INTRODUCTION

Hepatocellular carcinoma (HCC) is one of the important types of cancer prevalent worldwide mainly in developing countries. HCC is the third most common cause of mortality in cancer-related diseases throughout the world. It is estimated that around 600,000 deaths per year resulted due to the invasive nature of the HCC cells which finally enter into the metastatic stage. The patients diagnosed as an advanced stage in HCC state have a low survival rate as there are no suitable curative therapies for such patients. Hence, there is a urgent need in addressing the effective treatment options to treat HCC patients. Further, evaluating the underlying molecular mechanisms in HCC provides alternate therapeutic strategies which help in achieving more favorable clinical outcomes with less toxicity and thereby reducing the overall morbidity rates [1].

Traditional medicine plays an important role in treating different types of cancer. The least toxic nature, safety, and efficacy of these phytocompounds makes them potent drugs for targeting cancer-related issues. Bromelain, a cysteine protease isolated from the stem of pineapple has a wide range of pharmacological significance [2]. In our previous studies, we report the anti-cancer property of bromelain in HepG2 cell lines using various in vitro models. We also report the drug impediment apoptosis by up-regulation of p53 and inhibition of invasive nature of cancer cells by down-regulation of a beta-catenin gene on treating with bromelain. Finally, we reported that bromelain is an effective phytocompound in controlling the progression of cancer cells in HCC conditions [2].

In the present study, an attempt is made in understanding the possible role of bromelain on molecular mechanisms and pathways underlying in HCC state. We used the protein-protein interaction (PPI) network approach, prediction of target sites of bromelain to understand its role as an anti-cancer agent. We also predicted the core modulatory network of bromelain targets p53 and beta-catenin which are significantly related to the process of apoptotic signaling pathway and regulation of cell death, proliferation, and survival. To our comprehension, this study is reported for the first time which deals with the prediction of the mechanism of Bromelain in HCC condition using the in-silicon approach. The study provides the promising implementation of bromelain as a novel drug in treating HCC.

METHODOLOGY

Literature mining from public database

The protein interaction partners for p53 and beta-catenin involved in the progression of HCC were collected from National Center for Biotechnology Information. We collected data points and standardized the data points for our data analysis from the public database. We used Cytoscape 3.8.2 version plug-in for constructing a Protein-Protein interaction network. We constructed a pathway network using Biorender.com.

Data points collected

We collected data points for our data analysis from the public database. The data points for network analysis are represented according to the sample format.

Data standardization and enrichment

We used public databases to standardization for our data points. National Center for Biotechnology Information (NCBI) gene database was used to get the complete functional analysis of a gene to elucidate its biological properties. The database was used to get information on gene symbols, ID, gene functioning like cellular and molecular process. Along with this Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway was considered for functional analysis.

The data points are standardized as sample format as follows:

Keywords: Bromelain, Hepatocellular carcinoma, Protein-protein interaction-network, KEGG, p53, beta-catenin.

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ABSTRACT

Objective: The objective of the study was to understand biomolecular interactions of Bromelain and its networking with p53 and beta-catenin by a computational method of analysis in Hepatocellular carcinoma (HCC) condition.

Methodology: The protein interaction partners for p53 and beta-catenin involved in the progression of HCC were collected from National Center for Biotechnology Information. We collected data points and standardized the data points for our data analysis from the public database. We used Cytoscape 3.8.2 version plug-in for constructing a Protein-Protein interaction network. We constructed a pathway network using Biorender.com.

Results: The protein interactions concerning p53 and beta-catenin are identified and a network is constructed. A total of 18 and 34 nodes were identified which are involved in down-regulation and up-regulation of beta-catenin and a total of 30 and 27 nodes for homosapiens are identified which are involved in the downregulation and upregulation of the p53 gene. We identified different pathways which trigger and impact the p53 and Wnt/beta-catenin signaling pathways as potential target sites for Bromelain to arrest the progression of cancer.

Conclusion: In conclusion, our in silico studies anti-cancer activity of Bromelain in HCC relating its effect on apoptosis, cell differentiation, mesenchymal transition, p53 signaling, and Wnt/beta-catenin signaling pathways.

Keywords: Bromelain, Hepatocellular carcinoma, Protein-protein interaction-network, KEGG, p53, beta-catenin.
PPI network visualization and data analysis
We used Cytoscape 3.8.2 version plug-in for constructing the PPI network. The interactive networks for TP53, TP53-mut, β-catenin, β-catenin-mut, and bromelain were constructed based on the literature mining.

