Exploring the anti-proliferative activity of *Pelargonium sidoides* DC with *in silico* target identification and network pharmacology

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Abstract

Pelargonium sidoides DC (*Geraniacea*) is a medicinal plant indigenous to Southern Africa that has been widely evaluated for its use in the treatment of upper respiratory tract infections. In recent studies, the anti-proliferative potential of *P. sidoides* was shown and several phenolic compounds were identified as the bioactive compounds. Little, however, is known regarding their anti-proliferative protein targets. In this study, the anti-proliferative mechanisms of *P. sidoides* through *in silico* target identification and network pharmacology methodologies were evaluated. The protein targets of the 12 phenolic compounds were identified using the target-identification server PharmMapper and the server for predicting Drug Repositioning and Adverse Reactions via the Chemical-Protein Interactome (DRAR-CPI). Protein-protein and protein-pathway interaction networks were subsequently constructed with Cytoscape 3.4.0 to evaluate potential mechanisms of action. A total of 142 potential human target proteins were identified with the *in silico* target identification servers and 90 of these were found to be related to cancer. The protein interaction network was constructed from 86 proteins involved in 209 interactions with each other, and two protein clusters were observed. A pathway enrichment analysis identified over 80 Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways enriched with the protein targets and included several pathways specifically related to cancer as well as various signaling pathways that have been found to be dysregulated in cancer. These results indicate that the anti-proliferative activity of *P. sidoides* may be multifactorial and arises from the collective regulation of several interconnected cell signaling pathways.

Keywords: *Pelargonium sidoides*, anti-proliferative, target identification, network pharmacology, signaling pathways, kinase, PharmMapper, Cytoscape

Introduction

Pelargonium sidoides DC (*Geraniacea*) is a medicinal plant indigenous to South Africa and Lesotho that has traditionally been used for the treatment of tuberculosis as well as various stomach ailments, such as colic, diarrhea and dysentery [1]. It has, however, in more recent times, been developed into medicinal formulations like EPs® 7630 and Linctagon for the treatment of upper respiratory tract infections [1-5]. Laboratory-based studies have revealed that the beneficial effects of extracts prepared from the roots of *P. sidoides* can be attributed to their antibacterial, antiviral and immunomodulatory bioactivities [6-15]. Extracts have been shown to be rich in a variety of phenolic compounds such as gallic acid, highly oxygenated simple coumarins as well as proanthocyanidins [16].

In previous studies, we found anti-proliferative activity for a commercial radix mother tincture of *P. sidoides in vitro* and through a bioactivity-guided approach; we isolated seven fractions with significant anti-proliferative activity [17,18]. Liquid chromatography with tandem mass spectrometry detection (LC-MS/MS) analysis of these fractions revealed the presence of phenolic compounds gallic acid, koaburaside, proanthocyanidin dimers as well as a trimer, dihydroxycoumarin sulfates, trihydroxycoumarin as well as three turgorins namely leaf movement factor (LMF) 2, LMF3 and the periodic leaf movement factor 1 (PLMF1). A new phenolic acid glycoside sulfate (PGS) not previously shown in *P. sidoides* extracts was identified according to its mass fragmentation pattern. Although anti-proliferative activity has been shown for gallic acid and proanthocyanidins, it remains largely unknown how these compounds downregulate the expression of drug targets, such as cyclin-dependent kinases (CDK) 2 and 4, Bcl- 2, nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB), extracellular regulated kinase (ERK)1/2 as well as matrix metalloproteinase (MMP) 2 and 9 [19-22]. Furthermore, little is known regarding the potential anti-proliferative activity of the turgorins, phenolic glycosides and the coumarin sulfates and how these compounds may contribute to the observed anticancer activity of the fractions.

In silico target identification coupled with network pharmacological profiling has been gaining momentum and played an important role in the development of new computational methods to predict drug-target networks as well as drug discovery, drug re-purposing and drug-target validation in medicinal chemistry and pharmacology [23-30]. In the study of medicinal plants and their bioactive compounds, *in silico* target identification coupled with network pharmacological profiling has provided valuable insights into potential disease models, protein targets as well as mechanisms of action [31-35]. In the current study, we have implemented these methodologies in an attempt to identify the potential protein targets of the isolated compounds to provide insights into the anti-proliferative activity of *P. sidoides*.

Materials and methods

In silico target fishing

The two-dimensional structures of gallic acid, koaburaside, **6,7**-dihydroxycoumarin-**8**-sulfate (**67**DiOHC**8**S), **7**,**8**-dihydroxycouamrin-**6**-sulfate (**78**DiOHC**6**S), **6,7,8**-trihydroxycoumarin (**678**TriOHC), gallocatechin-gallocatechin dimer (GCGC), gallocatechin-gallocatechin-gallocatechin trimer (GCGCGC), catechin-gallocatechin (CGC), LMF2, LMF3, PLMF1 and PGS were created with Advanced Chemistry Development (ACD)/ChemSketch freeware version 12.02, 2010 [36] and the smiles notation representing each of the structures was generated. From the smiles notation, the 3D structure was generated and optimized with University of California, San Francisco (UCSF) Chimera version 1.11 (build 41097) [37,38] and saved as mol2 files. These mol2 files were subsequently uploaded to the PharmMapper and DRAR-CPI servers for *in silico* screening [39,40]. Following the screening, potential protein targets were identified using a cutoff fit-value of 4.000 together with a positive z-score for PharmMapper while for DRAR-CPI, a cutoff docking score of -50.000 with a z-score of -1.500 was used. Only human protein targets were considered. Literature sources, as well as the Disease and Gene Database [41,42], were consulted to determine whether the potential protein targets identified were related to cancer. The compound-target-cancer network was generated by Cytoscape version 3.4.0 [43], and the NetworkAnalyzer Application version 2.7 [44] was used to evaluate some basic network features.

