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# Evaluation of efficacy of selected *Bryophyllum pinnatum* phytochemicals in hepatocarcinogenic therapy through *in silico* approach

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One of the worst diseases in the world, hepatic carcinoma requires expensive medical care upfront. Around the world, *Bryophyllum pinnatum* is a plant that is used in folk medicine to treat a variety of illnesses. Because of its abundance of active therapeutic compounds, the plant is employed for its major pharmacological effects. As a result, it aids in the treatment of liver cancer, and *Bryophyllum pinnatum* can be used in place of more expensive medications. In hepatocarcinogenesis disease, the Pi3k-Akt pathway, downstream signalling pathway, MAPK pathway, and PDGF pathway in the liver is engaged in the invasion and spread of malignant cells. Methanolic, ethanolic, and aqueous extracts of the phytochemicals of *Bryophyllum pinnatum* were subjected to qualitative analysis, the results indicated that the yield of phytochemicals in the ethanolic extracts was higher than in the other two extracts. The bufadienolides, β-sitosterol and bryophyllin A, which possess chemotherapeutic potential, were chosen for our computational investigation to assess their ability to interact with key signalling molecules such as VEGFR2, IGFR, C-KIT, RET, Pi3K, C-Met, MEK-inhibitor, and PDGFR. UCSF Chimera X was utilised to optimise the recovered molecules and by using data from the protein databank. The interaction of bryophyllin A and β-sitosterol, of the plant with the selected signalling molecules was investigated using the molecular docking tool, CB-Dock. According to the docking analysis, Bryophillin A strongly interacted with Pi3K than the other proteins whereas β-sitosterol showed stronger interaction with C-met.

**Keywords:** Bryophyllin A, β-sitosterol, Down-stream signalling pathway, MAPK pathway, Molecular docking, PDGF pathway, Pi3k-Akt pathway

The highly aggressive form of cancer is hepatocellular carcinoma (HCC) for which there are no proven treatment options. Hepatocellular carcinoma, the most common primary liver tumour, typically develops after decades of chronic insults[1]. Numerous signalling pathways and molecular mechanisms have been found to be in charge of initiating and promoting HCC as a result of advancements in tumour biology and molecular genetic profiling. The Pi3K-PKB/Akt pathway highly conserved pathway where Pi3K (phosphatidylinositol 3-kinase) and C-kit (Tyrosine protein kinase/Receptor tyrosine kinase) are important proteins[2,3]. C-kit stimulates the growth of specific blood cell types by binding to a substance known as stem cell factor (SCF)[3,4]. The class of lipid kinases of Pi3K that helps regulate cell growth and transmit signals within cells by binding with the plasma membrane[3,5]. In the downstream signalling pathway, TGF-β controls a variety of cellular activities and downstream processes. MEK, RET, and VEGFR2 are the cycle's key molecules[3,6]. The TSAd-Src-Pi3K-PKB/AKT signalling pathway is triggered by KDR gene that encodes vascular endothelial growth factor receptor 2, which primarily controls endothelial cell survival[6]. Conversely, RET is triggered by attaching to both a non-signalling extracellular co-receptor (GDNF family receptor, GFRα) and a soluble ligand (GDNF, glial cell-line-derived neurotrophic factor)[7].

Proteins in a cell that are part of the mitogen-activated protein kinases (MAPK/ERK) pathway transmit a signal from a surface receptor to the DNA of the cell's nucleus[3,8]. IGFR which is the important factor in the cycle binds DNA and regulates transcription to affect the Pi3K/AKT pathway, which affects the growth of the cells leading to the metastasis of malignant melanoma cells[1,9].Activation of the PDGF/PDGFR signalling system is associated with cancer growth, cancer cell spread, invasion and angiogenesis[3,10]. In contrast, the C-Met inhibitor AMG 208 blocks both ligand-dependent and ligand-independent activation of C-Met and tyrosine kinase activity, which can lead to cell growth suppression in tumours that overexpress C-Met[11].

