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Research Article
Network Pharmacology Based Retrieval of Bioactive Ingredients of *Platycodon grandiflorus* and its Molecular Mechanism against Breast Cancer

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Abstract

Background and Objective: Breast cancer is one of the most frequent types of cancer that affects women worldwide. For their effective treatment which poses fewer side effects as compared to chemotherapy, herbs should be employed as therapeutic agents. *Platycodon grandiflorus*, a traditional herb of Chinese medicine is reported to be effective against many diseases such as cancer. Therefore, the pharmacological action of *P. grandiflorus* in treating breast cancer was investigated via the interactions of the herb with biological pathways and drug interactions. **Materials and Methods:** The ensuing literature search yielded the development of an ingredient-target database for *P. grandiflorus*, after which the targets relative to breast cancer were identified. STITCH database was used for the determination of PPIN, while cytoscape and its plugin ClueGO revealed the GO term pathway enrichment analysis. **Results:** The 10 compounds in *P. grandiflorus* and 58 targets were identified after which a screening of these compounds revealed 8 targets to be associated with breast cancer, including JUN, FOS, EGFR and MAPK8. 91 GO terms were elucidated using annotation clusters of gene functions as well as abundance value of targets. **Conclusion:** The study findings revealed the effective role of *P. grandiflorus* in the treatment of breast cancer, due to the action of its multiple compounds. The observed molecular mechanisms proposed in this study could be cellular response to cadmium ion, JUN phosphorylation, positive regulation of pri-miRNA transcription from RNA polymerase II promotor, response to estradiol and positive regulation of smooth muscle regulation.

Key words: *Platycodon grandiflorus*, STITCH, biological effects, cytoscape, breast cancer, mechanism of action, molecular targets

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Natural compounds have been in the spotlight in recent times for their potential therapeutic application against many clinical diseases and infections. Their potent activities demonstrate their phytochemical and pharmacological potentials which certainly give benefit to their widespread usage in medicine. Therefore, their use has been embraced as means of effective treatment of numerous medical disorders[1]. Many debilitating diseases such as cancer, chemotherapy and other potent synthetic drugs cause more harm than good, affecting the physical and mental health as well as social wellbeing of an individual, along with their families. In the recent era, people are turning towards herbal medicine which involves herbs for their therapeutic applications[2]. Among various therapeutic herbal practices, Traditional Chinese Medicine (TCM) has been perceived as a viable and contemporary alternative or auxiliary treatment to anti-cancer drugs and the adverse effects of chemotherapy and can also be used for the alleviation or mitigation of these side effects. *Platycodon grandiflorus* is a herb that is used globally for its wide range of applications, including its anti-inflammatory[3], anticancer[4,5], antioxidative[6,7] and anti-obesity effects[8]. Its bioactive compounds have been majorly reported to induce various effects such as apoptosis in malignant cells[9-11], decrease inflammation[12] and protect against toxicity in hepatocytes[13]. In recent years, the concept of network pharmacology has arisen from the systems biology approach, which has resulted in a paradigm shift from one drug and target to multiple drugs and targets, with their interaction increasing multi-fold[14]. Network pharmacology is an approach that focuses upon the biological interactions and their genetic counterparts which effectively play an active role in exerting the therapeutic effect of a herb once it interacts with the target proteins in the host. This approach is thereby very potent in deciphering the mechanistic mode of action of several individual herbs or a concoction of herbs, characteristically those that belong to TCM Pharmacopoeia, respectively[15]. Despite many studies attempting to elucidate the molecular basis of action of *P. grandiflorus,* the combined action of its multiple effective compounds and its therapeutic action remains to be known. As it is established that most of the medical conditions and diseases are attributable to the interplay of multiple components[16], thus, it is necessary for the determination of the mechanistic action of many herbs including *P. grandiflorus* to comprehend and develop its therapeutic role in treating various medical conditions such as cancer.

Breast cancer is one of the most frequent types of cancer affecting women globally[17]. It is reported that every 1 in 3 females is prone to develop breast cancer, but patients have a comparatively lesser mortality rate as compared to other forms of fatal cancer such as ovarian cancer. Nevertheless, there is always the risk of relapse as well as the emotional trauma from life-changing surgical procedures which may seriously endanger mental health[18]. More than 5% of the breast cancer cases are reported to be caused by genetic mutations inherited directly from parents or blood relatives, where the most common genes affiliated are *BRCA1* and *BRCA2*, respectively[19]. Women with the former genetic mutations are reported to have an increased breast cancer risk by 50-60%, while the latter increases the risk by more than 40%, respectively[20]. There are significant clinical as well as biological findings and observations that affect the general development of cancer at the molecular level[21,22], where various signalling processes enable the communication of cells with each other as well as the surrounding cellular environment [23,24]. Generally, breast cancer can be categorized into three categories, $ER^{+}/ERBB2^{-}$, $ERBB2^{+}/HR^{-/+}$ and $HR^{-}/ERBB2^{-}$[25]. These receptors carry out their role as Transcription Factors (TFs) to many genes which actively serve a key role in cellular growth and division, post their binding with growth hormones[26,27].