Protein interaction network and protein-pathway enrichment analysis

The gene symbols of the protein targets identified were submitted to the String Database [45,46] to evaluate the potential protein interactions amongst the targets. Only protein interactions with the highest confidence score (0.900) were considered. The protein interaction network was constructed with Cytoscape version 3.4.0 following the removal of all isolated nodes. To determine where the protein targets may play a role, pathway enrichment analysis of the KEGG pathways was performed with the String Database. Only the enriched pathways with a False Discovery Rate (FDR) of 0.001 or less were considered in the construction of the protein-pathway network with Cytoscape version 3.4.0.

Results and discussion

In silico target fishing

The 12 compounds identified from the bioactive fractions isolated from *P. sidoides* were screened *in silico* with PharmMapper and DRAR-CPI to elucidate the potential anti-proliferative protein targets of these compounds. Screening with the PharmMapper server

involves docking the compounds with pharmacophore models of the active site of protein targets while with DRAR-CPI, the compounds are docked into the protein pockets of the protein target [39,40].

The PharmMapper server revealed 53 different human potential protein targets for the compounds using a cutoff fit-value of 4.000 and a positive z-score value [35]. Some of the compounds were found to have high fit-values for different pharmacophore models representing the same protein target. This was observed for **67**DiOHC**8**S that showed high fit-values for four pharmacophore models of tyrosine-protein phosphatase non-receptor type 1 (PTPN1) and **78**DiOHC**6**S for three pharmacophore models of CDK2. Furthermore, some of the protein targets identified were found to be potential targets of more than one compound. This was observed for protein targets actin, alpha skeletal muscle 1 (ACTA1), cystathionine beta-synthase (CBS), cystic fibrosis transmembrane conductance regulator (CFTR), HRas, membrane metallo-endopeptidase (MME), 6-phosphofructo-2-kinase (PFKFB1), PTPN1, uridine-cytidine kinase 2 (UCK2).

The DRAR-CPI server revealed another 97 different potential human protein targets with a cutoff docking score of -50.000 and z-score values smaller than -1.5. As observed with the PharmMapper server, some of the protein targets identified were found to be potential targets of more than one compound. This was observed for 35 different protein targets. Some protein targets were also shared between the two servers as seen with **67**DiOHC**8**S and target CBS, PGS and glutathione S-transferase A1 (GSTA1) as well as target PTPN1 and potential inhibitors PLMF1 and **67**DiOHC**8**S. Taken collectively, 142 different human proteins were found to be potential targets of the 12 compounds (Fig. 1). PLMF1 and PGS were found to be the least selective with 49 and 47 potential targets, respectively. Both have a sulfated sugar moiety that may play an important role in forming intermolecular hydrogen bonds with the active site of the target proteins. On the other hand, the GCGCGC trimer, gallic acid and **678**TriOHC were found to be most selective with three, five and four potential targets, respectively. The GCGCGC trimer has a very large and bulky structure and this likely limits its access to the protein's active site while for gallic acid and **678**TriOHC, their relatively small structures with only a few hydrogen bond donors and acceptors likely limits their interactions with the protein's active site. It was observed that similar structures shared similar potential protein targets, and this could be seen from PLMF1 and PGS that shared 20 protein targets, LMF2 and LMF3 shared five, the two dihydroxycoumarin sulfates shared eight while the proanthocyanidin dimers shared four. Of the 142 potential human target proteins that were identified with the *in silico* target fishing servers, 90 were found to be related to cancer, based on literature sources as well as the Disease and Gene Database