The drugs for liver cancer approved by the Food and Drug Administration (FDA) include brivanib, which acts by preventing the newly generated blood vessels from supplying nutrients to tumours[12]. Several of the proteins activities are suppressed by regorafenib that is involved in the growth of the cancer cells, blood vessel formation and other processes that support tumour growth[13]. Sirolimus, also known as rapamycin, is a medication used in various medical fields, including organ

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transplantation, oncology, and dermatology. The mTOR pathway, which is involved in cell growth and proliferation is impaired by sirolimus[14], cabozantinib, sorafenib, atezoblizumab, futibatinib, imfinzi, nexavar, nivolumab, opdivo, pembrolizumab, trusetriq and so forth[15]. Side effects from cancer and cancer treatments can include anemia, bleeding, constipation, problems with fertility, hair loss, exhaustion, and more[16]. Nonetheless, natural substitutes are recommended, owing to the side effects of the usage of chemical drugs. Turmeric (*Curcuma longa*), green tea (*Camellia sinensis*), ginger (*Zingiber officinale*), thistle milk (*Silybum marianum*), *Salvia chinensis* (Benth), *Actinidia chinensis* (Planch plant) and *Bryophyllum pinnatum*[17] are some of the widely utilised natural sources in traditional medicine. *Bryophyllum pinnatum* contains astragalin, 3,8-dimethoxy-4,5,7trihydroxyflavone, friedelin, epigallocatechin-3-o-syringate luteolin,rutin, kaempferol, quercetin , quercetin3L-rhamonsido-L-arabino furanoside, quercetin-3-Odiarabinoside, kaempferol-3-glucoside , kaempferol-3-Oα-L-arabinopyranosyl (1 2)α-L-rhamno pyranoside , quercetin-3-O-α-L-arabino pyranosyl(1 2)α-L-rhamno pyranoside and 4',5-dihydroxy-3',8-dimethoxy flavone-7O-β-D-glucopyranoside. The plant contains α-amyrin, α-amyrinacetate, β-amyrin, βamyrinacetate, bryophollenone, bryophollone, taraxerol, Ψ-taraxasterol, friedelin, glutinol. The cardienolide and steroidal contents includes β-sitosterol, bryophyllol, bryophynol, bryophyllin B, bryophyllin A, bryophyllin C and bersaldegenin-3-acetate, bryotoxin A, bryotoxin B, bersaldegenin-1,3,5-orthoacetate, campesterol, isofucosterol, clionasterol, codisterol[18]. The two main bufadienolides that are extracted from leaves of *Bryophyllum pinnatum* are β-sitosterol and bryophyllin A[19,22]. Bryophyllin A is a bufadienolide compound that halts the G2/M and S phases of SKOV-3 cells in the cell cycle, induces depolarisation of the mitochondrial membrane, and demonstrated strong antiproliferative and cytotoxic activity[20]. β-sitosterol obstructs the signalling pathways of several cells. Pharmacological screening revealed these effects without appreciable toxicity. Liposome-mediated delivery of β-sitosterol is a promising demonstration[20]. In the present study, the phytochemical analysis was carried out in the *Bryophyllum pinnatum* leaves extracts and the the effect of β-sitosterol and bryophyllin A of *Bryophyllum pinnatum* on key proteins involved in hepatocarcinogenesis was evaluated by using computational tools.

## Materials and Methods

### Sample collection

The leaves of *Bryophyllum pinnatum* were collected from Hyderabad, India in the month of March-April, identified and authenticated by proper morphological analysis.

### Preparation of plant extracts

The *Bryophyllum pinnatum* leaves were washed thrice with water, dried and macerated into a fine paste. In three distinct conical flasks, the methanolic, ethanolic, and aqueous extracts were prepared by adding the macerated leaves in 80% methanol (1:3 ratio), 80% ethanol (1:3 ratio), and 100mL water (1:10 ratio) respectively. The extracts were kept for a duration of 72h at 25°C. The extracts were filtered individually using Whatman No. 1 filter paper and depending on the solvents employed, the filtrates were referred to as methanolic, ethanolic, and aqueous extracts. These extracts were stored at $4^0$C for phytochemical analysis[21].

### Qualitative assessments of phytochemicals

The extracts were tested for the presence of bioactive compounds by using the following standard methods.

#### Alkaloids

Following the addition of 1mL of concentrated picric acid to 2mL of plant extracts, the mixture was observed for the formation of a yellow precipitate, which would indicate the presence of alkaloids [23].

#### Flavonoids

Upon the addition of 1mL of 2N sodium hydroxide solution to 2mL of plant extract, the observation was made to see if any yellow colouration developed, indicating the presence of flavonoids[24].

