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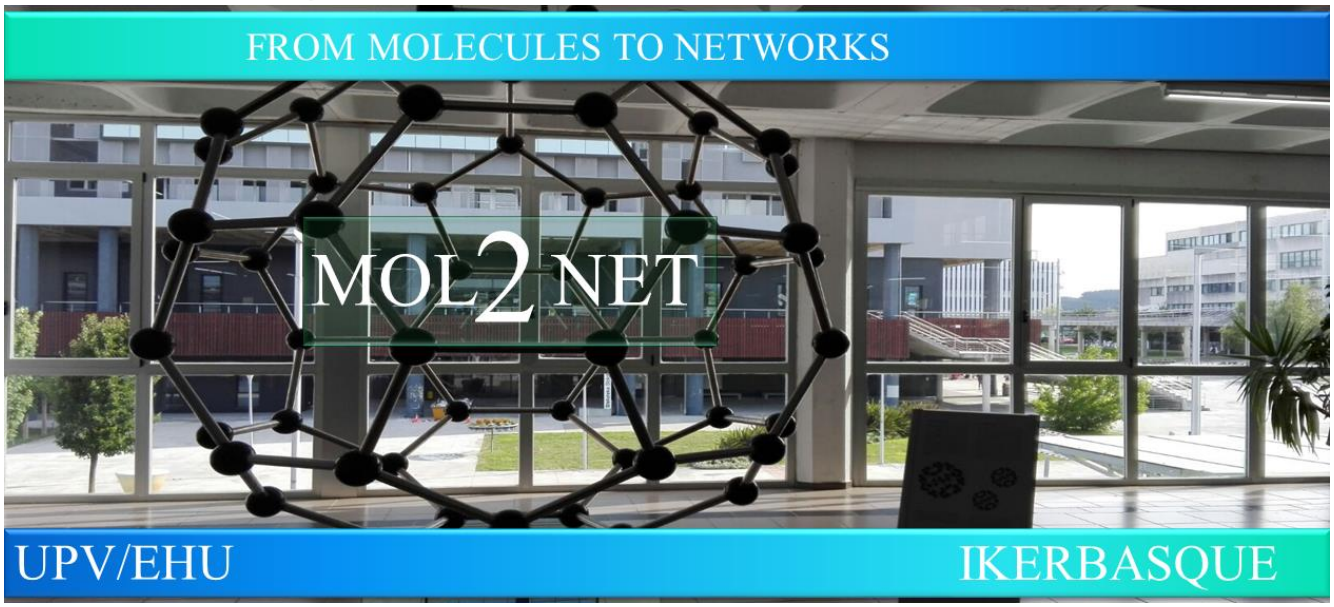
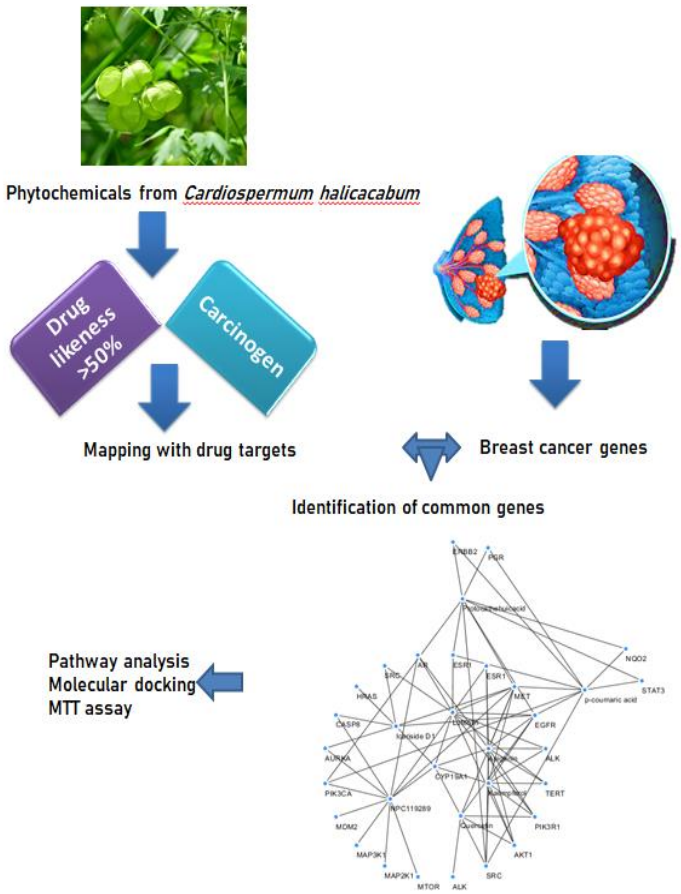
MOL2NET'21, Conference on Molecular, Biomedical & Computational Sciences and Engineering, 7th ed.