(Fig. 1). These *in silico* results provide support for the observed anti-proliferative activity of *P. sidoides* with *in vitro* studies.
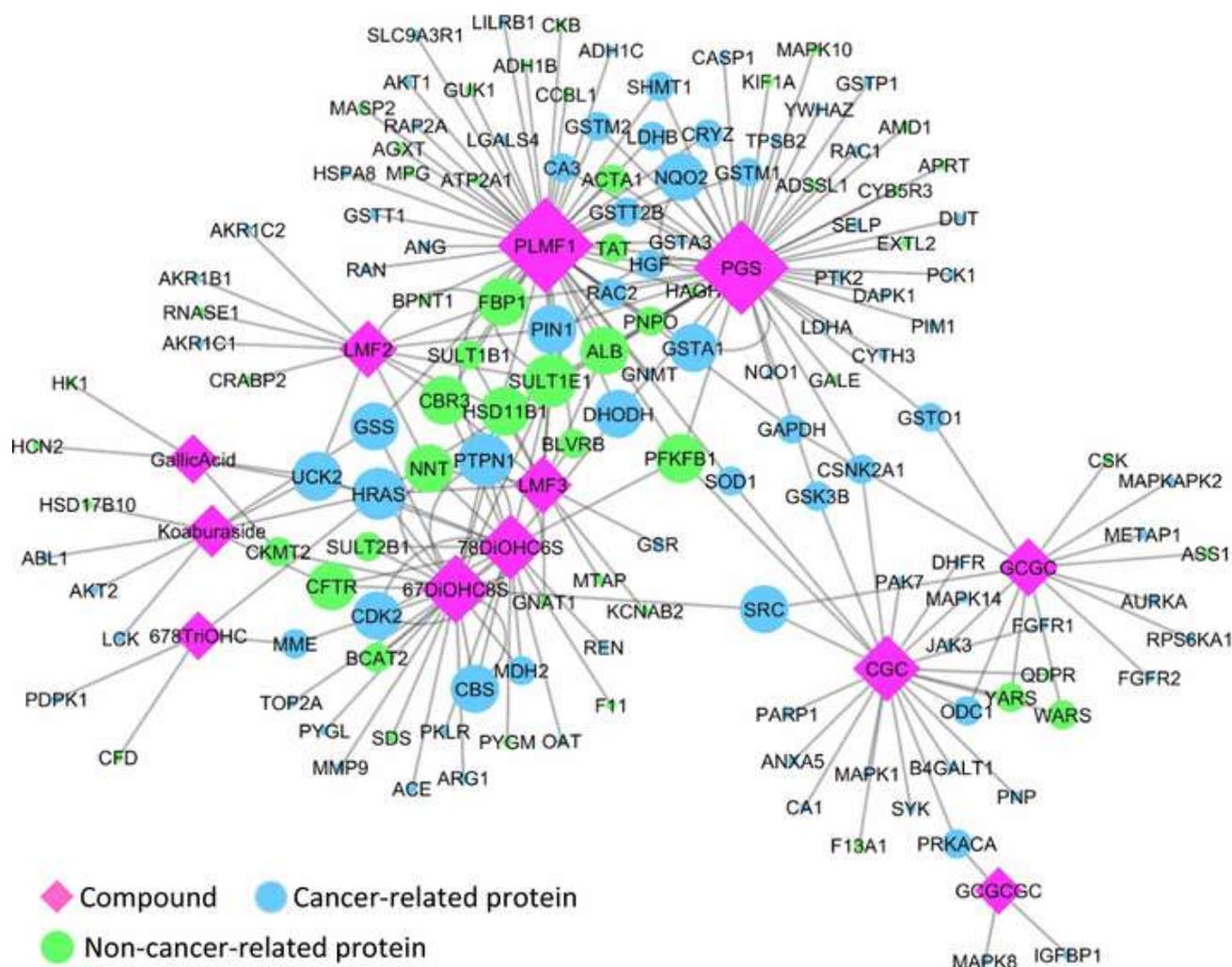
Fig. 1. Compound-target-cancer network. The network was generated with Cytoscape version 3.4.0 with the NetworkAnalyzer Application version 2.7. All protein targets are represented by their gene symbol. Size of compound markers reflects the number of targets for each compound, while size of protein marker reflects number of compounds that are possible inhibitors. Loops within the network indicate that the compound was a possible inhibitor for different models of the same protein target

Protein interaction network and pathway enrichment analysis

In order to explore where these protein targets may play a role, a protein interaction network as well as pathway enrichment analysis were performed [35]. The