ORIGINAL ARTICLE

Multi-target prediction to Detect the Anti-cancerous Potential of Sida cordifolia in Treating Breast Cancer

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ABSTRACT

Introduction: Breast cancer arises from the uncontrolled proliferation of breast cells, leading to tumour. Sida cordifolia, commonly known as Bala, an herbaceous plant widely used in traditional medicine, particularly in Ayurveda, due to its numerous medicinal properties. This study investigates the multi-target therapeutic mechanisms of S. cordifolia against breast cancer using network pharmacology.

Materials and Methods: Phytochemicals of S. cordifolia were extracted from the IMPPAT database, and their ADME (Absorption, Distribution, Metabolism, and Excretion) properties were evaluated using SwissADME database. The web tool SwissTargetPrediction identified phytochemical targets, and breast cancer targets were retrieved from the database Open Targets Platform. Shared targets were identified using the web tool Venny 2.1.0, and a PPI (Protein-Protein Interaction) network was generated via STRING database. Hub genes were analysed in Cytoscape 3.10.2 software, with KEGG (Kyoto Encyclopaedia of Genes and Genomes) and GO (Gene Ontology) was performed using the tool ShinyGO 0.80.

Results: The KEGG pathway analysis revealed five genes: Epidermal growth factor receptor (EGFR), Estrogen receptor 1 (ESR1), mammalian target of rapamycin (mTOR), Progesterone receptor (PGR), and tumour protein p53 (TP53) that directly participate in the breast cancer pathway. These genes were identified as core targets and are targeted by the phytochemicals present in S. cordifolia, including quinazoline, abietaic acid, malvalic acid, linoleic acid, and 20-hydroxyecdysone.

Discussion: This study highlights S. cordifolia's potential as a multi-target therapeutic agent against breast cancer, with key phytochemicals targeting critical genes involved in cancer progression. These findings suggest that S. cordifolia could be a valuable candidate for further research in breast cancer treatment.

KEYWORDS:
breast cancer, medicinal plants, phytochemicals, S. cordifolia, targeted genes

INTRODUCTION

Breast cancer is the main cancer-related mortality cause for women globally. Changes to breast cells can occasionally result in breast cancer.[1] Breast cancer typically begins in the cells lining the ducts, or the tubes that carry milk from the glands to the nipple.[2] Developed nations make up almost half of the cases worldwide. This tendency is mostly the result of Western lifestyle, which has been associated to low physical activity, bad food, diabetes, and excessive stress.[3] Usually, breast cancer starts out silently and only becomes apparent when a lump develops in the region of the breast or the disease spreads. Although newly developed lumps are frequently indicative of malignancy, most of them are not. Breast tumours that are firm, painless, and have uneven margins have an increased chance of becoming malignant. A skin modification such as a redness, dimpling, or rash can also happen, as well as changes in shape, texture, and size. There might be redness or itching near the nipples. Less frequent symptoms include breast soreness, fluid leakage from the nipples, and inflammatory breast carcinoma, which produces pain, redness, and inflammation.[4] Alternative treatment options are necessary since the therapies, such as hormone therapy and chemotherapy, can lead to side effects and resistance.[5] The effectiveness and safety of natural products especially, those derived from medicinal plants in cancer therapy have drawn attention.

S. cordifolia, often known as bala (family: Malvaceae), is a highly esteemed medicinal herb utilised in Ayurveda as well as other traditional medical systems in India and other countries. Bala is classified as a bulk-promoting herb (brahmhaniya), a tonic (balya), and a reproductive aid (prajasthapana) by the famous Ayurvedic physician Charaka. Bala is also considered by Charaka to be rejuvenating (rasayana) for the muscular system and muscle tissue.[6] *S. cordifolia* utilised as an antirheumatic, analgesic, antipyretic, laxative, diuretic, hypoglycaemic, nasal anti-congestant, aphrodisiac, anti-asthmatic, and hepatoprotective in Ayurveda medicine.[7] It has been studied for its anti-inflammatory properties in the treatment of cancer and in promoting liver regeneration.[8] However, a contemporary scientific viewpoint on the medicinal potential of this traditional herb is still unclear.