A networkpharmacology -based analysis of main pharmacological pathways of Cardiospermum Halicacabum acting on Human Breast Cancer with computational and experimental validation

Dhivya Shanmugarajan1 and Charles David 1.*

[1] *Department of Biotechnology, Vignan's Foundation for Science, Technology and Research (Deemed to be University), Guntur, Andhra Pradesh, India*



Abstract

The discovery of medicines based on natural products is thriving in the world. India is rich in medicinal plants and these plants are known to treat a variety of diseases. One among Cardiospermum halicacabum or balloon wine is a climbing plant. It belongs to family Sapindaceae and it's known for various pharmacological activities. In total 170 active ingredients were curated from Collective Molecular Activities of Useful Plants(CMAUP) platform and literature research are funnelled for >50% drug-likeness via BIOVIA Discovery studio small molecule tool. The OMIM, Therapeutic Targets database, DrugBank and GeneCard were used to establish a database of breast cancer targets. The interactive compound-gene target was created with Cytoscape software (Version 3.6.1). Further, the protein-protein interaction network was mapped with the STRING database, and the related protein interaction relationship was analyzed. KEGG enrichment and Gene Ontology functional analysis for main drug targets pathways are involved Estrogen signaling pathway,Ras signaling pathway,Proteoglycans in cancer. Finally, molecular docking of the active ingredients with the major drug target was carried out. Also, the cell line study was carried out in human breast cancer cell lines MCF7 to
investigate the level of the cytotoxicity.

Keywords: medicinal plants, compound-gene, protein-protein, active ingredients, cytotoxicity.

Introduction

Network pharmacology, a systematic analysis method, is used to analyze the interaction network of multiple parameters such as drugs/compounds, diseases, target proteins, and genes (Hopkins, 2007&2008). Networked pharmacology can decipher the mechanism of action of drugs from a holistic perspective, which focuses on changing the paradigm from " one drug, one target," to "target network for various therapeutics".Breast cancer is the most prevalent cancer and the leading cause of death for women worldwide (Yu Jin Kim et al., 2022). According to the World Health Organization's most recent statistics on cancer, 11.7% of all new instances of cancer were reported to be breast cancer in 2020 (World Health Organization: A Global report on breast cancer, 2020). Estimates indicate that 627,000 women lost their lives due to breast cancer in 2018. In 2019, there were projected to be 42,260 fatal cases and 271,270 new cases of breast cancer in the United States alone (DeSantis et al., 2019). In 2022, it is anticipated that 287,000 new cases of breast cancer would be reported (Grace L Wong et al., 2022). The mortality rate from breast cancer is higher in low- and middle-income countries as compared to high-income countries.

The differences in survival rates have been attributed to early detection through comprehensive mammographic screening and the availability of effective adjuvant systemic therapy for women in high-income nations (Ferlay et al., 2014). Breast cancer is a disease that affects both men and women, with 0. 8–1% of cases occurring in men (Fentiman et al., 2006).*Cardiospermum halicacabum* is a well-known medicinal plant that serves several functions. It is widely grown in Africa, America, and Asia and belongs to the Sapindaceae family (A. Guliya et al., 2010). It is commonly known as balloon vines. Its huge, black seeds with a white mark that resembles a stylized heart led to the generic name Cardiospermum, which translates to "heart seeds." The specific epithet halicacabum alludes to the inflated fruits, from which the popular name balloon vine is derived. It is taken from

the Greek word for a salt barrel. The roots of C. halicacabum have historically been used to treat epilepsy and anxiety problems (Rajesh Kumar et al., 2011).