protein interaction network in Fig. 2 was constructed with the String Database using the highest confidence score (0.900) and excluding all isolated nodes. Within this protein interaction network, two large clusters connected by glutathione S-transferase P (GSTP1) were observed. These clusters suggest that the bioactive compounds of *P. sidoides* are targeting proteins that are either part of the same protein complex or involved in the same pathway [47].
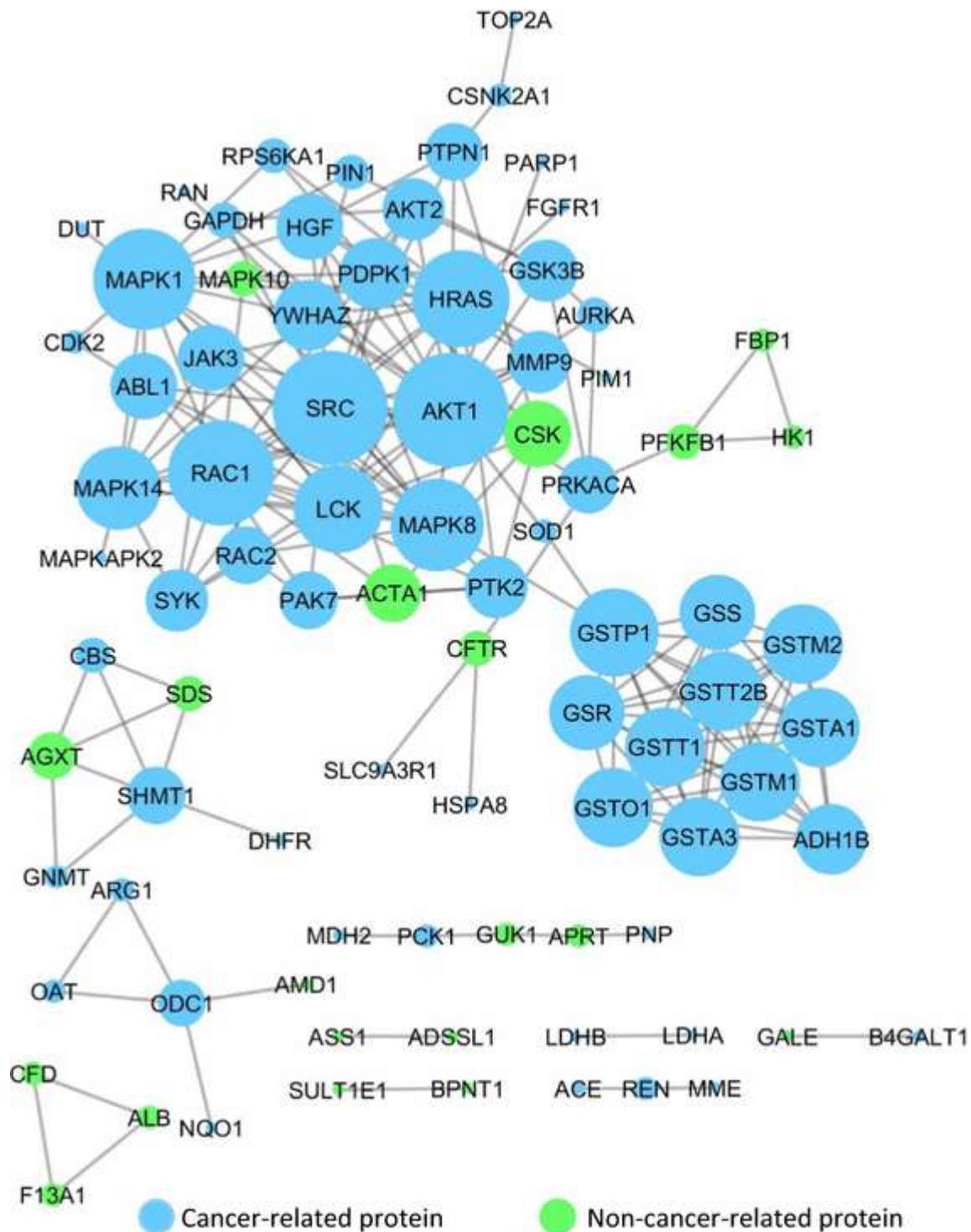
Fig. 2. Protein–protein interaction network. Protein–protein interactions were evaluated with the String Database at confidence level of 0.900. Following the removal of all isolated nodes, the protein–protein interaction network was generated with Cytoscape version 3.4.0 with the NetworkAnalyzer Application version 2.7. Size of protein marker reflects number of interactions with other proteins