#### Phenols

To 1 mL of plant extract, 1 mL of water and 1-2 drops of ferric chloride were added, and the mixture was tested for a dark greenish colour, which suggests the presence of phenols[25].

#### Resins

1mL of concentrated sulfuric acid and a few drops of acetic anhydride were added to 1mL of plant

extract, and colour formation from orange to yellow indicated the presence of resins[26].

Saponins

2mL of plant extract, 5mL of distilled water, and 3–4 drops of olive oil were added and the mixture was thoroughly mixed. The formation of foam on the solution's surface is a sign that saponins are present [27].

Steroids

Upon addition of 2mL of chloroform and 1mL of concentrated sulfuric acid to 5mL of plant extract, an observation was done to check the formation of red colour, a sign of the presence of steroids[28].

Sterols

5mL of plant extract, 2mL of chloroform and 1mL of concentrated sulfuric acid were added to check for the formation of a reddish-brown colour, signifying the presence of sterols[29].

Tannins

The plant extract was diluted with distilled water and mixed with three drops of 5% ferric chloride. The resulting solution was observed for the formation of a dark green or black colour, indicating the presence of tannins[29].

**Retrieval of target and ligand molecules**

RCSB PDB (protein data bank) provided the 3D structures of VEGFR2, IGFR, C-KIT, RET, Pi3K, C-Met, MEK-inhibitor, and PDGFR[30,31]. Three-dimensional structures of β-sitosterol and Bryophyllin A' were obtained from PubChem[32].

**Prediction of drug potency of the ligands**

AdmetSAR and Lipinski filter were used to analyse the drug potencies. Absorption, distribution, metabolism, excretion, and toxicity (ADMET) are essential features for the creation of industrial chemicals, medicines, food additives and consumer goods. The rule of five (RO5), also referred to as Pfizer's rule of five or Lipinski's rule of five, is a broad method for assessing drug likeliness or figuring out whether a chemical compound with a particular pharmacological or biological activity has physical and chemical characteristics like molar refractivity, hydrogen bond donors, mass, hydrogen bond acceptors, LogP[7].

**Prediction of active site binding residues in the proteins of interest**

Computed Atlas of Surface Topography of proteins (CASTp) a web service, was utilised to predict the active sites of the proteins of interest. This server provides online tools for defining, measuring and detecting the geometric and topological properties of protein structures[33,34].

**Energy optimisation of ligand and the proteins of interest**

The next-generation interactive visualisation tool, UCSF ChimeraX, assists in stabilising our target proteins by eliminating water molecules and undesirable ions before docking. Also, the ligands have undergone comparable optimization[35].

**Docking of ligand and the proteins of interest**

Molecular docking was carried out using CB-Dock. This protein-ligand docking technique utilises auto dock vina to recognise binding sites, determine the dimensions & centre and modify the docking as per the query ligand[36].

## Results and Discussion

**Phytochemical analysis of the extracts of *Bryophyllum pinnatum* leaves**

The phytochemical analysis showed that, compared to methanolic and aqueous extracts, most phytochemicals were found in the ethanolic extract. Table 1 displays the findings of the qualitative phytochemical analysis in the three extracts.

**Drug likeliness analysis of ligands**

As shown in Table 2, β-sitosterol satisfies four of the lipinski properties excluding hydrogen bond donors. In contrast, bryophyllin A satisfies all of the

Table 1— Phytochemicals of varied concentrations present in the *Bryophyllum pinnatum* leaves

| Phytochemicals | Methanolic extract | Ethanolic extract | Aqueous extract |
|---|---|---|---|
| Alkaloids | +++ | ++ | + |
| Flavonoids | ++ | + | +++ |
| Phenols | ++ | ++ | + |
| Resins | - | - | +++ |
| Saponins | +++ | +++ | +++ |
| Steroids | ++ | +++ | + |
| Sterols | ++ | +++ | - |
| Tannins | +++ | +++ | +++ |

Table 2 — Comparative analysis from Lipinski of our desired ligands, Bryophyllin A and β-sitosterol

| Description | Range | Bryophyllin A | β-sitosterol |
|---|---|---|---|
| Mass | <500 | 472 | 312 |
| Hydrogen bond donors | <5 | 2 | 5 |
| Hydrogen bond acceptors | <10 | 3 | 8 | 6 |
| logP | 5 | 2.127301 | 0.053101 |
| Molar refractivity | 40-130 | 115.631561 | 77.145782 |
| Rules obeyed out of 5 | | 5 | 4 |