In this study, the mode of action of *P. grandiflorus* against breast cancer was elucidated by a network pharmacology approach. At present, limited data is available for the anti-cancerous potential of *P. grandiflorus,* where its mechanism of action remains to be seen. Furthermore, the predictive analysis using molecular docking aims to determine the potential action of its active compounds in their interaction with possible therapeutic targets of the herb. This study presents a suitable premise to ascertain the potential ability of the herb for its other potentially therapeutic abilities. Therefore, it is necessary to investigate the said mechanism of action, with specific attention given to the multi-target interaction of *P. grandiflorus*.

MATERIALS AND METHODS

Study area: This network pharmacological investigation was conducted from 01.09.2020-31.03.2021 in the Computational Lab, Chongqing Engineering Research Center of Antitumor Natural Drugs, Chongqing, China.

In this study, the first step comprised of the retrieval of the chemical targets and the prediction of those targets to be found in human beings, respectively. The effective role of *P. grandiflorus* in treating breast cancer was perceived in a PPI network using the STITCH database, which was the next step of the study, followed by the GO term enrichment analysis via cytoscape, respectively.

Extraction of chemical constituents and target projection: All ingredients of *P. grandiflorus* were searched on the TCMSP database after which the target proteins and genes specific to breast cancer were searched on the KEGG pathway database[28].

Construction of PPI network and statistics: The protective role and pharmacological action of *P. grandiflorus* and its bioactive compounds with the associated targets and biological pathways were ascertained using the STITCH database (v. 5.0)[29], which is a comprehensive online database containing the knowledge of more than 2000 organisms and around 10 million proteins and their interactions. To investigate the interaction of *P. grandiflorus* compounds with its target proteins, the PPIN was constructed and analyzed.

GO term enrichment analysis: The therapeutic potential of associated proteins was revealed through GO term enrichment analysis, which was carried out using cytoscape (plugin ClueGO, confidence level = 0.05) (Version 3.4.0)[30,31]. The parameters set for the analysis were as follows: medium network, two-sided hypergeometric test (with Bonferroni correction). Eventually, the functional network was analyzed through an algorithmic organic layout.

RESULTS

Extraction of chemical constituents and target projection: Ten chemical compounds in *P. grandiflorus* were retrieved. All these compounds have the properties of medicinal agents as their molecular weight and drug likeliness (DL, %) values are >200 and 0.1, respectively. Moreover, the values of their Oral Bioavailability (OB, %) are more than 10, which indicates that all these agents could be readily absorbed through the oral route. The literature review demonstrated the presence of 58 targets for the herb (Table 1), which, according to reported literature, could be used for treating breast cancer. Out of 58 targets, 8 (Glutathione S-transferase P, epidermal growth factor receptor, 72 kDa type IV collagenase, prostaglandin G/H synthase 2, Heat shock protein HSP 90, Transcription factor AP-1, estrogen receptor and cathepsin D) were found to be present in humans, which were standardized using UniProt (Table 2). According to the literature, all these protein targets have a prominent role in the pathogenesis of breast cancer.

Network construction and statistics: An intricate PPI network was constructed using the STITCH database, which consisted of the subsequent association of 9 protein targets, with a medium network probability score of 0.400, with 17 nodes and 87 edges. The resultant PPI network is comprised of functional interactions, where the nodes and edges represented the target proteins and/or their associated genes and the interactions between different genes, respectively. Details of the constructed network demonstrated its p-value to be negligibly small (4.66e-15), which shows that the developed network has a significant contribution to the action of *P. grandiflorus*. Small p-value denotes the non-random selection and the significant number of edges in a network,