It has been demonstrated that an ethanol extract of *C. halicacabum* leaves possesses anti-inflammatory properties against carrageenan-induced rat paw edema (J. Sadique et al., 1987). C. halicacabum plant has been reported to have analgesic and vasodepressant properties (C. Gopalakrishnan et al., 1976). Rats exposed to pyrexia caused by yeast can be treated with an ethanol extract of *C. halicacabum* (V. V. Asha et al., 1999). The ethanol extract of *C. halicacabum* possesses anti-ulcer properties against ethanol-induced stomach ulcers in rats (M. S. Sheeba et al., 2006). Wistar rats that have been given streptozotocin to produce diabetes respond favorably to the ethanol extract of *C. halicacabum* (Chinnadurai Veeramani et al., 2008). In this current study, the network pharmacology approach was implemented to investigate the *Cardiospermum halicacabum* active ingredients against breast cancer computationally and experimentally.

Methods

Compound data curation and screening

The active ingredient of the plant *Cardiospermum halicacabum* was retrieved from the Collective Molecular Activities of Useful Plants (CMAUP) database provides the collective landscape information of multiple targets, activity profile, ontologies and biological pathways(https://bidd.group/CMAUP/index.html) and literature study. The collective compounds are funneled for RO5 violation or oral drug availability using the small molecule tool of BIOVIA Discovery studio.

Gene Target acquisition related to disease effects

Breast cancer prevalent genes or drug candidates are searched and retrieved from various databases like OMIM database (https://www.omim.org/), Therapeutic Targets database (http://bidd.nus.edu.sg/BIDD-Databases/ TTD/TTD.asp), GeneCard database (https://www.genecards.org/),and DrugBank database (https://www. drugbank.ca). Database search results are combined, and duplicate targets are removed to retrieve all drug targets of breast cancer.

Selected Compounds mapped with Target Genes

The 1D format SMILES of screened compounds are mapped with target genes using SwissTargetPrediction (www.swisstargetprediction.ch). The overlapping genes of breast cancer were identified and visualized by a Venn diagram, plotted using a free online platform (https://bioinformatics.psb.ugent.be/webtools/Venn/)

Cytoscape network Construction Between Overlapping Genes and compounds

The network of the interactions was constructed, analyzed, and visualized by Cytoscape ver. 3.6.1 (https://cytoscape.org/).The main active ingredients and gene hubs of *Cardiospermum halicacabum* against breast cancer were selected based on the degree value of genes or compounds. The higher degree value of genes or compounds is considered an important compound or gene for the therapeutic effect.

Overlapping Genes for Pathway Enrichment Analysis with computational and experimental validation

The overlapping genes were subjected to Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis for Visualization and Annotation using DAVID Bioinformatics Resources(https://david.ncifcrf.gov/) with the “Homo sapiens” setting. Consequently, KEGG pathway enrichment results decipher the potential molecular mechanisms of Cardiospermum halicacabum against breast cancer. Finally, the ethanolic extract of Cardiospermum halicacabum was used to study the cytotoxicity effect against The human breast cancer cell line MCF7 to investigate the cytotoxicity effect(Tohkayomatee etal., 2022) and COCKER docking in BIOVIA Discovery studio.

Results

In total 170 active ingredients were curated from the Collective Molecular Activities of Useful Plants(CMAUP) platform and literature research is funneled for >50% drug-likeness via the BIOVIA Discovery studio small molecule tool. Further, carcinogen screening results 10 compounds categorized as flavonoids(Apigenin, kaempferol, Luteolin,Quercetin), steroid(Estrone), phenolic acids(protocatechuic acid and p-coumaric acid), piperidine alkaloid(N-Methylpelletierine), glycosyl (Icariside D1) and NPC119289 shows the interaction of 29 overlapping genes between all these compounds. The network analysis includes 43 nodes and 83 edges.