Construction of pathway for p53 and β-catenin
We constructed a pathway network using Biorender.com [4] (https://biorender.com/) keeping the KEGG pathway as reference [5] (https://www.genome.jp/kegg-bin/show_pathway?hsa05225) to understand the probable role of associated pathways in regulating the expression of p53 and β-catenin and indirectly affecting HCC diseased state.

RESULTS
PPI network
The Cytoscape plug-in version 3.8.2 is used for performing PPI networking. The protein interactions with respect to p53 and β-catenin are identified and the network is constructed. A total of 18 and 34 nodes were identified which are involved in down-regulation and up-regulation of β-catenin respectively. The 34 nodes identify are involved potentially in the progression of HCC (Figs. 1 and 2). Similarly, a total of 30 and 27 nodes for homosapiens are identified which is involved in the down-regulation and up-regulation of the p53 gene. On the other hand, 2 nodes and 1 node are identified for Mus musculus in down-regulation and up-regulation of p53 (Figs. 3 and 4). The network analysis also includes the study of interaction patterns in PPI involved in the mutation of p53 and CTNNB1 protein and the networking is visualized by Cytoscape. The data points involved in targeting the mutant TP53 (TP53mut) and beta-catenin (CTNNB1mut) were collected. Potential 4 nodes and 3 nodes were identified in affecting mutation in β-catenin (Fig. 5) and p53 (Fig. 6). The data mining also reports the suppression of TP53mut by cinnamon oil and garlic oil. These identified genes act as potential nodes in regulating the disease progression in the HCC state.

Effect of Bromelain on various pathways affecting Wnt/β-catenin signaling pathway and p53-dependent apoptotic pathway
The data points involved in the various pathways which affect Wnt/β-catenin signaling pathway and p53-dependent apoptotic pathway are standardized. Keeping the KEGG pathway for HCC as reference (hsa05225), the Biorender tool was used to create different pathways. Wnt/β-catenin signaling pathway is activated and inactivated form is shown in the figure (Figs. 7 and 8) and similarly, p53-dependent apoptotic pathway in is depicted in Figs. 9 and 10. The effect of Bromelain in these pathways which regulates p53 and β-catenin gene expressions is predicted in the HCC condition (Table 1).

Effect of Bromelain on different types of cancer
The data points for the anti-cancer activity of bromelain in different types of cancer are collected and standardized in MESH form. The MESH ID represents the following type of cancer on which bromelain has shown anti-cancer activity: DO15179: Colon rectal cancer; C060192: Breast cancer; D010190: pancreatic cancer; D006258: HCC; C5876657: Gastric cancer; C587667: Prostate cancer; D012878: Skin tumor; D001943: breast neoplasm; and C091991: breast adenocarcinoma (Fig. 11).

DISCUSSION
Bromelain is an important dietary protein isolated from the stem and fruit of the pineapple. The studies have shown the significant effect of bromelain in treating allergy, inflammation, and cancer, as an immunomodulator which makes protein an important therapeutic molecule. Previously our study reports the anti-cancer activity of bromelain in HepG2 cell lines and provides insight as an effective agent in treating HCC. We reported that in HCC, Bromelain exerts multiple effects in controlling the progression of cancer [6]. Our study reports that Bromelain is involved in inhibiting cell proliferation by arresting the cell cycle, induces cell death by triggering apoptotic pathway, reduces the invasiveness of cancer cells, and controls the progression of cancer to the metastatic stage in HepG2 cell lines. We report upregulation of p53 and down-regulation of β-catenin on treating with bromelain which indicates its role in modulating the associated molecules which are involved in their signaling pathways.
The study reports IC10 0.54 μM and IC25 0.72 μM concentration of Bromelain can induce anti-cancer activity in HepG2 cell lines [6].

In the present study, we used the in silico approach to explore the molecular mechanism of bromelain in understanding its role in HCC from a systemic viewpoint.

Using the PPI-network approach we identified genes that play a key role in regulating signal transduction mechanism by p53. This mechanism is involved in the regulation of cell death and brings about an intrinsic mode of the apoptotic signaling pathway. The PPI network also identified key genes which are involved in the regulation of cell death and brings about an intrinsic mode of the apoptotic signaling pathway.

The study predicts that bromelain can act as a regulatory molecule where the study shows the cell cycle arrest and cell death leading to apoptotic pathway through TP53-dependent pathway in HepG2 cell lines [6].