Network pharmacology, an emerging field that integrates multiple scientific disciplines, explores the interactions

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between diverse compounds and various biological targets and pathways.[9] Using a network pharmacology approach, this study methodically uncovered multiple targets and pathways by which *S. cordifolia* offers its anti-cancerous properties. This study reveals the plant's regulatory function in pathways associated to breast cancer by identifying significant phytochemicals and their core targets. The findings validate the use of *S. cordifolia* as an intricate and effective treatment for breast cancer and provide a thorough understanding of the possible therapeutic advantages of the plant.

MATERIALS AND METHODS

Identification of S. cordifolia's phytochemicals

The phytochemical-based IMPPAT database (IMPPAT - accessed on 25 June 2024)[10,11] was utilised to retrieve the phytochemicals present in *S. cordifolia*. The Simplified Molecular Input Line Entry System (SMILES) is the standard way for depicting the chemical structures of phytochemicals. It was acquired using PubChem to identify possible target molecules (PubChem - accessed on 25 June 2024).

Absorption, Distribution, Metabolism, and Excretion (ADME) prediction

The ADME of the phytochemicals was estimated using the web tool SwissADME (SwissADME - accessed on 25 June 2024).[12] According to their high gastrointestinal (GI) absorption and 0.3 or higher oral bioavailability threshold, the phytochemicals of *S. cordifolia* were selected.

Target identification of S. cordifolia

SwissTargetPrediction (SwissTargetPrediction – accessed on 25 June 2024) is an online tool that forecasts the targets of phytochemicals. It has been updated with the canonical SMLES of ADME-qualified phytochemicals.[13] The targets were selected based on a probability score of 0.1 or higher, and the resulting targets were designated for further investigation.

Breast cancer associated target identification

Open Targets Platform, which was accessed on 25 June 2024, contains information on predicted and annotated targets related to human illnesses.[14] This database has been used to get molecular targets associated with breast cancer. A global score of 0.3 or above was used to filter the targets of breast cancer. The Venn diagram drawing tool, Venny 2.1.0 (Venny 2.1.0 - accessed on 25 June 2024),[15] was utilised to identify the overlapping targets in order to acquire the common targets of *S. cordifolia's* phytochemicals and breast cancer-related targets. Cytoscape 3.10.2 was utilised to create a plant-phytochemicals-target-disease network.

Construction of Protein-Protein Interaction network and HUB genes

The PPI network was created once the screened common targets were loaded into the protein-protein association networks analyser tool, STRING 12.0 (STRING – accessed on June 25 2024).[16] The species was specified as "*Homo sapiens*," 0.400 was chosen as medium confidence level and all other parameters were left unchanged. In order to get core targets for network analysis, the collected PPI results were imported into Cytoscape 3.10.2 (Cytoscape - accessed on 19 May 2024). It facilitates the visualisation of complex networks and attribute data integration. Using the CytoHubba plugin's MCC (Maximum Clique Centrality) topological analysis technique, the hub genes inside the intricate PPI network were identified.

Functional enrichment analysis

On the hub genes, the KEGG pathway enrichment analysis and GO studies were performed using Shiny GO 0.80, a graphical enrichment analysis tool (ShinyGO 0.80 - accessed on June 25 2024).[17] The results have been shown using a bar plot. The only species accepted was "*Homo sapiens*." The output of enriched GO terms for molecular function (MF), biological process (BP), cellular component (CC) was displayed using bar plot. Using 0.05 as False Discovery Rate (FDR) limit, bar plots representing the distribution of genes linked to each process were produced based on these data. The -log10FDR, fold enrichment, and gene counts for the GO analysis were displayed in the bar plot. Depending on the total number of genes associated with each pathway, the KEGG pathway analysis found pathways relevant to the targets.

RESULTS

Target identification

From Twenty phytochemicals were identified for *S. cordifolia* from the IMPPAT database and fifteen phytochemicals meet the requirements for ADME screening. Those fifteen phytochemicals' canonical SMILES were submitted for target prediction using the Swiss Target Prediction online tool. A total of 211 targets have been found to have a probability score of 0.1 or greater. 1107 targets related to breast cancer were retrieved from the Open Targets Platform based on a global score of 0.3 or higher. To find the overlapping genes, the Venny 2.1.0 database was integrated with the disease-targeted genes of breast cancer and the phytochemical-targeted genes of *S. cordifolia*. From the Venn diagram, fifty-three common targets were identified and used for further investigation (Fig. 1). The complex data was better understood by using Cytoscape 3.10.2 to produce a degree-sorted circular arrangement for a systematic depiction of the plant-phytochemical-target-disease network (Fig. 2).