Table 3 — Important properties from admetSAR of Bryophyllin A and β-sitosterol

| Classification | Bryophyllin A | | β-sitosterol | |
|---|---|---|---|---|
| | Value | Probability | Value | Probability |
| Ames mutagenesis | - | 0.68 | - | 0.9 |
| Acute oral toxicity | - | 0.4207 | - | 0.4287 |
| Blood brain barrier | - | 0.625 | + | 0.575 |
| Biodegradation | - | 0.925 | - | 0.825 |
| CaCO2 permeability | - | 0.7758 | + | 0.5385 |
| Human intestinal absorption | + | 0.9791 | + | 1 |
| Human oral bio-availability | - | 0.6 | + | 0.5286 |
| P-glycoprotein substrate | - | 0.5557 | + | 0.827 |
| Carcinogenicity (binary) | - | 0.94 | - | 0.97 |
| Carcinogenicity (trinary) | Non required | 0.5458 | Non required | 0.5888 |
| CYP inhibitory promiscuity | - | 0.9659 | - | 0.5244 |

lipinski properties. The admetSAR study showed that β sitosterol can cross the blood-brain barrier, shows Cancer coli-2(Caco-2) permeability, be orally bioavailable, and act as a substrate for P-glycoproteins, whereas bryophyllin A shows the opposite results for the aforementioned criteria. The results for the other metrics for bryophyllin A and β sitosterol were found to be identical, as shown in Table 3.

**Active site binding residues analysis in the ligands**

The active site residues in β-sitosterol and bryophyllin A, were examined using CASTp (Table 4). A variety of amino acids can be found in the active site residues, including acidic amino acids like glutamic acid and aspartic acid, basic amino acids like lysine, arginine, and histidine, aliphatic amino acids like glycine, alanine, asparagine, isoleucine, leucine, proline, and valine, aromatic amino acids like phenylalanine, tyrosine, and tryptophan, sulphur-containing amino acids like cysteine and methionine, and aliphatic amino acids like serine and threonine.

**Docking analysis**

Through docking studies, it was observed that bryophyllin A has established four hydrophobic contacts, four hydrogen bonds, and one weak hydrogen bond with Pi3K. Bryophyllin A exhibited the highest molecular interactions with 645[LEU], 431[ASP], 437[VAL], 683[ARG], 638[GLN], 428[ASN], 648[GLN], 135[GLU], and 679[THR]. β-sitosterol formed nine hydrophobic contacts with 1108[ALA], 1110[LYS], 1127[GLU], 1130[ILE], 1157[LEU], 1200[PHE], 1221[ASP], 1222[ASP] and 1222[PHE] amino acid residues of Pi3K. In Fig. 1, among the two phytochemicals studied in this work, bryophyllin A exhibited the strongest interactions with Pi3K and IGFR. The binding affinities of