The Wnt/β-catenin pathway plays an important role in the progression of tumors at different stages in HCC conditions. The Wnt/β-catenin pathway also called a canonical pathway; activation and involvement form an important focus area for researchers in developing drug targeted therapies. Research shows that in most HCC patients there is a high expression of β-catenin which further enters into the canonical pathway. In normal conditions, the β-catenin level is highly regulated and is frequently degraded by the proteasomal machinery ensuring the inactivation or the off-state of the canonical pathway. In HCC condition in association with the other genes and pathways this mechanism is lost leading to the up-regulation and translocation of β-catenin from cytoplasm to nucleus wherein gets associated with the other factors and activates Wnt/β-catenin signaling. On activation of this pathway, the downstream genes of the Wnt genes are expressed such as Cyclin D1, EMT, and c-Myc which are involved in the invasion, migration, and cell cycle activation process of cancer cells thereby enhancing

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**Table 1: Predicted target sites of bromelain**

| Gene symbol | Protein name                                      |
|-------------|--------------------------------------------------|
| Hbp1        | HMG-Box transcription factor 1                   |
| Srebf1      | Sterol regulatory element-binding transcription  |
|             | factor 1                                         |
| FOXP4       | Forkhead box protein P4                          |
| GAS2        | Growth arrest-specific protein 2                 |
| PARP        | Poly (ADP-ribose) polymerase 1                   |
| CASP3       | Caspase3                                         |
| CASP8       | Caspase8                                         |
| CASP9       | Caspase9                                         |
| Rab10       | Ras-related protein                              |
| ASPP2       | Apoptosis-stimulating of p53 protein 2           |
| AKT         | Protein kinase B                                 |
| TREM2       | Triggering receptor expressed on myeloid cells 2 |
| PI3K        | Phosphatidylinositol 3-kinase                    |
| GST21       | Glutathione S-Transferase                        |
| AJAP1       | Adherens junction protein 1                      |
| ZEB1        | Zinc Finger E-box                                |
| GSK-3β      | Glycogen synthase kinase 3                       |
| CK1a        | Casein Kinase 1 alpha                            |
| BCL-2       | Apoptosis regulator BBrd2                        |
| BAX         | Bcl2-associated X protein                        |
the progression of HCC and leading to a metastatic state. With this background known β-catenin forms a key transcription factor to target and understand its involvement in the interaction with other molecules in upregulating the Wnt/β-catenin pathway.
In our previous studies, an attempt has been made to study the effect of Bromelain, a phytocompound, on the gene and protein expression of β-catenin and p53 in HepG2 cell lines using various in vitro tools. The study discussed and concludes that bromelain at its IC10 and IC25 concentration downregulates the gene expression of β-catenin and up-regulates p53 in HepG2 cell lines which further is correlated with the protein expression studies. In the present study using the in silico approach, an attempt is made to understand the interaction patterns of β-catenin and p53 with another protein that is involved in the apoptotic pathway mediated by p53 expression and Wnt/β-catenin signaling pathway mediated by the expression of β-catenin. Finally, the probable role of Bromelain is predicted in p53 signal transduction and Wnt/β-catenin signaling pathway in curtailing the spread of the disease through the bioinformatics approach.

Wnt/β-catenin signaling pathway in curtailing the spread of the disease through the bioinformatics approach.

The research has reported that some genes are involved in the down-regulation of the β-catenin level, its activation, and interactions with the other molecules and blocks the Wnt/β-catenin signaling pathway. This mechanism restricts the migration and invasive properties of cancer cells and inhibits the metastatic progression in HCC to a considerable extent. TREM2 or triggering receptor expresses on myeloid cell 2 activates PI3K which further induces phosphorylation of AKT. The active form of AKT triggers GSK-3β to activate β-catenin to undergo phosphorylation. The phosphorylated form of β-catenin is recognized by the proteosomal degradation system and degrades the β-catenin inhibiting pathway through the PI3K/Akt/β-catenin.
pathway [7]. The important mechanism for the activation of the canonical pathway is the translocation of β-catenin from the cytoplasm to the nucleus. This process is blocked by AJAP1 [8] and the effector response of this is downregulation of the ZEB1 gene which is involved in the EMT. P7TP3 down-regulates Wnt/β-catenin signaling pathway and the gene are controlled by miR-182-5p [9]. The research predicted that DKK3 is the potential target gene for γ-miR-626. The inactivation of DKK3 will reduce the Wnt/β-catenin signaling and suppressed the spread of tumor in HCC conditions [10]. A similar effect is observed with the gene GSTZ1-1 [11].