Protein-Protein Interaction network construction and Hub gene screening

A PPI network was created for the fifty-three shared targets of *S. cordifolia* and breast cancer by employing the STRING database, with <-1.0e-16 as the enrichment p-value. The network consists of 399 edges (interaction) and 53 nodes (gene) with an average node degree of 15.1 (Fig. 3a). Edge thickness in the PPI network indicates the strength of the data support. The PPI network was analysed using the CytoHubba plugin to identify the top 10 most important genes, and the MCC method was used (Fig. 3b). Hub genes have a greater degree of interaction significance, suggesting a significant impact on the condition of the disease. The topmost ten hub genes are TP53, EGFR, BCL2, ESR1, BCL2L1, mTOR, MDM2, AR, PGR, and CCNA2.

Functional enrichment analysis

GO and KEGG analyses are performed on the ten hub genes

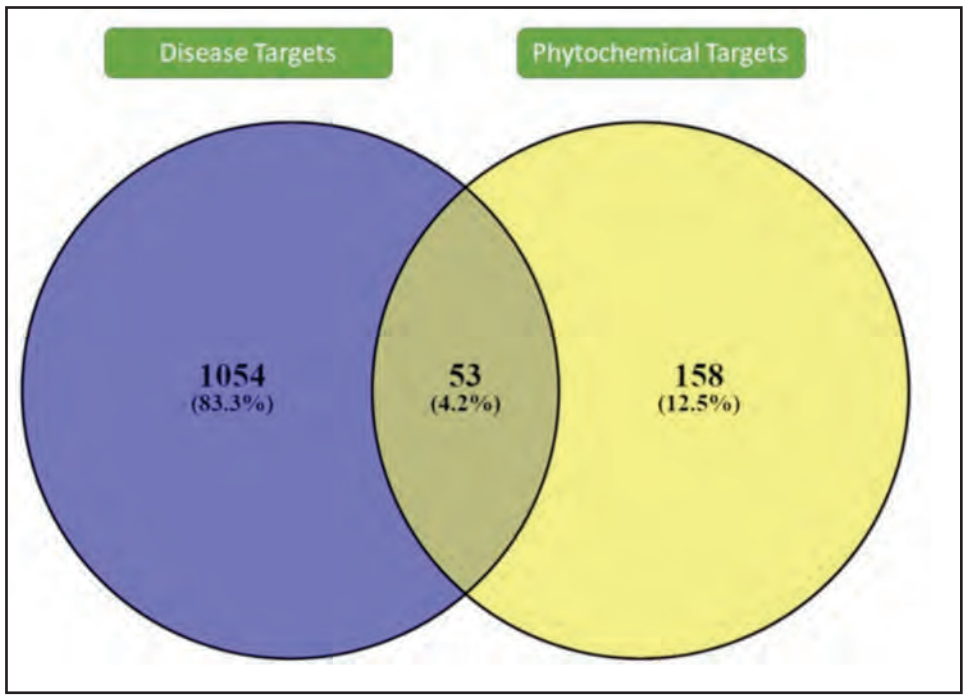

Fig. 1: Venn diagram illustrating the overlap between disease targets and phytochemical targets. The blue circle represents the 1,054 disease-targets, while the yellow circle represents the 158 phytochemical-targets. The intersection of the two circles shows 53 shared targets