Table 4 — Target molecules and active site binding residues

| Protein | Active site binding residues |
|---|---|
| VEGFR2 | 814[ASP],817[CYS],826[LYS],827[TRP],840[LEU],848[VAL],866[ALA],868[LYS],870[LEU],881[ALA],882[LEU],884[SER],885[GLU],888[ILE],889[LEU],890[ILE],892[ILE],893[GLY],894[HIS],898[VAL],899[VAL],900[ASN],901[LEU],914[VAL], 916[VAL],917[GLU],918[PHE],919[CYS],920[LYS],922[GLY],922[ASN],1019[LEU],1024[CYS],1025[ILE],1026[HIS],1026[HIS],1027[ARG],1035[LEU], 1044[ILE],1045[CYS],1046[ASP],1047[PHE],1048[GLY],1049[LEU] |
| IGFR | 981[PHE],982[SER],983[ALA],984[ALA],985[ASP],986[VAL],1005[LEU],1006[GLY],1007[GLN],1008[GLY],1009[SER],1010[PHE],1011[GLY],1013[VAL],1031[ALA],1033[LYS],1034[THR],1045[ILE], 1046[GLU],1047[PHE],1049[ASN],1050[GLU],1051[ALA],1052[SER],1053[VAL],1054[MET],1055[LYS],1056[GLU],1063[VAL],1079[MET],1080[GLU],1081[LEU],1082[MET],1085[GLY],1086[ASP],1126[LEU],1129[ASN],1130[LYS],1131[PHE],1133[HIS],1134[ARG],1135[ASP],1140[ASN],1141[CYS], 1142[MET],1152[GLY],1153[ASP],1154[PHE],1155[GLY],1156[MET],1157[THR],1158[ARG],1159[ASP],1160[ILE],1161[TYR]. |
| C-KIT | 557[TRP],599[ALA],600[PHE],622[LYS],632[THR],633[GLU],636[ALA],637[LEU],640[GLU],790[HIS],791[ARG],792[ASP],810[ASP],812[GLY],813[LEU],815[ARG],816[ASP],817[ILE],818[LYS],822[TYR],828[ASN],829[ALA],830[ARG],831[LEU],832[PRO],846[TYR]. |
| RET | 728[LYS],730[LEU],731[GLY],732[GLU],733[GLY],734[GLU],735[PHE],736[GLY],737[LYS],738[VAL],756[ALA],758[LYS],759[MET],775[GLU],804[VAL],805[GLU],806[TYR],807[ALA],808[LYS],809[TYR],810[GLY],811[SER],814[GLY],815[PHE], 818[GLU],872[HIS],874[ASP],878[ARG],879[ASN],881[LEU],891[SER],892[ASP],893[PHE],894[GLY],895[LEU],896[SER]. |

(*contd.*)

Table 4 — Target molecules and active site binding residues (*contd.*)

| Protein | Active site binding residues |
|---|---|
| Pi3K | 107[ASN],109[GLU],110[GLU],113[LEU],116[GLU],151[VAL],154[ARG],155[ASP],162[ARG],165[TYR],166[VAL],167[TYR],168[PRO],169[PRO],170[ASN],171[VAL],172[GLU],173[SER],174[SER],176[GLU],177[LEU],178[PRO],180[HIS],181[HIS],182[TYR],185[LEU],213[HIS],253[LYS],254[VAL],255[CYS],257[CYS],258[ASP],259[GLU],260[TYR],265[TYR],268[SER],269[GLN],271[LYS],272[TYR],274[ARG],275[SER],276[CYS],277[ILE],278[MET],279[LEU],281[ARG],282[MET],284[ASN],286[MET],288[MET],293[LEU],296[GLN],297[LEU],298[PRO],299[MET],300[ASP],+301[CYS],302[PHE],303[THR],304[MET],305[PRO],535[SER],537[ARG],538[ASP],539[PRO],540[LEU],541[SER],543[ILE],563[GLU],566[PRO],567[LYS],570[LEU],593[ILE],594[LYS],596[GLU],597[GLN],600[GLU],603[ASP],605[6ASN],606[TYR],624[THR],625[ASP],626[ASP],627[LYS],629[SER],630[GLN],631[TYR],632[LEU],633[ILE],660[ASN],661[GLN],662[ARG],663[ILE],665[HIS],666[PHE],670[HIS],692[CYS],693[ARG],694[ALA],695[CYS],696[GLY],697[MET],698[TYR],699[LEU], 700[LYS],701[HIS],752[LEU],753[SER],755[LEU],756[ASN],757[PRO],758[ALA],759[HIS],760[GLN],787[ASP],788[ILE],792[LEU],793[LEU],794[PHE],796[ASN],797[ASN],798[GLU],799[ILE],811[MET],814[LEU],815[GLN],818[ARG],819[ILE],821[GLU],822[ASN],822[ILE],825[GLN],826[ASN],832[ARG],833[LEU],834[LEU],835[PRO],836[TYR],837[GLY],838[CYS],839[LEU],849[GLU],850[VAL],852[ARG],924[LYS],928[GLN],994[HIS],996[ASN],997[LEU],1000[ASN],1003[SER],1016[PHE]. |
| C-MET | 1084[ILE],1085[GLY],1086[ARG],1087[GLY],1089[PHE],1090[GLY],1091[CYS],1092[VAL],1108[ALA],1110[LYS],1112[LEU],1120[GLU],1122[GLN],1124[PHE],1127[GLU],1128[GLY],1130[ILE],1131[MET],1134[VAL],1140[LEU],1155[VAL],1157[LEU],1158[PRO],1159[TYR],1160[GLY],1195[LEU],1200[PHE],1201[VAL],1202[HIS],1203[ARG],1211[MET],1220[VAL],1221[ALA],1222[ASP],1222[PHE],1224[LEU],1227[ARG],1228[ASP]. |
| MEK-INHIBITOR 1 | 74[LEU],75[GLY],76[ALA],77[GLY],78[ASN],79[GLY],82[VAL],95[ALA],97[LYS],99[ILE],115[LEU],127[VAL],141[ILE],143[MET],144[GLU],145[HIS],146[MET],149[GLY],150[SER],152[ASP],153[GLN],188[ARG],190[LYS],192[LYS],194[SER],195[ASN],197[LEU],207[CYS],208[ASP],209[PHE],210[GLY],VAL[211],212[SER],215[LEU],216[ILE],219[MET],220[ALA],225[GLY],226[THR],227[ARG],220[MET],224[ARG]. |