With the pathway enrichment analysis, over 80 KEGG pathways with FDR values of less than 0.001 were found to be enriched with the protein targets. Fourteen pathways relating specifically to cancer were identified and included pathways in cancer, proteoglycans in

cancer, chemical carcinogenesis, colorectal cancer, prostate cancer, pancreatic cancer, renal cell carcinoma, endometrial cancer, viral carcinogenesis, melanoma, non-small cell lung cancer, acute myeloid leukemia, chronic myeloid leukemia and glioma (Table 1). Several signaling pathways were also identified and some of these pathways have been found to be dysregulated in cancer as has been observed with the ErbB, vascular endothelial growth factor (VEGF), mitogen-activated protein kinase (MAPK), Ras, phosphatidylinositol-3-kinase (PI3K)-Akt, Rap1, 5'-adenosine monophosphate-activated protein kinase (AMPK), Wnt, focal adhesion, hypoxia inducible factor (HIF-1) and mammalian target of rapamycin (mTOR) signaling pathways [48-58]. Dysregulation of these pathways has been found to be associated with increased cell proliferation, cell survival, inhibition of apoptosis, cell migration and invasion as well as angiogenesis.

Table 1. Enriched KEGG pathways related to cancer

Pathway ID	Pathway description	Proteins	FDR
Cancer-related pathways			
hsa05200	Pathways in cancer	15	1.24e-10
hsa05205	Proteoglycans in cancer	13	1.52e-10
hsa05204	Chemical carcinogenesis	9	2.71e-10
hsa05210	Colorectal cancer	8	1.56e-09
hsa05215	Prostate cancer	8	2.86e-08
hsa05212	Pancreatic cancer	7	6.29e-08
hsa05211	Renal cell carcinoma	7	8.31e-08
hsa05213	Endometrial cancer	6	4.79e-07
hsa05203	Viral carcinogenesis	9	5.13e-07
hsa05218	Melanoma	6	2.74e-06
hsa05223	Non-small cell lung cancer	5	1.52e-05
hsa05221	Acute myeloid leukemia	5	1.62e-05
hsa05220	Chronic myeloid leukemia	5	4.62e-05
hsa05214	Glioma	4	0.000466
Cancer-related signaling pathways			
hsa04012	ErbB signaling pathway	11	2.55e-12
hsa04370	VEGF signaling pathway	10	2.55e-12
hsa04510	Focal adhesion	14	5.31e-12
hsa04010	MAPK signaling pathway	14	7.24e-11
hsa04014	Ras signaling pathway	13	1.52e-10
hsa04151	PI3K-Akt signaling pathway	14	1.87e-09
hsa04915	Estrogen signaling pathway	8	5.27e-08
hsa04015	Rap1 signaling pathway	10	1.24e-07
hsa04152	AMPK signaling pathway	7	3.83e-06
hsa04310	Wnt signaling pathway	7	8.32e-06
hsa04066	HIF-1 signaling pathway	6	2.17e-05
hsa04150	mTOR signaling pathway	5	2.19e-05
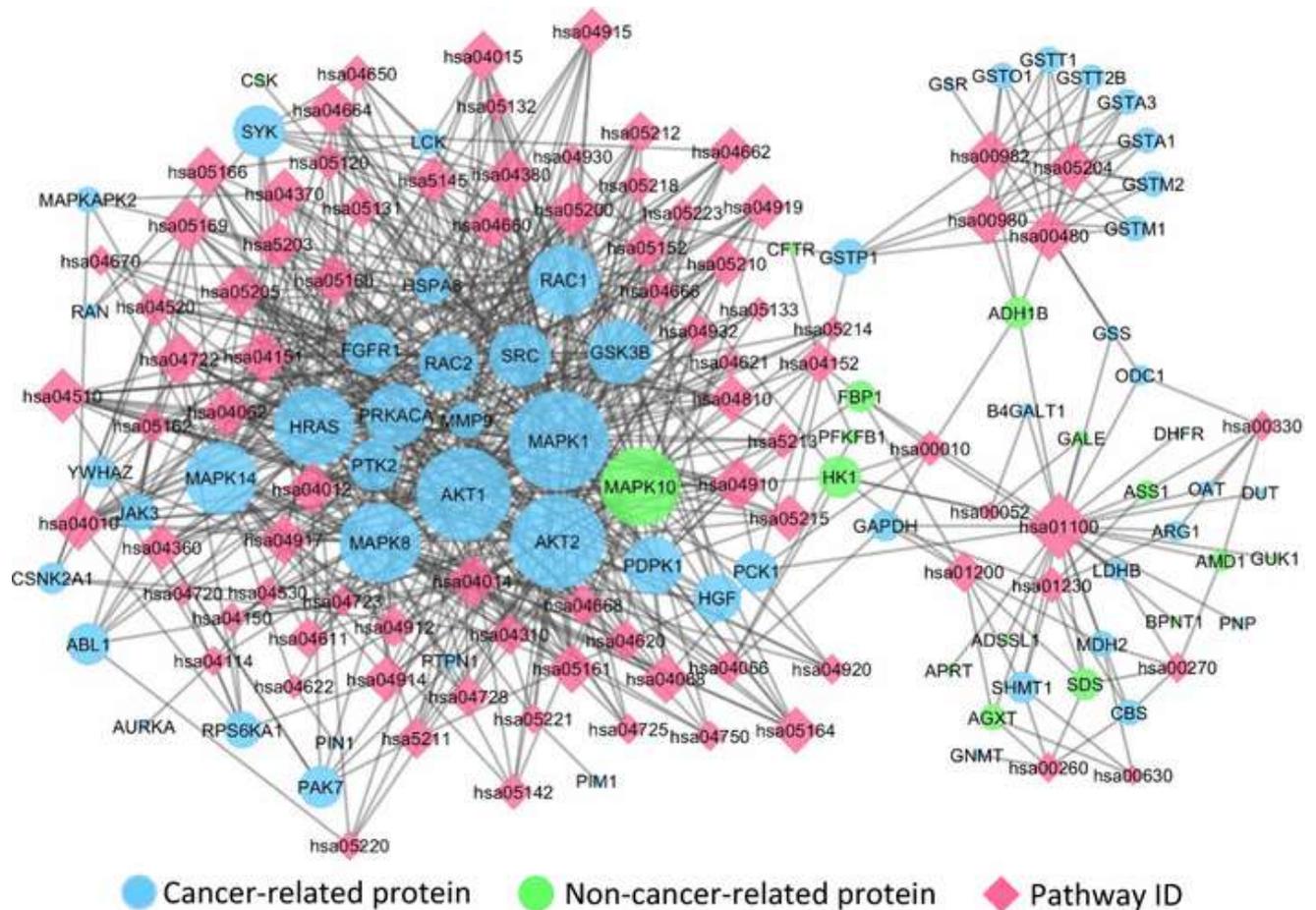
Fig. 3. Protein–pathway interaction network. Pathway enrichment analysis of the KEGG pathways was evaluated with the String Database. The protein–pathway interaction network was constructed by Cytoscape version 3.4.0 with the NetworkAnalyzer Application version 2.7. Only pathways that were found to be enriched with a FDR of 0.001 were included. Pathways are represented by their KEGG pathway ID. Size of the protein markers reflects the number of pathways that are associated with, while the size of the pathway marker reflects the number of the protein targets that are involved in that specific pathway

Inspection of the protein targets involved in these pathways revealed that some of these targets are involved in multiple pathways suggesting that these pathways are interrelated. This can be seen in Fig. 3 where a group of about 25 proteins namely phosphoenolpyruvate carboxykinase (PCK1), hepatocyte growth factor (HGF), Pak7, fibroblast growth factor receptor (FGFR), Rac 1 and 2, focal adhesion kinase (FAK), 3-phosphoinositide-dependent protein kinase 1 (PDPK1), Akt 1 and 2, glycogen synthase kinase-3 beta (GSK3β), Ras, Abl1, Src, cAMP-dependent protein kinase catalytic subunit alpha (PRKACA), MMP9, Heat shock cognate 71 kiloDalton protein (HSPA8), mitogen-activated protein kinase-activated protein kinase 2 (MAPKAPK2), Lck, Syk, casein kinase II subunit alpha (CSNK2A1) as well as the MAPKs 1, 8, 10 and 14 that were all found to be involved in the majority of the pathways identified. Several of these protein targets are concerned with protein kinase activity (GO0004672) involving the phosphorylation of amino acid residues in proteins