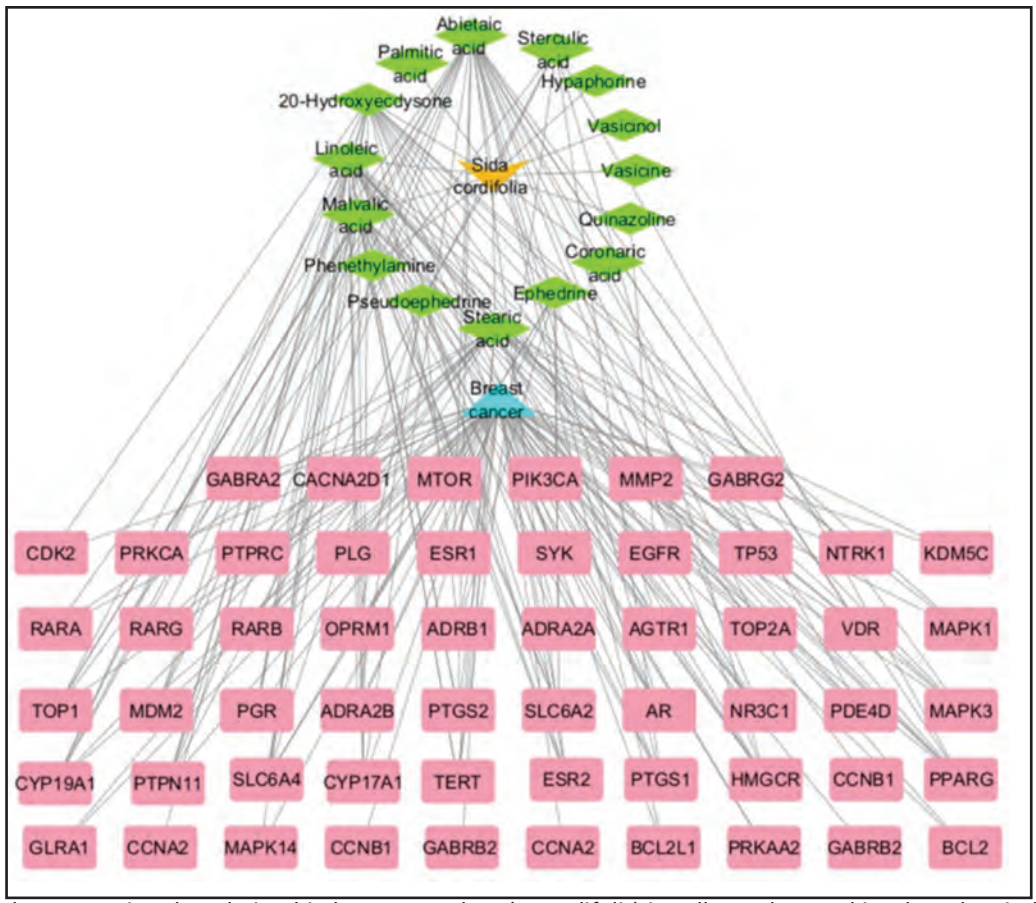

Fig. 2: A network representing the relationship between a plant (*S. cordifolia*) in yellow colour and its phytochemicals (green nodes), common targets for both phytochemicals and disease (peach nodes), and the disease (blue nodes) is depicted