Table 1: The retrieved chemical constituents in *Platycodon grandiflorus*

ID	Compounds	Mol (wt)	OB (%)	DL
MOL001689	Acacetin	284.28	34.97	0.24
MOL004355	Spinasterol	412.77	42.98	0.76
MOL004580	cis-Dihydroquercetin	304.27	66.44	0.27
MOL005996	2-O-methyl-3–O-β-D-glucopyranosyl platycogenate A	739.01	45.15	0.25
MOL000006	Luteolin	286.25	36.16	0.25
MOL006015	3-O-laminaribiosylplatycodigenin methyl ester_qt	536.83	21.86	0.65
MOL006022	α-Spinasterol-β-D-glucoside	560.90	21.31	0.67
MOL006026	Dimethyl-2-O-methyl-3-O-a-D-glucopyranosyl platycogenate A	739.01	39.21	0.25
MOL006066	Polygalacic acid	506.80	20.07	0.70
MOL006070	Robinin	592.60	39.84	0.71

Mol (wt): Molecular weight, OB: Oral bioavailability, DL: Drug likeliness

Table 2: Protein targets of *Platycodon grandiflorus* ingredients

Target Drugbank ID	Target names
904	Glutathione S-transferase P
844	Epidermal growth factor receptor
707	72 kDa type IV collagenase
290	Prostaglandin G/H synthase 2
1939	Heat shock protein HSP 90
1629	Transcription factor AP-1
136	Estrogen receptor
1243	Cathepsin D

Table 3: Node degrees of various nodes

Node	Degree	Node	Degree	Node	Degree
CBL	6	CTSD	5	EGFR	15
ESR1	12	FOS	15	GRB2	9
GSTP1	6	JUN	16	MAPK10	6
MAPK8	14	MAPK9	10	MMP2	11
NCOA3	6	PTGS2	10	SHC1	9
STAT3	12	TIMP2	4		

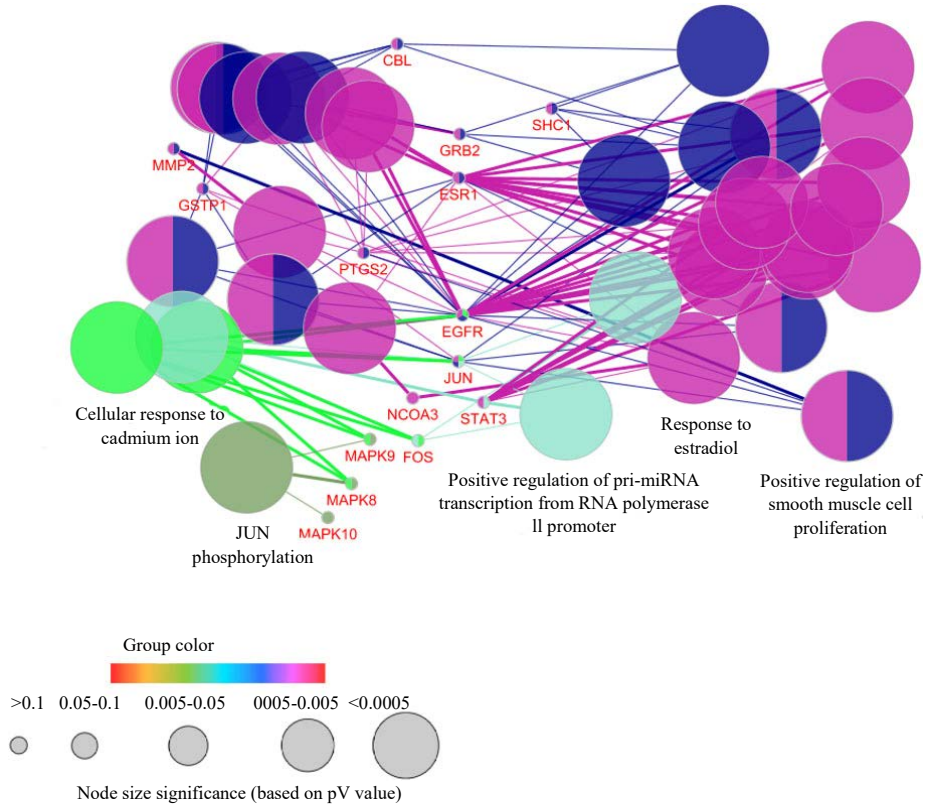

Fig. 1: Extraction of functionally-grouped networks for targets of *Platycodon grandiflorus*, obtained via ClueGO

respectively. The number of edges for the network was found to be 31 at randomly chosen nodes. Such a large number of edges indicates that an appreciable number of protein targets work together in the exhibition of the therapeutic effect of *P. grandiflorus*. The constructed PPI network demonstrated the average node degree and clustering coefficient to be 9.76 and 0.823, respectively. The clustering coefficient indicates the acquired proteins work in the form of clusters, rather than chains form. Moreover, 9 hubs were found due to their nodal degree being higher than the average i.e., 2.67 where JUN was found to have the highest node degree. It was followed by other proteins such as FOS, EGFR and MAPK8, respectively. The protein JUN possesses the highest node degree (i.e. 16), which indicates that this protein largely interacts with other protein targets in the body and contribute to the therapeutic action of *P. grandiflorus*. Other important proteins targets which play a crucial role in the action of *P. grandiflorus* are FOS, EGFR and MAPK8 (i.e. 15, 15 and 14). The node degree of each target protein associated with the PPIN is given in Table 3.