(GO0006468) suggesting that phenolic compounds as those identified in *P. sidoides* may specifically target the protein kinases (serine/threonine and tyrosine kinases) involved in signal transduction.
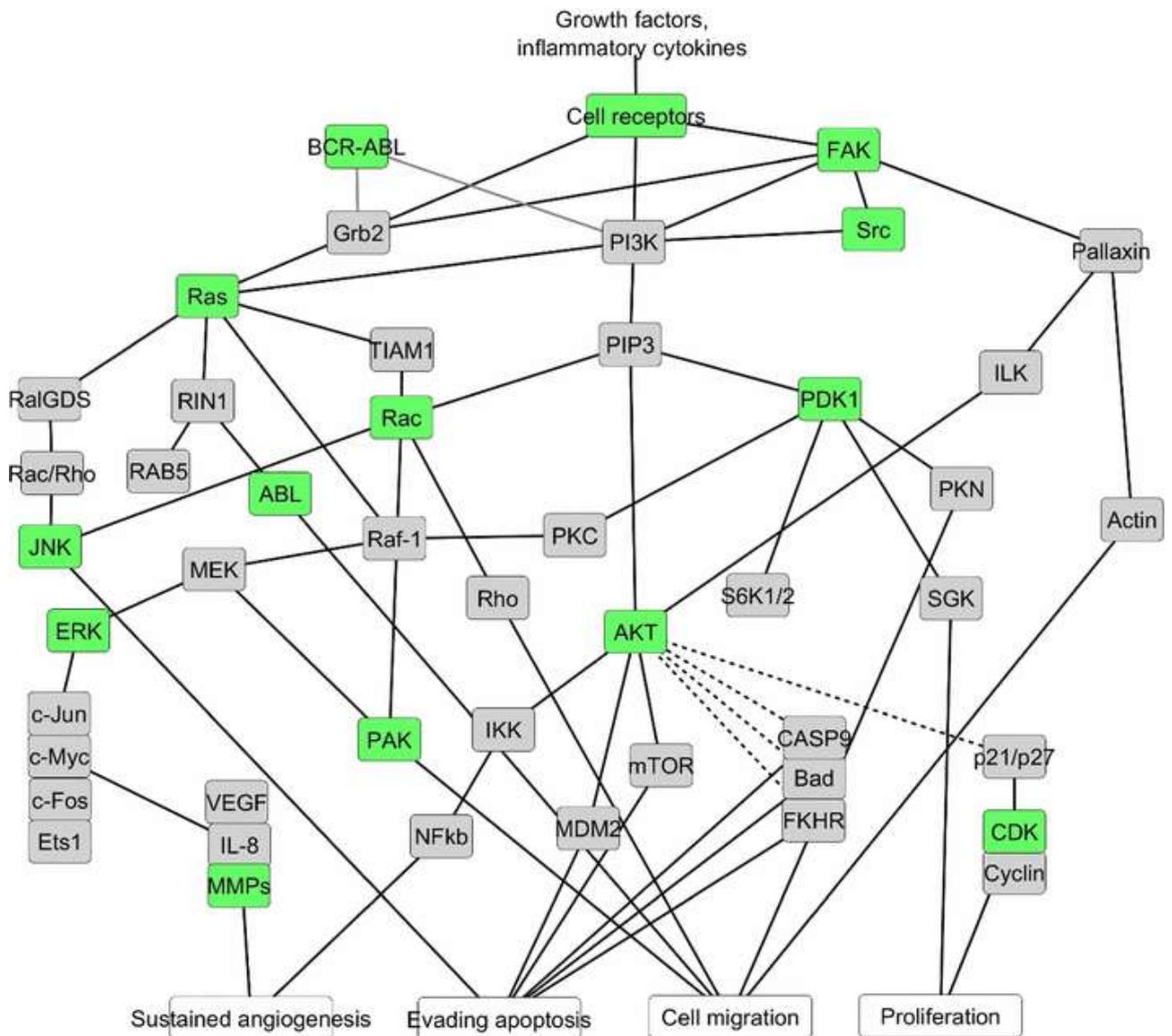
Fig. 4. Potential regulation of PI3K-Akt and Ras/MAPK pathways by the phenolic compounds of *P. sidoides*. Pathways were adapted from hsa05200 Pathways in cancer from the KEGG Database. Protein targets highlighted in green represent the potential targets of the *P. sidoides* compounds

The interplay between these pathways in cancer pathogenesis can be seen in Fig. 4 and the protein targets of the bioactive compounds of *P. sidoides* have been highlighted. Regulation of these pathways by the bioactive compounds of *P. sidoides* should result in the experimentally observed cell growth inhibition, cell cycle arrest and induction of apoptosis. There is evidence in the literature supporting the observed results here. Phenolic compounds similar to those that were identified in *P. sidoides* like gallic acid,

proanthocyanidins and hydroxylated simple coumarins have shown, following treatment of cancer cells *in vitro*, altered expression of PI3K, Akt, Ras, Rac1, MMP9, NF-κB, FAK, CDK2, MAPK-ERK and c-Jun N-terminal kinase (JNK) resulting in cell growth inhibition, cell cycle arrest and apoptosis [19,22,59-61].

The cell signaling pathways identified here are also under oxidative control as has been observed with the PI3K-Akt and the Janus Kinase (JAK)- signal transducers and activators of transcription protein (STAT) signaling pathways as well as the Src protein kinases that can then go on to activate the PI3K-Akt, Ras/Raf/MAPK and STAT pathways [62-67]. The bioactive compounds identified from *P. sidoides* were also shown to have significant antioxidant activity *in vitro* [17]. Thus, *P. sidoides* besides regulating these signal transduction pathways through possible direct interaction with the concerned protein kinases, may also regulate these same pathways by scavenging the reactive oxygen and nitrogen species that can also activate these pathways.