(*contd.*)

Table 4 — Target molecules and active site binding residues (*contd.*)

| Protein | Active site binding residues |
|---|---|
| PDGFR | 97[ASN],98[SER],86[MET],87[ASN],88[MET],89[THR],90[GLU],91[PHE],92[PRO],93[ARG],94[TYR],111[PHE],112[ASP],115[SER],116[THR],117[GLN],119[ARG],120[LYS],121[PRO],122[ALA],124[TYR],145[THR],146[VAL],147[PRO],222[ARG],223[ASN],225[PHE],226[ARG],58[GLN],59[GLN],59[TYR],60[PRO],61[MET],62[SER],63[GLU],93[ALA],95[THR],96[GLY],97[LEU],99[THR], 106[GLN],107[THR],109[GLU],110[ASN],111[GLU],112[LEU],113[GLU],114[GLY],116[HIS],118[TYR],129[VAL],130[PRO],131[LEU],132[GLY],133[MET],134[THR],135[ASP],136[TYR],137[LEU],151[ARG],201[ILE]. |

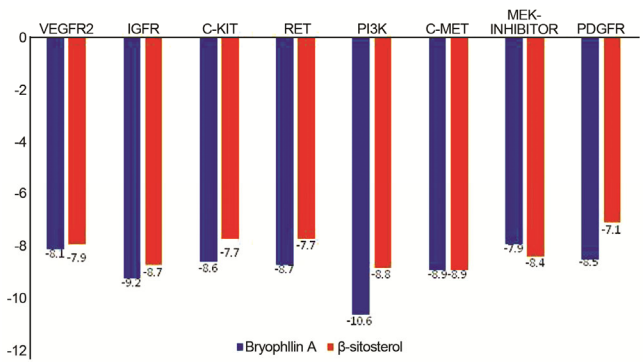


Fig. 1 — Molecular bonding affinity between the phytochemicals and the receptors.

bryophyllin A to the aforementioned receptors were -10.6 Kcal/mol and -9.2 Kcal/mol, respectively. Pi3K activity is involved in growth, invasion, and metastasis of the cancer cells. Bryophyllin A binds tightly to the pleckstrin-homology (PH), FYVE, Phox (PX), C1, C2 or other lipid-binding domains thereby helping in preventing the metastasis of the cancer cells. IGFR converts into an active IGFR by undergoing phosphorylation. Bryophyllin A binds to the active IGFR and may stop the Akt1 signalling process to prevent malignant melanoma cells from growing and spreading. β-sitosterol exhibited the strongest interactions with C-met and Pi3K. The binding affinities of β-sitosterol with the aforementioned receptors were -8.9 Kcal/mol and -8.8 Kcal/mol, respectively. B-sitosterol binds to β chain of the C-met, decreases its tyrosine kinase activity and blocks both ligand-dependent and ligand-independent activation thereby preventing the growth, invasion, and metastasis of the cancer cells. Pi3K activity, which is involved in the growth, invasion, and

metastasis of the cancer cells binds to β sitosterol C1, C2 or other lipid-binding domains which helps in preventing the metastasis of the cancer cells. *Bryophyllum pinnatum* is widely present in Africa, India, Brazil, China and all other tropical nations[37]. Throughout India, the plant species is mostly found in the northern regions of Bengal [38]. This plant is highly adaptable to cultivation and may thrive in almost any soil-based media in tropical and subtropical regions. However, biotechnological techniques such as plant tissue culture, organogenesis, plant regeneration, micropropagation and plant suspension-cultured cells can be used to cultivate the plant species in laboratories[39]. Previous studies have reported the presence of various bioactive in the leaves of *Bryophyllum pinnatum* such as alkaloids, flavonoids,

