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From silico to benchtop: cosmosiin as a PD-1/PDL-1 immune checkpoint inhibitor revealed through DFT, network pharmacology analysis, and molecular docking integrated experimental verification

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This study investigated the *anti*-PD-1/PD-L1 inhibition potential of the flavonoid cosmosiin against breast cancer (BC) using computational chemistry, network pharmacology, bioinformatics, and validated by experimental assays. Execution of Density Functional Theory (DFT) calculations was achieved by GAUSSIAN 09 with the 6-311G/B3LYP formalism to assess cosmosiin's physicochemical properties. Potential targets of cosmosiin were identified through SuperPred, Stitch, and SwissTargetPrediction databases, while BC-allied targets were sourced by accessing GeneCards. Overlapping analysis using Venny 2.0 identified 25 common targets between 38 targets of cosmosiin and 1314 targets of breast cancer. CytoHubba analysis highlighted 10 hub genes, with PTGS2, NFKB1, and CDK5 being the most significant. Molecular docking revealed stable binding of cosmosiin to CDK5 (−8.5 kcal mol^{-1}), NFKB1 (−7.6 kcal mol^{-1}), and PTGS2 (−9.6 kcal mol^{-1}), confirmed by molecular dynamics simulations. GEPIA analysis linked the expression of these genes to survival outcomes and disease stage in breast cancer. Experimentally, cosmosiin was tested on MCF-7 (breast cancer) and MCF-10 (non-tumorigenic) cells. Cytotoxicity was evaluated using the MTT assay, showing dose-dependent viability reduction in MCF-7 cells with minimal impact on MCF-10 cells, thus exhibiting selective cytotoxicity. Phase-contrast microscopy revealed altered morphology in treated MCF-7 cells. Annexin V/PI flow cytometry confirmed increased early and late apoptosis, while EdU incorporation assays indicated decreased DNA synthesis and reduced proliferation. Transwell assays demonstrated up to 81% inhibition of cell migration at higher concentrations. Western blotting validated downregulation of CDK5, NFKB1, and PTGS2, aligning with computational predictions. These findings highlight selective, multi-targeted anticancer activity of cosmosiin, particularly through PTGS2, NFKB1, and CDK5, supporting its therapeutic potency for breast cancer with favorable effects on apoptosis, proliferation, and cell migration, while correlating with survival and immune infiltration outcomes.

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1. Introduction

Breast cancer is a complex, multifaceted disease originating in breast tissue cells. It is a kind of metastatic cancer that may disseminate to several organs, including the lungs, bones, liver, and brain, rendering it incurable. However, early detection can lead to a good prognosis and high survival rates. BC is frequently diagnosed among US women. Additionally, it leads in UK, with 55 000 new cases annually (15%), a figure expected to increase by 2% by 2035.[1,2] Breast Cancer predominantly affects women, with incidence rates increasing significantly with age including the diagnosis in about >80% of instances for those over 50 years. According to cancer statistics for 2023, the number of new BC cases has increased to 297 780 among

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