The glutathione pathway (hsa00480) was also found to be enriched with an FDR of 1.36e-08, and it was not surprising that some of the compounds were found to be potential substrates of the GSTs. The GSTs are enzymes that conjugate glutathione to xenobiotics for detoxification [68]. Several anticancer drugs have been found to be substrates of the GSTs, and the GSTs have been implicated in chemotherapeutic drug resistance development. The *P. sidoides* compounds may thus through their interaction with the GSTs, deplete glutathione and reduce the excretion of these drugs thus prolonging their therapeutic effects. This depletion may be beneficial for drugs that have a short half-life but harmful if a drug has a long half-life as toxicity may then become a factor with the longer exposure times. Besides their role in detoxification pathways, the GSTs have been found to be upregulated in several cancer types and have been found to play a role in the regulation of the MAP kinase pathway by inhibiting JNK-induced apoptosis [68]. The *P. sidoides* compounds thus by targeting the GSTs may result in an anti-proliferative effect by preventing the GSTs from inhibiting JNK and allowing the cells to undergo stress-induced apoptosis. This speculation suggests that a combination therapy of *P. sidoides* extract with anti-cancer drugs may be particularly effective, by draining away glutathione, thus prolonging the half-life of the drugs, and inhibiting GSTs so that JNK-induced apoptosis of the cancer cells may proceed unhindered.

Also of particular interest, was that these pathways are activated by the inflammatory mediators, cytokines and growth factors, and extracts of *P. sidoides* has previously shown anti-inflammatory activity *in vivo* [14]. Besides inflammation, the protein targets and activation of these signaling pathways are also found to play important roles during innate

immune responses to generate a defense to invading pathogens. *P. sidoides* extracts have also been found to exert immunomodulatory activity [6,9,10]. The enriched pathways concerned with the innate immune responses include Fc epsilon RI signaling pathway, leukocyte transendothelial migration, chemokine signaling pathway, Toll-like receptor signaling pathways, natural killer cell mediated cytotoxicity, Fc gamma R-mediated phagocytosis, nucleotide-binding oligomerization domain (NOD)-like receptor signaling pathway and retinoic acid-inducible gene 1 (RIG-I)-like receptor signaling pathway (Table 2). The results presented here may thus also suggest the inflammatory pathways regulated by *P. sidoides* extracts as well as provide evidence for the immunomodulation associated with *P. sidoides* extracts. Also, the immune system has been shown to play an important role in cancer progression and drugs such as thalidomide and pomalidomide that can inhibit the action of some of the inflammatory mediators and enhance the activity of immune effectors have proven useful in the treatment of cancer [69-71]. Thus, the immunomodulatory activity of *P. sidoides* may also account for its anti-proliferative activity.

Table 2. Enriched KEGG pathways related to immune function and infection

Pathway ID	Pathway description	Proteins	FDR
Immune-related pathways			
hsa04664	Fc epsilon RI signaling pathway	11	3.18E-13
hsa04062	Chemokine signaling pathway	11	3.16E-09
hsa04660	T cell receptor signaling pathway	9	4.24E-09
hsa04662	B cell receptor signaling pathway	8	6.19E-09
hsa04620	Toll-like receptor signaling pathway	7	1.55E-06
hsa04668	TNF signaling pathway	7	2.01E-06
hsa04666	Fc gamma R-mediated phagocytosis	6	8.96E-06
hsa04650	Natural killer cell mediated cytotoxicity	6	5.68E-05
hsa04621	NOD-like receptor signaling pathway	4	0.000342
hsa04670	Leukocyte transendothelial migration	5	0.000428
hsa04622	RIG-I-like receptor signaling pathway	4	0.000707
Pathways related to infection			
hsa05169	Epstein-Barr virus infection	13	3.63E-11
hsa05160	Hepatitis C	9	3.60E-08
hsa05131	Shigellosis	7	4.14E-08
hsa05161	Hepatitis B	9	6.50E-08
hsa05145	Toxoplasmosis	8	1.91E-07
hsa05120	Epithelial cell signaling in Helicobacter pylori infection	6	2.01E-06
hsa05164	Influenza A	8	2.88E-06
hsa05152	Tuberculosis	8	3.38E-06
hsa05166	HTLV-I infection	9	5.57E-06
hsa05142	Chagas disease (American trypanosomiasis)	6	1.52E-05
hsa05162	Measles	6	7.35E-05
hsa05132	Salmonella infection	5	9.68E-05
hsa05133	Pertussis	4	0.000707

The results of this study highlight not only the potential polypharmacological nature of the *P. sidoides* compounds evaluated here but also the complexity of treating a disease like cancer. Cancer is a complex multi-step disease involving mutations and genomic instability leading to uncontrolled cell growth, resistance to cell death, angiogenesis, invasion, inflammation and immune evasion [72]. Over the years, drug discovery has moved from the idea of a "single drug single target" to a "single drug multiple targets" idea [73-75]. Although the polypharmacological nature of drug compounds may be viewed as an undesirable feature due to the possibility of adverse reactions, looking at the complexity of cancer as a disease this "single drug multiple targets" idea or "multiple drugs multiple targets" may be better suited to its therapeutic treatment. This is observed with the use of multi-kinase inhibitors like sunitinib, axitinib, lapatinib and sorafenib that target several protein kinases as well as combination therapy in cancer treatment strategies [74].

