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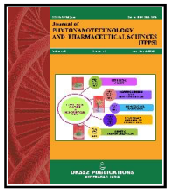

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Investigation of pharmaceutical properties and drug likeness score of punicic acid using bioinformatics tools

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Abstract

Punicic acid (PuA) is a type of conjugated linolenic acid (CLnA), commonly found in pomegranate seed oil, bitter gourd seed oil, and snake gourd seed oil. The aim of this study was to determine the molecular targets, *in silico* drug-like properties, and potential interactions of PuA through network-based pharmacological approaches. In this study, PuA was entered into the ChEBI and PubChem databases, and the possible interacting genes and proteins of PuA were predicted using DIGEP-Pred, Gene Cards, DisGeNET, and PharmGKB. The drug-likeness properties and toxicity characteristics of PuA were assigned using the SwissADME and ProToxII databases. Afterward, the STRING database and Cytoscape software were used to clarify the role of possible interacting proteins to create a protein-protein interaction (PPI) network. The KEGG enrichment database was also used for mapping pathways at the molecular level. The predicted pharmacological activities of PuA showed that it is an inhibitor of cyclooxygenase 1, phosphatidyl-glycerophosphatase and alkylacetylglycerophosphatase, protector of mucomembranous, antagonist of the hormone, scavenger of hydroxyls and free radicals, and regulator of lipid metabolism at (Pa)>0.7. According to the pharmacokinetic properties and drug-likeness analysis, PuA could be a potential drug candidate with avalue of –0.30, in addition to good brain barrier permeability and gastrointestinal absorption. Moreover, the results of toxicity analysis revealed that PuA did not cause any detectable toxicity with an LD_{50} of 3200 mg/kg. A total of 36 protein-codinggenes were identified as likely interacting targets of PuA, and PTGS2, IL6, PPARG, GSR, PPARA, PPARG, CAT, SLC2A4, CCL2, and GAPDH were selected as core targets in the PPI network (confidence score = 0.4). Based on the KEGG enrichment of pathways, a total of 129 different signaling pathways was identified as possible pathways regulated by PuA. Among them, the HIF-1signaling pathway, transcriptional dysregulation in cancer, AGE-RAGE signaling pathway in diabetic complications, FOXO signaling pathway, microRNAs in cancer, PI3K-Akt signaling pathway, thyroid hormone signaling pathway, the PPAR signaling pathway, and IL-17 signaling pathway were indicated as the major signaling pathways associated with PuA-regulated proteins (FDR<0.05). The preliminary results of this study support the beneficial effects of PuAon human health, including its antileukemia, anticancer, antiobesity, anti-inflammatory, antioxidant, and antineoplastic properties. Accordingly, the combination of PuA and network-based pharmacology has the potential to reveal the therapeutic and molecular mechanisms of PuA.

1. Introduction

Plant secondary metabolites are natural compounds produced by plants that are not involved in the primary functions of the plant, such as growth and development. These compounds have been found to have important medicinal properties and can be used in the treatment of various diseases. For example, some secondary metabolites have been found to have anti-inflammatory properties that can be useful in the treatment of conditions such as arthritis (Epifano *et al*., 2007; Velu *et al*., 2018; Gezici and Sekeroglu, 2019).

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Other secondary metabolites have been found to have anticancer properties, which can be used in the development of new cancer treatments. Accordingly, the study of plant secondary metabolites is an important area of research in the field of medicine, as it has the potential to lead to the development of new and effective treatments for a wide range of diseases (Hussein and El-Anssary, 2019; Sekeroglu and Gezici, 2019; Singh *et al*., 2021; Wu *et al*., 2022).

Punicic acid (PuA) is a type of conjugated linolenic acid (CLnA) found in various plant species. The most common plant sources of punicic acid are pomegranate seed oil, bitter gourd seed oil, and snake gourd seed oil belonging to the Punicaceae, Bignoniaceae, Rosaceae, Curcubitaceae, and Euphorbiaceae families. PuA is known for its anti-inflammatory, anticancer properties, antidiabetic, and antioxidant, making it a popular ingredient in natural medicine and supplement products. It has also been shown to improve lipid metabolism and help reduce the risk of cardiovascular disease by

reducing cholesterol levels and improving insulin sensitivity. There is also evidence to suggest that punicic acid may be beneficial in treating metabolic disorders such as obesity and type 2 diabetes (Bassaganya-Riera *et al*., 2011; Aruna *et al*., 2016; Khajebishak *et al*., 2019; Franczyk- arów *et al*., 2023).