phenols, resins, saponins, steroids, sterols and tannins. A series of tests were conducted to prove the presence of the above phytochemical constituents[40]. The biological and pharmacological effects of the phytochemical constituents showed anti-inflammatory, analgesic, antiallergy, anticancer, antidiabetic, antihypersensitive, antileishmanial, antimicrobial, antifungal, urolithic, gastroprotective, hepatoprotective, antioxidant, nephron-protective and wound healing activities[41]. Previous study reported the inhibitory efficacy of *Bryophyllum pinnatum* on cervical cancer in humans [42]. Based on these findings, we further found that β sitosterol and bryophyllin A contributed to the inhibition of liver cancer cells' ability to develop metastases. Research on application of bryophyllin A to COVID-19 cytokine storm therapy was conducted earlier[43]. Based on these reports we carried out our present work to demonstrate computationally the role of *Bryophyllum pinnatum* in mitigating the hepatocarcinognesis in humans.

The potential of *Bryophyllum pinnatum* as a therapy for hepatocarcinogenesis was studied by targeting Pi3K-AKT, downstream signalling, MAPK, and PDGF pathways. As a part of phytochemical analysis, the ethanolic extract of *Bryophyllum pinnatum* has predominantly showed the presence of the phytochemical constituents as compared to the other two extracts namely methanolic and aqueous extracts, while the resins were found in the aqueous extract only.

The drug potency analysis tools revealed that both β-sitosterol and bryophyllin A showed the drug likeliness properties. Bryophyllin A was found to be non-substrate of P-glycoprotein whereas β-sitosterol was found to be the substrate of P-glycoprotein indicating that β-sitosterol could be effluxed from the cell by P-glycoprotein and decreases its availability than bryophyllin A. P-glycoprotein substrate is one of the most widely suited transporters involved in drug resistance and drug-drug interactions. P-glycoprotein is expressed in many organs such as the small intestine, blood-brain barrier, kidney and liver[44]. Both Bryophyllin A and β-sitosterol did not show CYP inhibitory promiscuity. A noninhibitor of CYP450 means that the molecule will not hamper the biotransformation of drugs metabolised by CYP450 enzyme[45].

From the molecular interaction studies, we can interpret, bryophyllin A has a higher binding affinity value at -10.6kcal/mol and β-sitosterol has its highest binding affinity value at -8.9kcal/mol. An *in vivo* study proves that the leaves of *Bryophyllum pinnatum* have the potential of lowering the metastasis in the tumour cells of liver. A group of rats were infected with N-diethylnitrosamnine which induced hepatic injury. They later showed significant rise in the levels of triglycerides, cholesterol and few liver enzymes such as ASP, ALT and GGT. After medicating them with the leaf extracts, these parameters were brought back to normal, indicating the beneficial effects of administration of aqueous extracts of *Bryophyllum pinnatum* during DENA-induced hepatocarcinogenesis[45]. Contradictory to our assumptions, bryophyllin A has the strongest binding affinity compared to β-sitosterol. Further experimental investigations are to be conducted to validate further the potency of β-sitosterol and Bryophyllin A as antihepatocarcinogenic agents. A qualitative and quantitative phytochemical analysis of wide range of phytochemicals could have been conducted but due to financial constraints, qualitative phytochemical analysis was performed. In this article, we only studied the effect of two ligands on limited signalling proteins involved in hepato carcinogenesis using bioinformatics tools. A future study can be conducted by utilising more target proteins from signalling systems involved in upregulation of hepatocarcinogenesis. Although, our study proved the lowering/reversal of hepatocarcinogenesis, we were unable to conduct an *in vivo* experimental procedure due to low/no feasibility.

## Conclusion

In our study, qualitative phytochemical screening of *Bryophyllum pinnatum* leaves extracts was

evaluated. Bryophyllin A and β-sitosterol were found to suppress Pi3K and IGFR activity and showed potential as therapeutic agents for hepatocarcinogenesis. Our study paved the way for demonstrating the benefits of 'The never die Plant' – *Bryophyllum pinnatum* in mitigating hepatocarcinogenesis.

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**Conflicts of interest**

The authors declare no conflicts of interest.

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