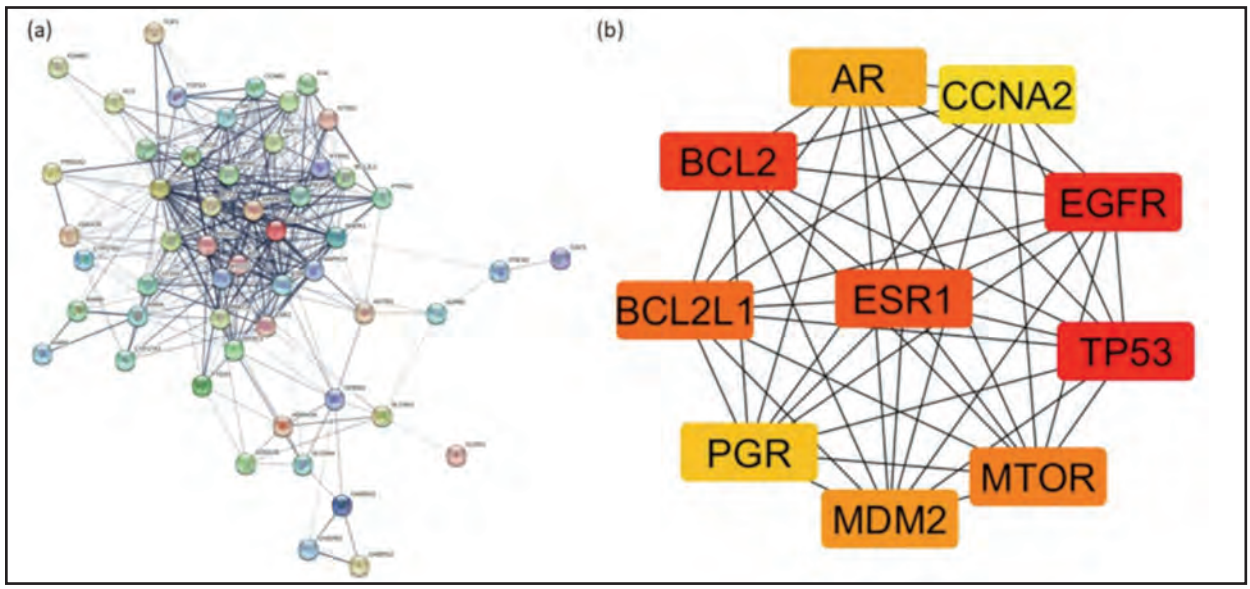

Fig. 3: (a) Protein-Protein Interaction network of the shared genes in *S. cordifolia* and breast cancer. (b) The top 10 hub genes obtained from the shared targets

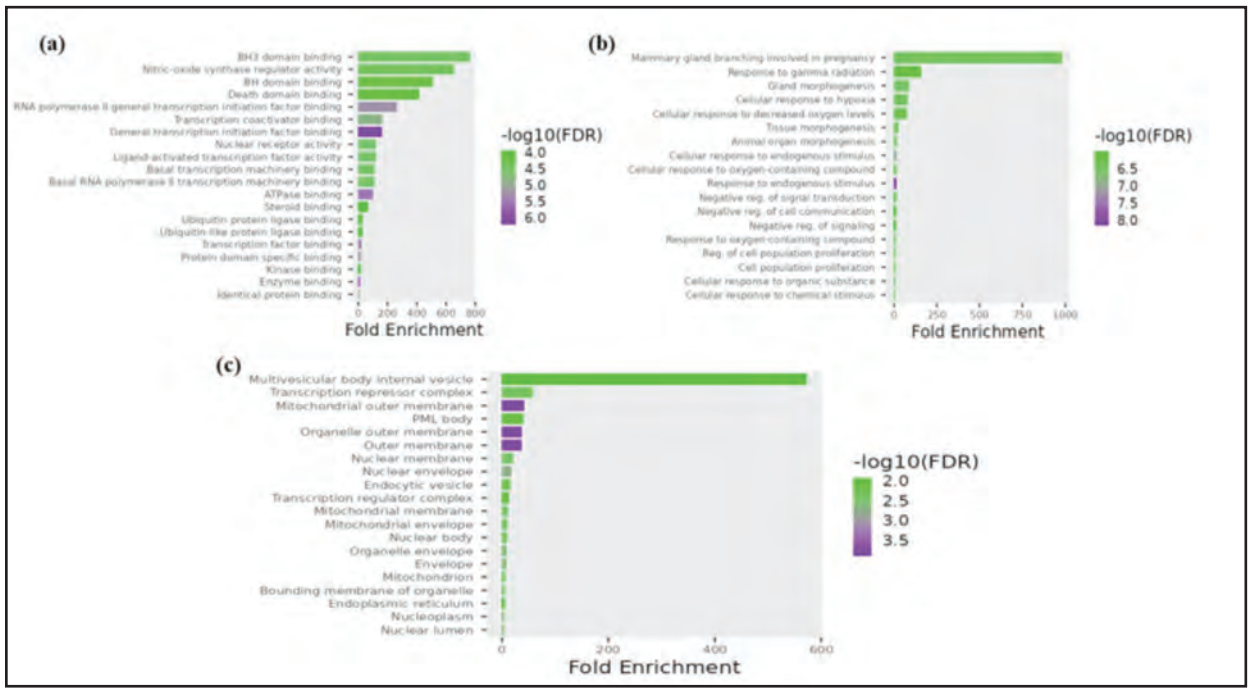

Fig. 4: Bar plot depicting the gene ontology of the predicted targets were utilized. These plots showcase the molecular function (a), biological process (b), and cellular component (c) are the segment terms from gene ontology analysis