GO term enrichment analysis: The target proteins of *P. grandiflorus* were assessed via. ClueGO software and its enrichment analysis. The biological action of *P. grandiflorus* belonged to 91 GO terms, which were then categorized into 5 sub-groups, which were associated with cellular response to cadmium ion, JUN phosphorylation, positive regulation of pri-miRNA transcription from RNA polymerase II promotor, response to estradiol and positive regulation of smooth muscle regulation (Fig. 1), where these mechanisms are significantly associated with the molecular mechanism of

P. grandiflorus against breast cancer. All these GO terms have a direct or indirect association with the different pathophysiological phenomena which induce breast cancer. It indicates that the use of *P. grandiflorus* likely has the potential of breast cancer therapy.

DISCUSSION

The role of *P. grandiflorus* in Traditional Chinese Medicine (TCM) has been in the spotlight for its effective role in the treatment of many disorders such as inflammation, obesity and cancer. Its role in the treatment of cancer has been reported by various studies but the mechanistic action of many herbs is unknown due to the unrevealed molecular mechanisms of multiple compounds and their interplay with various target proteins. In this study, the mechanistic mode of action was studied for the determination of the therapeutic role of *P. grandiflorus* against breast cancer. This multi-step study comprised of many steps, including the retrieval of chemical compounds, the prediction of target compounds as well as the construction of the PPI network, as well as the enrichment analysis and molecular docking. The literature survey yielded the presence of 58 proteins, which were screened to elucidate 9 proteins that were related to breast cancer in human beings. The GO term analysis helped to confirm the interaction of *P. grandiflorus* with the biological processes and pathways in the host related to breast cancer.

The presence and role of various compounds found in *P. grandiflorus*, such as acacetin, spinasterol, cis-dihydroquercetin, luteolin, robinin and platycodin D in the treatment of cancer have been reported extensively over many years. Acacetin is a flavonoid that is widely sought to be effective in treating inflammatory disorders[32,33]. Acacetin was reported to contribute to the inhibition of the cell cycle in carcinoma cells, along with cell death and caspase activation and mitochondrial death signalling[34-36]. In carcinoma cells, acacetin demonstrated inhibition of cellular growth and contributed to the decrease in the survival of breast carcinoma cells[37]. This mechanism was observed in a recent study, where acacetin facilitated the inhibition of cellular growth via activating the ROS-JNK pathway in cancerous cells[38]. It also demonstrated to stall the movement of breast carcinoma cells via the mechanism of cellular attenuation and adhesion generation, respectively[39]. Moreover, it was observed to reduce the expression of BCL-2, prompting the release of the cell apoptosis factor and in an increase in the Reactive Oxygen Species (ROS), inducing cell death. This ability to downregulate BCL-2 is facilitated by the depletion of nuclear NF-κB by acacetin at molecular level[40]. Luteolin has been widely reported to act as a potential anti-cancerous agent. In breast carcinoma cells, luteolin caused the suppression of STAT3, EGFR and Akt in cell lines, via the downregulation of their major signalling pathways[41]. Luteolin actively induced apoptosis in breast cancer cells[42,43], stall the migration of cancer cells *in vitro*, where the compound might also suppress intravasation of breast cancer cells through the lymphatic barrier[44]. It is also reported to cause downregulation of VEGF for the inhibition of metastasis in MFC-1cell lines[45,46]. Spinasterol isolated from *Ganoderma resinaceum* demonstrated anti-tumour activity against breast cancer cell lines by inhibiting the proliferation of the cells, thus stalling their survival[47]. The proliferation of MCF-7 cell lines was observed to be inhibited via the anti-proliferative action of spinasterol[48]. Platycodin D is a terpenoid isolated from *P. grandiflorus* and is observed to be active in treating cancer cells via inhibition of cellular invasion and reduction of MMP and mRNA expression. Moreover, it was observed to downregulate the phosphorylation of ERK, JNK, Akt/TOR signalling pathway, induce NF-κB inhibition, ultimately suppressing the expression of EGFR[49,50]. Bone loss induced by breast cancer was also mitigated by the facilitation of apoptosis and blockage of FOS and NFAT1 in osteoclasts[51].

