintrong from traditional use toward potential clinical application while addressing the current gaps in the understanding of its pharmacological properties. Therefore, this integrated approach could facilitate the translation of Sintrong from traditional medicine into evidence-based oncology applications.

Conclusion

This study comprehensively integrates network pharmacology and experimental validation to investigate the anticancer potential of Sintrong against luminal breast cancer. Ethyl acetate fraction exhibits significant cytotoxicity against T47D cells (EC_{50} = 28.07 ± 0.675 µg/mL), comparable to doxorubicin, and is selectively toxic to cancer cells. Network-based analyses identified bioactive compounds (carvacrol and *β*-caryophyllene) that potentially modulate key cancer-related targets: PTGS1, PPARA, and CNR2. While the presence of pyrrolizidine alkaloids necessitates rigorous safety evaluation, the multi-target profile and selective cytotoxicity position Sintrong as a promising candidate for further development as an adjunct treatment for luminal breast cancer.

Conflicts of Interest

The authors declare no conflicts of interest.

Authors' Declaration

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

Acknowledgments

The authors are grateful to Universitas Muhammadiyah Pekajangan Pekalongan (UMPP) for providing laboratory facilities, funding support (grant number D43TW009672), and an enabling research environment.

References

1. F Bray 1, M Laversanne, H Sung, J Ferlay, RL Siegel, I Soerjomataram, A Jemal. Global cancer statistics 2022: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2024;74(3):229–63. Doi:10.3322/caac.21834
2. Yunmeng Z, Yuting J, Siwen L, Jingjing L, Jie W, Qianyun J, Xiaomin L, Hongyuan D, Zhuowei F, Ya L, Yacong Z, Zhangyan L, Fangfang S, Fengju S, Lei Y, Hong L, and Yubei Huang . Global burden of female breast cancer: new estimates in 2022, temporal trend and future projections up to 2050 based on the latest release from GLOBOCAN. Journal of the National Cancer Center. 2025; 5 (3): 287-296. Doi: 10.1016/j.jncc.2025.02.002.
3. Mohammadpour S, Soleimanpour S, Javan-Noughabi J, Gallehzan NA, Aboutorabi A, Jahangiri R. A systemmatic literature review on indirect costs of women with breast cancer. Cost Effectiveness and Resource Allocation. 2022 ;20(1):68. Doi:10.1186/s12962-022-00408-6Doi:10.1186/s12962-022-00408-6
4. Duggan C, Trapani D, Ilbawi AM, Fidarova E, Laversanne M, Curigliano G, F Bay, BO Anderson. National health system characteristics, breast cancer stage at diagnosis, and breast cancer mortality: a population-based analysis. Lancet Oncol. 2021;22(11):1632–42. Doi:10.1016/S1470-2045(21)00462-9Doi:10.1016/S1470-2045(21)00462-9
5. Obidiro O, Battogtokh G, Akala EO. Triple Negative Breast Cancer Treatment Options and Limitations: Future Outlook. Pharmaceutics. 2023;15(7):1796. Doi:10.3390/pharmaceutics15071796Doi:10.3390/pharmaceutics15071796
6. Mills JN, Rutkovsky AC, Giordano A. Mechanisms of resistance in estrogen receptor positive breast cancer: overcoming resistance to tamoxifen/aromatase inhibitors. Curr Opin Pharmacol. 2018;41:59–65. Doi:10.1016/j.coph.2018.04.009Doi:10.1016/j.coph.2018.04.009
7. Shaikh AY, Shih JA. Chemotherapy-Induced Cardiotoxicity. Curr Heart Fail Rep. 2012; 9(2):117–27. https://pubmed.ncbi.nlm.nih.gov/24023601/https://pubmed.ncbi.nlm.nih.gov/24023601/
8. Yang J, Chen WY, Fu Y, Yang T, Luo XD, Wang YH. Medicinal and edible plants used by the Lhoba people in Medog County, Tibet, China. J Ethnopharmacol. 2020; 249:112430. Doi:10.1016/j.jep.2019.112430Doi:10.1016/j.jep.2019.112430
9. Zhang S, Liu K, Liu Y, Hu X, Gu X. The role and application of bioinformatics techniques and tools in drug discovery. Front Pharmacol. 2025;16. Doi:10.3389/fphar.2025.1547131Doi:10.3389/fphar.2025.1547131
10. Yulyana A, Amin C, Simanjuntak P, Abdillah S, Rohman A, Mugiyanto E. Assessing the Antimetabolite Activity of Anthocyanins in Cantigi Fruits from Two Conservation Sites in Indonesia. Indonesian Journal of Pharmacy. 2023; 34(3), 450–459. Doi:10.22146/ijp.8788
11. Falodun, A., Uzoekwe A. S., and Shengxiang Q. Phytochemical, Anticancer and Antioxidant Evaluation of Potential Chemical Consituents of Calliandria Surinamensis. Nig J. Biotech. Vol. 21 (2010) 55 – 59 . Doi: 10.4314/njb.v21i1
12. Mohanraj K, Karthikeyan BS, Vivek-Ananth RP, Chand RPB, Aparna SR, Mangalapandi P. IMPPAT: A curated database of Indian Medicinal Plants, Phytochemistry And Therapeutics. Sci Rep. 2018;8(1):4329. Doi:10.1038/s41598-018-22631-zDoi:10.1038/s41598-018-22631-z
13. McWilliam H, Li W, Uludag M, Squizzato S, Park YM, Buso N,. Analysis Tool Web Services from the EMBL-EBI. Nucleic Acids Res. 2013;41(W1):W597–600. Doi:10.1093/nar/gkt376Doi:10.1093/nar/gkt376
14. Zoete V, Daina A, Bovigny C, Michielin O. SwissSimilarity: A Web Tool for Low to Ultra High Throughput Ligand-Based Virtual Screening. J Chem Inf Model. 2016;56(8):1399–404. Doi:10.1021/acs.jcim.6b00174