The studies report different genes involved in different pathway mechanisms to activate β-catenin and regulate the Wnt/β-catenin signaling pathway. The upstream factors affecting β-catenin can be effective receptors for targeting the drugs. A member of protein disulfide isomerase which is known as Thioredoxin domain-containing protein 12 (TXNDC12) is involved in affecting the invasion and migration property of HCC cells in in vitro conditions. TXNDC12 up-regulates ZEB1, which further initiates EMT ZEB1 is the major effector molecule of β-catenin. TXNDC12 binds with β-catenin and translocates it from the cytoplasm to the nucleus. In the nucleus, the β-catenin gets associated with the other genes and initiates the Wnt/β-catenin signaling pathway in which one of the downstream genes is ZEB1. By ZEB1-mediated EMT, there is a rapid increase in the invasion and migratory rate of HCC cells [12]. The studies showed that activation of the Wnt/β-catenin signaling pathway, interaction, and stabilization of β-catenin and TCF is important. PES1 gene is involved in this process through β-catenin/TCF signaling stabilizes the interaction of β-catenin and TCF [13]. The same type of study revealed that CARF is also involved in the stabilization process between β-catenin and TCF where CARF blocks Icat from binding to β-catenin and helps β-catenin to interact with TCF [14]. A novel oncogene SGK3 up-regulates the progression of HCC by SGK3/GSK-3β/β-catenin pathway. Here, in the presence of PI3K inhibitor SGK3 phosphorylates GSK-3β on serine 9. This phosphorylated GSK-3β inactivates the proteosomal degradation machinery by the level of β-catenin in the cytosol increases which under the influence of other genes translocates into the nucleus [15]. MIR-5188 interacts with FOXX1 which induces the translocation of β-catenin thereby activating Wnt genes EMT and c-Jun. c-Jun shows a positive feedback loop by involving in the expression of miR-5186 by the miR-5188-FOXX1/β-catenin-c-Jun feedback loop pathway [16]. The studies have revealed that EphB4 is overexpressed in HCC tissues. EphB4 activates β-catenin to enter into a canonical pathway through EphB4/β-catenin-dependent manner [17]. LINCO1278 suppresses miR-1258 which results in the active expression of Smad2/3. Smad2/3 stabilizes β-catenin in the cytoplasm and helps in the process of translocation to the nucleus where β-catenin interacts with TCF and activates Wnt genes through β-catenin/TCF-4-LINC01278-miR-1258-Smad2/3 feedback loop pathway [18]. MFH9 brings about ubiquitination and degradation of GSK-3β which in turn blocks the degradation of β-catenin. PHF19 is involved in the inactivation of the β-catenin degradation system, activates the β-catenin/TCF interaction, and facilitates the canonical pathway [19]. The gene PHF19 on activating Wnt/β-catenin expresses an important inflammatory cytokine IL-6 through the β-catenin/IL6 signaling axis [20]. The mechanism of degradation of β-catenin proteosomal machinery and its stabilization is exhibited by IGTB5 through miR-186-5p/IGTB5-β-catenin pathway [21]. PSPCI1 [22], PRIC1 [23], and PEK5 [24] through Wnt/β-catenin pathway activating Wnt gene thereby enhancing metastasis in HCC. FXXD2-AS1 binds to EZH2 epigenetically and silences EZH2. The silencing of EZH2 further represses DKK1 and thereby the cells observe Wnt/β-catenin signaling pathway [25]. MiR-182-5p inhibits the degradation system of β-catenin and negatively regulates FOXO3a activating Wnt/β-catenin pathway [26]. MEIS2C potentially binds to CDC73 which is a dephosphorylated molecule that is involved in the activation of β-catenin [27]. LINCO0210 binds to CTNNBI1P1 which is an inhibitory molecule of β-catenin. The binding makes CTNNBI1P1 lose its inhibitory property against β-catenin [28]. UBE2M is an oncogene involved in the translocation process of β-catenin from the cytoplasm to the nucleus where it activates the downstream expression of the gene cyclin D1 through β-catenin/cyclin D1 signaling [29]. TP5 is the potential binding partner to TCFL2. The activated TCFL2 interacts and stabilizes with β-catenin. The research reports that in HCC patients there is overexpression of the miR-23b gene. The important direct target to this gene is ST7L. ST7L binds to the carboxyl-terminal region of AKT and inhibits the AKT/GSK3β/β-catenin pathway [30]. All these mechanisms activate β-catenin target to involve in Wnt/β-catenin pathway and results in the progression of HCC. Therefore, the studies show that the activation of the Wnt/β-catenin pathway is mainly by restricting the degradation of β-catenin by proteosomal degradation system, translocation of β-catenin accumulated in the cytoplasm to the nucleus, and enhancing the interaction of β-catenin with TCF. This mechanism forms the basis for the target to inhibit the activity of β-catenin.