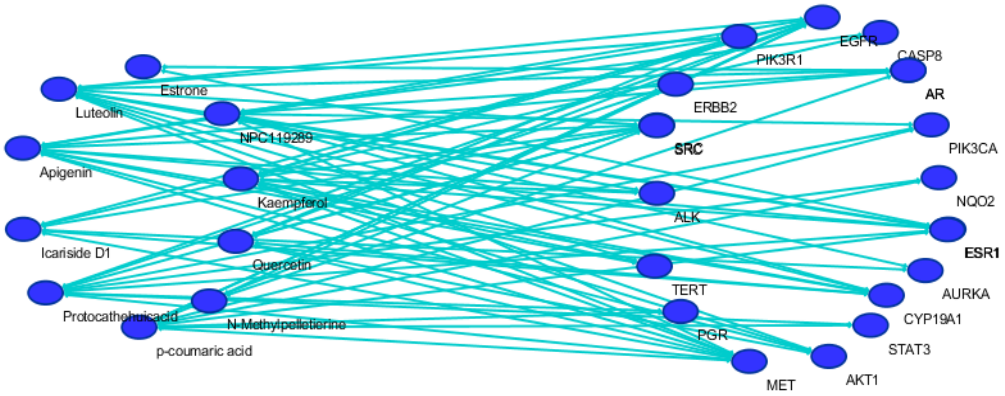


Fig 1. Compounds- Gene interactions of *Cardiospermum halicacabum*

It was simple to plot and distinguish the contribution difference of 10 screened compounds and 29 genes to *Cardiospermum halicacabum* against breast cancer. EGFR and MET, connected to eight compounds and ESR1 and SRC connected to seven compounds were considered the hub gene of *Cardiospermum halicacabum* against breast cancer. The enrichment analysis of genes is populated in the pathway (Fig 2)

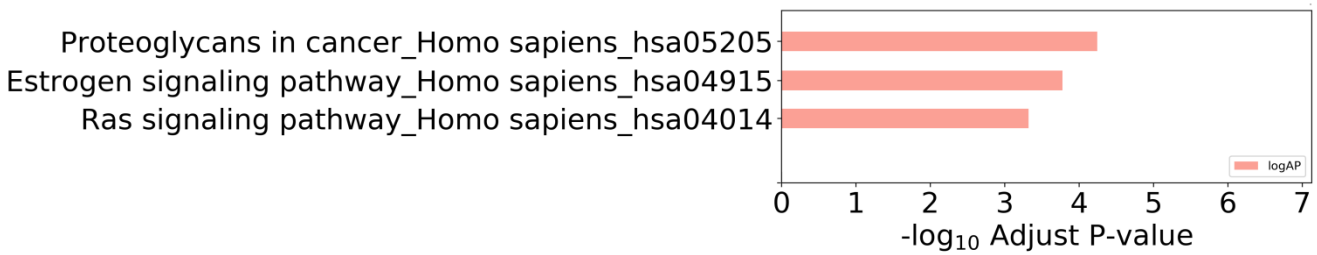


Fig 2. Enriched KEGG Pathways of Target Proteins

Among them, EGFR and ESR1 were chosen as drug targets for docking due to their high prevalenace role in many breast cancer and receptor-liagand interaction analysis was carried out using CDOCKER a molecular dynamics simulation docking tool. The receptors (EGFR and ESR1) and compounds were prepared and docked to observe the atom-level interaction.

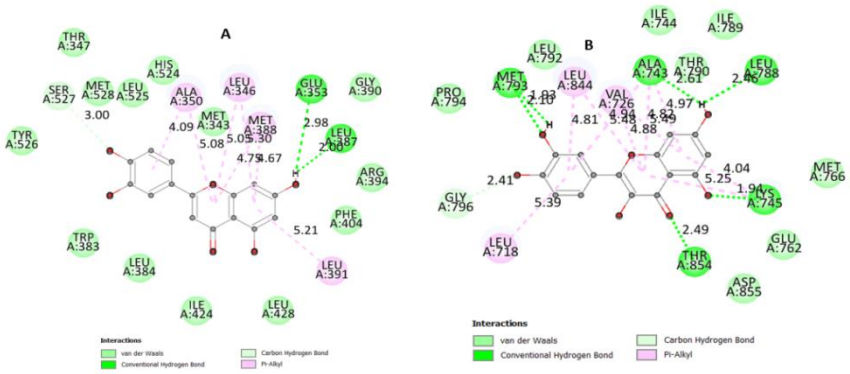


Fig 3. Atom-atom interaction of receptor-liagnd complex A: ESR1 docked with Luteolin, B: EGFR docked with Quercetin

Luteolin and Quercetin CDOCKER energy and interaction energy are -33.717 Kcal/mol, -40.3917 Kcal/mol, and -39.216 Kcal/mol , -43.6313 Kcal/mol binding affinity with active site residues Leu 346, Thr347, Ala350, Arg394(ESR1((Dhivya &Charles, 2022))) and Leu 718, Ala743, Lys745, Leu788, Thr790, Met793, Gly796 respectively. Finally, the MCF-7 cell line ethanol extract shows IC50 value of 141.2 µg/ml. Hence, the therapeutic potential of *C. halicacabum* was analyzed and validated both computationally and experimentally.

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