15. Szklarczyk D, Nastou K, Koutrouli M, Kirsch R, Mehryary F, Hachilif R,. The STRING database in 2025: protein networks with directionality of regulation. Nucleic Acids Res. 2025;53(D1):D730–7. Doi:10.1093/nar/gkae1113Doi:10.1093/nar/gkae1113
16. Mugiyanto E, Adikusuma W, Irham LM, Huang WC, Chang WC, Kuo CN. Integrated genomic analysis to identify druggable targets for pancreatic cancer. Front Oncol. 2022;12. Doi:0.3389/fonc.2022.989077Doi:0.3389/fonc.2022.989077
17. Kanehisa M, Furumichi M, Sato Y, Matsuura Y, Ishiguro-Watanabe M. KEGG: biological systems database as a model of the real world. Nucleic Acids Res. 2025;53(D1):D672–7. Doi:10.1093/nar/gkae909Doi:10.1093/nar/gkae909
18. Liao Y, Wang J, Jaehnig EJ, Shi Z, Zhang B. WebGestalt 2019: gene set analysis toolkit with revamped UIs and APIs. Nucleic Acids Res. 2019 ;47(W1):W199–205. Doi:10.1093/nar/gkz401Doi:10.1093/nar/gkz401
19. Elizarraras JM, Liao Y, Shi Z, Zhu Q, Pico AR, Zhang B. WebGestalt 2024: faster gene set analysis and new support for metabolomics and multi-omics. Nucleic Acids Res. 2024;52(W1):W415–21. Doi:10.1093/nar/gkae456Doi:10.1093/nar/gkae456
20. Kongsaeree P, Prabpai S, Sriubolmas N, Vongvein C, Wiyakrutta S. Antimalarial Dihydroisocoumarins Produced by *Geotrichum* sp., an Endophytic Fungus of *Crassocephalum c repidioides*. J Nat Prod. 2003;66(5):709–11. Doi:10.1021/np0205598Doi:10.1021/np0205598
21. Hegazy MEF, Ohta S, Abdel-latif FF, Albadry HA, Ohta E, Paré PW,. Cyclooxygenase (COX)-1 and -2 Inhibitory Labdane Diterpenes from *Crassocephalum mannii*. J Nat Prod. 2008;71(6):1070–3. Doi:10.1021/np800017xDoi:10.1021/np800017x
22. Zollo PHA, Kuiate JR, Menut C, Bessiere JM. Aromatic Plants of Tropical Central Africa. XXXVI. Chemical Composition of Essential Oils from Seven Cameroonian *Crassocephalum* Species. Journal of Essential Oil Research. 2000;12(5):533–6. Doi:10.1080/10412905.2000.9712152Doi:10.1080/10412905.2000.9712152
23. Fidyt K, Fiedorowicz A, Strządała L, Szumny A. β -caryophyllene and β -caryophyllene oxide—natural compounds of anticancer and analgesic properties. Cancer Med. 2016;5(10):3007–17. Doi:10.1002/cam4.816Doi:10.1002/cam4.816
24. Blücher C, Stadler SC. Obesity and Breast Cancer: Current Insights on the Role of Fatty Acids and Lipid Metabolism in Promoting Breast Cancer Growth and Progression. Front Endocrinol (Lausanne). 2017;8. Doi:10.3389/fendo.2017.00293Doi:10.3389/fendo.2017.00293
25. Yao P, Liu Y. Terpenoids: Natural Compounds for Non-Alcoholic Fatty Liver Disease (NAFLD) Therapy. Molecules. 2022;28(1):272. Doi:10.3390/molecules28010272Doi:10.3390/molecules28010272
26. Pérez-Gómez E, Andradas C, Blasco-Benito S, Caffarel MM, García-Taboada E, Villa-Morales M,. Role of Cannabinoid Receptor CB2 in HER2 Pro-oncogenic Signaling in Breast Cancer. J Natl Cancer Inst. 2015;107(6). Doi:10.1093/jnci/djv077Doi:10.1093/jnci/djv077