in order to provide more insight into their functions. A bar plot has been created to show the targeted protein's GO (molecular function, biological process, and cellular component,) (Fig. 4). On the X-axis, the fold enrichment is shown. The Y-axis displays the process names and each bar's size, and colour correspond to the -log10FDR and gene count. The targeted gene's biological process found to be mostly engaged in the mammary gland branching involved in pregnancy and response to gamma radiation. The targeted gene's cellular component indicated their involvement in the multivesicular body internal vesicle. The targeted genes play a role in BH3 domain binding, nitric-oxide synthase regulator activity, and BH domain binding according to their molecular function. KEGG pathway enrichment analysis, with an FDR cut-off criterion of 0.05, has been used to identify the topmost 20 biological pathways network for the core targeted genes. The hub genes are actively participating in cancer pathway, endocrine resistance, and breast cancer pathway were selected as the pathways with the highest significant gene count. The total number of genes involved in a pathway is represented by the size of the pathway node in the pathway network. The number of overlapping genes is

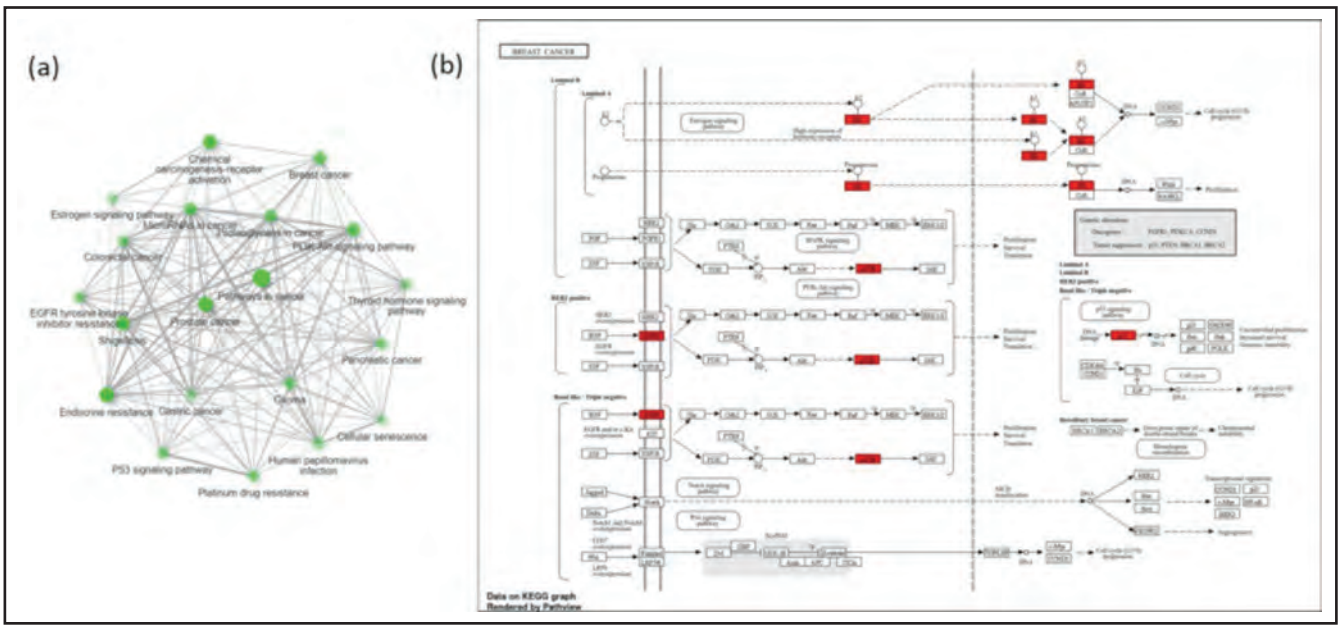

Fig. 5: (a) Top 20 KEGG pathway network of the targeted genes. (b) Breast cancer pathway, displays the core targets in red colour was created using the web tool ShinyGO.0.80

shown by the thickness of the edges (Fig.5a). In the breast cancer pathway five gene such as EGFR, ESR1, mTOR, PGR, and TP53 are directly involved were considered as the core targets. The core targeted genes were highlighted as red colour in the breast cancer pathway (Fig. 5b). Five phytochemicals found in *S. cordifolia* target the five core targeted genes: EGFR by Quinazoline, ESR1 by Abietaic acid, mTOR by 20-Hydroxyecdysone, Malvalic acid, Linoleic acid and 20-Hydroxyecdysone by PGR, and TP53 by Abietaic acid.