The majority of the compounds evaluated here were predicted to have more than 10 potential protein targets, with the exception being gallic acid, the trihydroxycoumarin as well as the bulky GCGCGC proanthocyanin trimer. The main type of protein targets of these compounds were the protein kinases that were found to be involved in Ras-MAPK and PI3K-AKT signaling pathways (Fig. 4). The *P. sidoides* compounds can potentially not only affect the activity of several upstream regulators of these two pathways Ras, Src, FAK, FGFR and BCR-ABL but also several of the downstream effectors of these two pathways. This is noteworthy because some of these downstream effectors can be activated by other proteins such as Protein Kinase C, JAK, cAMP and CDC42 (not shown, see KEGG pathways hsa04151 and hsa04010). This will thus lead to not only the attenuation of the Ras-MAPK and PI3K-AKT signaling pathways but also possibly the attenuation of other signaling pathways. Also, there is much cross-talk between the Ras-MAPK and PI3K-AKT signaling pathways and studies have shown the down regulation of the one pathway results in an upregulation of the other, thus the downregulation of both pathways may be needed for effective treatment strategies [76].

The importance of the potential polyphamacological nature of the *P. sidoides* compounds is particularly evident when looking as the tumor microenvironment. The Ras-MAPK and PI3K-AKT signaling pathways play not only a role in the proliferation and growth of the tumor cells but also the surrounding cells in the tumor environment like the endothelial cells, fibroblasts and cells of the immune system [77]. As an example, activation of these pathways by the binding of growth factors or cytokines to their respective receptor on the cell surface of the tumor cell will promote the proliferation and growth of the tumor cells [78]. The tumor cells also through the activation of these pathways will also result in the production and secretion of growth factors and cytokines that give important signals to the surrounding cells of the

tumor microenvironment. Upon the release of VEGF by the tumor cells and their subsequent binding to the VEGFR on endothelial cells and pericytes, the Ras-MAPK and PI3K-AKT pathways will be activated to stimulate the proliferation and survival of these cells as well as their migration through the activation of FAK and MAPK 14 (also both targets for the *P. sidoides* compounds) [57,79]. Thus, the *P. sidoides* compounds besides inhibiting the growth of the tumor cells through regulation of the PI3K-AKT and Ras-MAPK pathways may also inhibit the process of angiogenesis through the attenuation of the PI3K-AKT and Ras-MAPK pathways in the cells of the vascular system. The VEGF signaling pathway (hsa04370) was found to be enriched for the protein targets of the *P. sidoides* compounds (Table 1).

Similarly, the release of growth factors from the tumor cells can act on the surrounding cells of the extracellular matrix (ECM) like the fibroblasts and through the activation of the PI3K-AKT and Ras-MAPK pathways, promote their proliferation, survival, migration and subsequent ECM remodeling allowing for tumor migration [80]. Also, regulation of these two key pathways by the *P. sidoides* compounds should have an anti-inflammatory effect by inhibiting the release and production of inflammatory cytokines and growth factors from immune cells like the macrophages and neutrophils that are associated with the tumor microenvironment and drive tumorigenesis [62,77]. This is evident from the immune related pathways that were found to be enriched with the PI3K-AKT and Ras-MAPK protein targets of the *P. sidoides* compounds (Table 2).

Thus, through the collective regulation/inhibition of these protein targets by the bioactive compounds of *P. sidoides* that are involved in parallel and interlinked pathways and are important to several cell types found in the tumor microenvironment, a significant effect on not only tumor cell proliferation (as observed with our *in vitro* studies) but on the tumorigenesis process as a whole should be observed with *in vivo* studies. The *P. sidoides* compounds need not necessarily be strong binders of these protein targets but by potentially binding to and affecting the activity of multiple proteins and weakening their interactions with each other, the *P. sidoides* compounds should efficiently attenuate these signaling pathways resulting in reduced cancer cell proliferation, migration and evasion [73]. Also, by inhibiting several parallel signal transduction pathways, there may also be less of a chance of resistance development [74]. The polypharmacological nature of compounds and in particular phenolic compounds also suggest why these compounds show beneficial effects in the treatment of a wide variety of diseases. This is observed with green tea and its primary bioactive compounds the catechins that have been shown in previous studies to have beneficial effects in the prevention and treatment of diseases like cancer, Alzheimer's, cardiovascular diseases, diabetes and infections [35].

Conclusions

In this study, *in silico* target identification coupled with network pharmacology have provided important insights into the anti-proliferative activity of *P. sidoides* and further provided support for the bioactivity observed *in vitro*. The major protein targets identified were shown to be protein kinases involved in signal transduction events particularly those concerned with the Ras/MAPK and PI3K-Akt signaling pathways that have been found to be dysregulated in cancer. The results also provided insight into the anti-inflammatory and immunomodulatory activity that has been found *in vivo* to be associated with *P. sidoides* extracts. These results suggest that the anti-proliferative activity of *P. sidoides* may be multifactorial and arises from the collective regulation of several interconnected cell signaling pathways. It remains to be seen whether further *in vitro* and *in vivo* studies will reflect the *in silico* results presented here.

Acknowledgements

Declaration of interest

All authors declare no conflict of interest.

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