27. Ellert-Miklaszewska A, Grajkowska W, Gabrusiewicz K, Kaminska B, Konarska L. Distinctive pattern of cannabinoid receptor type II (CB2) expression in adult and pediatric brain tumors. Brain Res. 2007;1137:161–9. Doi:10.1016/j.brainres.2006.12.060Doi:10.1016/j.brainres.2006.12.060
28. Toyang NJ, Verpoorte R. A review of the medicinal potentials of plants of the genus Vernonia (Asteraceae). J Ethnopharmacol. 2013;146(3):681–723. Doi:10.1016/j.jep.2013.01.040Doi:10.1016/j.jep.2013.01.040
29. Kalač P, Kaltner F. Pyrrolizidine alkaloids of European Senecio/Jacobaea species in forage and their carry-over to milk: A review. Anim Feed Sci Technol. 2021;280:115062. Doi:10.1016/j.anifeedsci.2021.115062Doi:10.1016/j.anifeedsci.2021.115062
30. Casado N, Morante-Zarcero S, Sierra I. The concerning food safety issue of pyrrolizidine alkaloids: An overview. Trends Food Sci Technol. 2022;120:123–39 . Doi:10.1016/j.tifs.2022.01.007Doi:10.1016/j.tifs.2022.01.007
31. Fu PP, Xia Q, Lin G, Chou MW. Pyrrolizidine Alkaloids—Genotoxicity, Metabolism Enzymes, Metabolic Activation, and Mechanisms. Drug Metab Rev. 2004;36(1):1–55. Doi:10.1081/DMR-120028426Doi:10.1081/DMR-120028426
32. Li N, Xia Q, Ruan J, P. Fu P, Lin G. Hepatotoxicity and Tumorigenicity Induced by Metabolic Activation of Pyrrolizidine Alkaloids in Herbs. Curr Drug Metab. 2011;12(9):823–34. Doi:10.2174/138920011797470119Doi:10.2174/138920011797470119
33. Chojkier M. Hepatic sinusoidal-obstruction syndrome: toxicity of pyrrolizidine alkaloids. J Hepatol. 2003;39(3):437–46. Doi:10.1016/S0168-8278(03)00231-9Doi:10.1016/S0168-8278(03)00231-9
34. Zhuge Y, Liu Y, Xie W, Zou X, Xu J, Wang J. Expert consensus on the clinical management of pyrrolizidine alkaloid-induced hepatic sinusoidal obstruction syndrome. J Gastroenterol Hepatol. 2019;34(4):634–42. Doi:10.1111/jgh.14612Doi:10.1111/jgh.14612
35. Aryal B, Raut BK, Bhattarai S, Bhandari S, Tandan P, Gyawali K,. Potential Therapeutic Applications of Plant-Derived Alkaloids against Inflammatory and Neurodegenerative Diseases. Evid Based Complement Alternat Med. 2022;2022:1–18. Doi:10.1155/2022/7299778Doi:10.1155/2022/7299778
36. Molyneux RJ, Gardner DL, Colegate SM, Edgar JA. Pyrrolizidine alkaloid toxicity in livestock: a paradigm for human poisoning? Food Additives & Contaminants: Part A. 2011;28(3):293–307. Doi:10.1080/19440049.2010.547519Doi:10.1080/19440049.2010.547519
37. Popov SA, Sheremet OP, Kornaukhova LM, Grazhdannikov AE, Shults EE. An approach to effective green extraction of triterpenoids from outer birch bark using ethyl acetate with extractant recycle. Ind Crops Prod. 2017;102:122–32. Doi:10.1016/j.indcrop.2017.03.020Doi:10.1016/j.indcrop.2017.03.020
38. Boobis A, Gundert-Remy U, Kremers P, Macheras P, Pelkonen O. In silico prediction of ADME and pharmacokinetics. Eur J Pharm Sci. 2002;17(4–5):183–93. Doi:10.1016/S0928-0987(02)00185-9Doi:10.1016/S0928-0987(02)00185-9