DISCUSSION

The multitargeted therapeutic effect of *S. cordifolia* on breast cancer has been clarified, exposing the complex nature of its therapeutic processes, through utilising the technique of network pharmacology. Using this method, five core targeted genes were identified according to their direct involvement in breast cancer pathway.[18] These genes are EGFR, ESR1, mTOR, PGR, and TP53. Epidermal growth factor receptor (EGFR) is abundantly shown in breast cancer, particularly triple-negative breast cancer (TNBC). It is a key player in controlling and maintaining the biological traits of breast cancer, including stemness, proliferation, invasion, and metastasis.[19] Patients with oestrogen receptor-positive (ER-positive) breast carcinoma are much more likely to benefit from endocrine therapy and typically have a reduced early-stage risk of recurrence than those with ER-negative breast cancer. Patients who test positive for ER are specifically between 7 and 8 times more probable to benefit from such therapy. On the other hand, mutations within the Estrogen receptor 1 (ESR1) ligand-binding region in metastatic breast carcinoma (MBC) may result in resistance to endocrine treatment. The overall survival statistics of ER-positive patients are negatively impacted by the 20% of individuals who may have ER expression loss in their metastases. As a result, resistance in ER-positive cancer of the breast persists despite the use of ovarian suppression medications and aromatase inhibitors in premenopausal women, who attempt to deprive the malignancy of its ligands. Consequently, in order to stop the course of the disease and develop efficient treatment plans, early identification, and precise evaluation of ESR1 level are essential.[20] The mammalian target of rapamycin (mTOR) is often implicated in breast cancer; preclinical research has demonstrated that it may be inhibited, and there are currently several Phase I to III clinical trials evaluating the effectiveness of mTOR inhibitors in both solid tumours and breast cancer.[21] The crucial function of mTOR in breast cancer is highlighted by the reported reduction in mTOR activity in the MCF7 breast carcinoma cell line after treatment with lactoferrin (Lf). In breast cancer, mTOR, a crucial regulator of cell metabolism and growth, is usually hyperactive, which promotes tumour development and therapy resistance. Since mTOR expression is downregulated in response to Lf, it is possible that Lf affects mTOR signalling and hence inhibits mTOR activity, which might hinder the development and proliferation of cancer cells. This outcome emphasises how effective it may be to target mTOR pathway in breast cancer therapy plans.[22] Progesterone receptor (PGR) promoters A and B is frequently expressed weakly in invasive breast cancer, and DNA methylation at these promoters is closely linked to lower protein production of ERα and PR.[23] Compared to the oestrogen receptor (ER), the progesterone receptor may provide a more accurate indicator of endocrine responsiveness. PR is becoming more widely acknowledged as a critical marker in breast cancer. Approximately forty percent of ER-positive tumours are resistant to endocrine therapy, even though ER positivity is correlated with a favourable response to such therapies. While ER is a better predictor of the likelihood of recurrence in primary breast cancer, PR, the by-product of oestrogen action, may offer a more accurate indicator of therapy success. Thus, assessing PR in addition to ER may improve patient outcomes and allow for more customised treatment strategies.[24]

Approximately 20-40% of breast cancer patients encompass mutations in the tumour protein p53 (TP53) gene, which are thought to be crucial early stages of the condition and are affected by the size and stage of the tumour.[25] The network pharmacology study incorporated herbal applications that are widely used in biomedical practices.[26-28]

CONCLUSION

The pharmacological action of S. cordifolia as a multitarget therapeutic drug for breast cancer is well understood through this study. The combination of ADME prediction, identifications of core targets, functional enrichment analysis provides an extensive basis to comprehend the medicinal properties of *S. cordifolia*. The phytochemicals satisfy ADME requirements demonstrate their potential efficacy for oral administration. The relationship between these phytochemicals and breast cancer were demonstrated by GO and KEGG pathway analysis. The results of the research indicate that *S. cordifolia* is a versatile breast cancer treatment option. Further, experimental study is required to conform the curative prospective, safety, and efficacy of this medicinal plant.

CONFLICT OF INTEREST

The authors declare that no conflict of interest would prejudice the impartiality of this scientific work.

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