The research reports that miR-21 blocks Hbp1 which further regulates p53 to CCNB1, CCND1, Srebf1 [31]. GAS2 triggers p53-GAS2-Caspase cascade via p53 dependent apoptotic pathway [32], miR-3196 inhibits FOXP4 through p53-dependent, miR-3196-mediated-FoxP4 pathway [33] to induce cell death through p53 mediated apoptotic pathway in HCC condition.

Similarly, many genes are involved in the down-regulating p53 signal transduction process. CD44 phosphorylates AKT which activates MDM2 termination of p53 through PI3K/AKT/MDM2 pathway. The studies reports, expression of miR-21 and CD147 degrades p53 [34]. Inhibition of p53 by EGF through PI3K/AKT/ERK pathway [35], miR-23a activates TRIB1 which blocks p53 and activates CTNNB1 gene and regulates the expression of c-Myc, MMP7 and EMT [36], CMS down-regulates HMGBl which in turn down-regulates p53 [37], deacetylation of p53 by SIRT7 through SIRT7-p53 dependent pathway [38], Lg5 target gene PDFCD5 programmed cell death protein which inhibits p53 via PDCD5/p53 signaling axis [39], IRX5 up-regulates cyclin D1 through p53 signaling pathway [40] inhibits the activity of p53. All the above-mentioned pathways inhibit the p53-mediated apoptosis in the HCC conditions.

In our in vitro studies, we observed that bromelain downregulated the expression of β-catenin and upregulation of p53 in HepG2 cell lines. The above-mentioned genes are predicted to be potential target sites for bromelain that might have inhibited the progression of HCC by affecting β-catenin in enhancing its degradation process or inhibiting the translocation process and thus downregulating Wnt/β-catenin signaling pathway and up-regulating p53 mediated apoptotic pathway by activating the apoptotic proteins and the proteins involved in the cell cycle.

Bromelain is reported to up-regulate Casp-3, 8, and 9. PARP-1 in colon-rectal cancer through caspase-independent apoptotic pathway [41]. downregulates GREM-1, IL-1β, IL-4, NfkB1, PTGS2 in breast cancer [42], downregulates ACSL-4 and upregulates ASCL-4 in human colorectal cancer [43]. Casp-3, Casp-9, Bax, and Trp53 is upregulated and Bcl-2, Ptg2, Nkb1, Nkbia, Mapk14, and Akt are down-regulated in skin tumor [44]. In the other study, Bromelain is reported to inhibit gastric carcinoma by cleaving TP53, upregulating CYCS, and downregulating BCL-2, AKT1, MUC1 through transcription-independent p53 apoptosis [45] which is a deviation from the present study. Overall the role of bromelain and its effect on the gene expression of other transcription factors are studied in various cancer conditions. This provides an insight into the significance of bromelain in apoptotic mediated cell death by interfering with the various types of apoptotic proteins in different cancerous conditions. Considering these facts we suggest that bromelain might have been exerting its effect on other genes apart from p53 and β-catenin in controlling the progression of HCC. In in silico studies play a key role in predicting the suitable target site for the drug under study in the cell [46].

CONCLUSION

Our in silico studies anti-cancer activity of bromelain in HCC relating its effect on apoptosis, cell differentiation, mesenchymal transition, p53 signaling, Wnt/β-catenin signaling pathways. We here identify PPI
network module which can be predicted as targets for bromelain which helps in the inhibition of progression of cancer to metastatic state in HCC condition. We also identified different pathways which trigger and impact the p53 and Wnt/β-catenin signaling pathways as potential target sites for bromelain to arrest the progression of cancer. Further, biological experiments are needed to verify the present in silico study.

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AUTHOR CONTRIBUTION
SSM studied the effect of bromelain on p53 and β-catenin protein expression and signaling pathway by in silico approach. Validation and writing were done by SSM and TBN.

CONFLICT OF INTEREST
The authors declare no conflict of interest.

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