In the PPI network, the main protein hubs were found to be JUN, FOS, MAPK8 and EGFR, which have already been reported to be affiliated with the development and regulation (positive or negative) of breast cancer. The phosphoprotein c-JUN, a significant part of the transcription factor activation protein known as AP-1 in mammals is encoded by c-JUN proto oncogene whose complexes are formed by JUN and JUN/FOS dimers and heterodimers, respectively[52]. It interacts with other subunits such as NF-κB subunit p65 as well as NFAT[53]. JUN is active during the invasive form of breast cancer, which further coincides with elevated microvessel density[54]. When the protein is induced abundantly, it regulates various downstream mechanisms, like the development, survival and proliferative action of cancerous cells[55,56]. c-JUN is phosphorylated with the help of MAP kinases which further are attributable for cell death and migration, respectively[57,58]. Epidermal Growth Factor Receptor (EGFR) is a growth factor that is found to be overexpressed in cancer and is responsible for the induction of cellular proliferation. This family of growth receptors are frequently affiliated with the pathophysiology of breast cancer and their abundant expression in the human body is often synonymous with a bleak prognosis in patients, respectively[59,60]. Almost all types of EGFR undergo overexpression, especially in inflammatory breast cancer[60,61]. The EGFR family found in human beings is comprised of

four associated trans-membrane receptors which contain a ligand-binding domain, as well as an intracellular tyrosine kinase receptor domain. It is responsible for activating many pathways which are crucial in carrying out various biological processes[62]. Along with cellular proliferation, EGFR is responsible for affecting adhesion as well as the movement of the cell, offering protection against cellular apoptosis and promotion of angiogenesis at a molecular level[63]. Activated members of the EGFR family are implicated in the invasion of epithelial breast cancer cells *in vitro*, which facilitates cellular polarity and other aspects of cell differentiation[64]. One aspect of EGFR's abundant expression is via amplifying the EGFR gene, as this mechanism has been observed in many cancers such as those of brain, spinal cord, lung, breast and gastric cancer, respectively[65-67]. Though previously rare with the occurrence of breast cancer, this type of gene amplification is observed in meta-plastic cases of breast cancer (>20%)[68-70].

GO term enrichment analysis demonstrated the extracted GO terms to be directly associated with breast cancer. Five GO terms, such as cellular response to cadmium ion, JUN phosphorylation, positive regulation of pri-miRNA transcription from RNA polymerase II promotor, response to estradiol and positive regulation of smooth muscle regulation were found to be prominent. Cadmium, a toxic heavy metal classified as a human carcinogen by US EPA[71], tends to accumulate and persist in the environment and is known to cause various serious health problems. The linkage between cadmium exposure or intake and breast cancer is evident by widespread studies sprawling decades[72,73]. The malignant breast cancers showed an elevated level of cadmium, in comparison with benign breast cancers, respectively, suggesting a significant link between cadmium and ERα positive breast cancers, increasing the risk of breast cancer to aggravated malignancy and cell proliferation[74-76]. The study findings of a recent study demonstrated that persistent cadmium exposure to MCF-7 cells resulted in elevated growth, motility and enhanced invasive properties, which was suggestive of the degradative action of cadmium in promoting cancer by altering JUN, FOS and ERα interactions through the overexpression of chemokines[77,78].

The current study proposes the mechanistic action that *P. grandiflorus* fosters in the treatment of breast cancer. The various biological pathways, as well as the proteins involved in its mechanistic action, shed light on the protective role the herb exerts in human beings against this type of fatal cancer. Furthermore, the docking analysis of the efficacious binding of its compounds with the target protein indicates the therapeutic role of *P. grandiflorus* against breast cancer.

CONCLUSION

From the findings, it is concluded that compounds of *Platycodon grandiflorus* such as acacetin, spinasterol, cis-dihydroquercetin, luteolin, robinin and platycodin D interact with various proteins such as JUN, FOS, EGFR and MAPK8 and their mutual interplay is found to be associated with breast cancer manifestation and its treatment using *Platycodon grandiflorus*, indicating the significance of the synergistic relationship of the herbal compounds with the target proteins. Further studies such as docking and molecular dynamic simulation can help to validate the potential action of *P. grandiflorus* in treating breast cancer.

SIGNIFICANCE STATEMENT

This study proposed the action mechanism of *Platycodon grandiflorus* in treating and managing breast cancer that can be beneficial for its preclinical trials. This study will help the researchers to uncover the critical areas of breast cancer treatment that many researchers were not able to explore. Thus, a new theory on the potential role of *Platycodon grandiflorus* against breast cancer may have arrived at the suggestion of testing the potential of this finding in animal models.

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