Bioinformatics tools and network-based pharmacology are powerful tools for identifying and validating potential disease targets. Bioinformatics tools provide an integrated view of biological systems, allowing researchers to identify key pathways and genes that may be involved in disease. Network-based pharmacology is a field of research that involves analyzing the interactions between drugs and the molecules in the human body. Network-based pharmacology takes this analysis a step further by predicting how drugs may impact these biological networks. By combining bioinformatics and network-based pharmacology, researchers can identify novel disease targets and develop more effective treatments for a wide range of diseases (Berger and Iyengar, 2009; Oulas *et al*., 2019; Gezici, 2022). Researchers have used network-based pharmacology to investigate the potential therapeutic benefits of phytochemicals. By studying the interactions between phytochemicals and various proteins in the body, researchers have identified potential targets for drug development. This research could lead to the development of new treatments for a variety of health issues, including cancer, diabetes, neurodegenerative diseases, and cardiovascular disease. These tools have already been successfully applied in drug discovery for cancer, cardiovascular disease, and infectious diseases, and are likely to become even more important in the future as the amount of biological data continues to grow (Gezici and Sekeroglu, 2021a; Gezici and Sekeroglu, 2021b; Kumar *et al*., 2023).Thus, we aimed to determine the molecular targets, drug-likeness properties, and potential interactions of punicic acidusinga network-based pharmacology approach. The combination of punicic acid and network-based pharmacology has the potential to uncover the therapeutic and molecular mechanisms of punicic acid.

2. Material and Methods

2.1 Chemical structure and pharmacological properties

The Chemical Entities of Biological Interest (ChEBI) database, a part of ELIXIR Core Data Resources, was used asa dictionary of molecular entities and chemical properties of PuA (Hastings *et al*., 2016). PubChem database, a public chemical database at the National Center for Biotechnology Information (NCBI) of the National Library of Medicine (NLM), an institute within the U.S. National Institutes of Health (NIH), was used to obtain chemical structure and pharmacological properties of PuA, as well as the ChEBI database (Kim *et al*., 2016; Kim *et al*., 2023).

2.2 Pharmacokinetic properties and drug likeness analysis

Swiss ADME and ProToxII were used to determine drug-likeness possibilities and toxicity properties of PuA, respectively (Daina *et al*., 2017; Banerjee *et al.,* 2018; Daina *et al.,* 2019). The targets of punicic acid were identified using DIGEP-Pred (Prediction of drug-induced changes of gene expression profile) based on the structural formula of PuA (Lagunin *et al*., 2013).

2.3 Prediction of targets by gene set enrichment analysis

GeneCards, The Human Gene Database, was used to evaluate probable interacting genes of PuA. Based on this database, top interacting genes were analyzed using unique GeneCards identifiers (GC ids), provided by the GeneLoc Algorithm (Harel *et al*., 2009; Fishilevich *et al*., 2016). DisGeNET (version 7.0) database and the pharmacogenomics knowledge base (PharmGKB) were employed to reveal data about disease-associated genes and variants from multiple sources (Thorn *et al*., 2013; Piñero *et al*., 2020).

2.4 Construction protein-protein interaction (PPI) network

STRING database and Cytoscape software were used for visualization of the role of probable interacting genes and proteins associated with PuA. PPI network mapping was conducted on punicic acid and protein targets using the Retrieval of Interacting Genes database with the species limited to “homo sapiens” and a confidence score > 0.4 (Wu *et al*., 2009; Athanasios *et al*., 2017).

2.5 KEGG enrichment analysis

KEGG (Kyoto Encyclopedia of Genes and Genomes) is an integrated database of genes and genomes used for mapping pathways at the molecular level. KEGG enrichment analysis was performed for the construction network regulated by PuA (Aoki-Kinoshita and Kanehisa, 2007; Kanehisa *et al*., 2017).

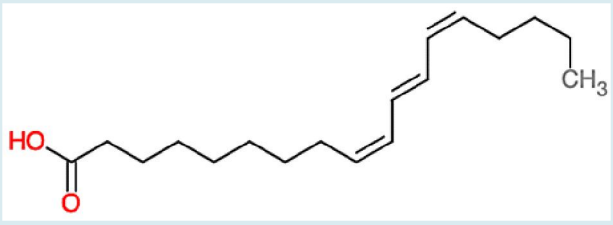
3. Results

3.1 Chemical compositions and prediction of drug-induced changes

Punicic acid is also known as trichosanic acid or pumicate with a molecular formula $C_{18}H_{30}O_2$ while its molar mass is 278.43 g/mol. PuA is a polyunsaturated fatty acid that is categorized as a conjugated linolenic acid, which is a polyunsaturated omega-6 and 18-carbon long fatty acid, with two CC double bonds at the 9- and 12-positions. Punicic acid is derived from linoleic acid (18:2Δcis9, cis12) by a fatty acid conjugate which converts a cis-Δ12 double bond into a conjugated trans-cis-double-bond system. The synonyms of PuA are trichosanoic acid, punicate, (9Z,11E,13Z)-octadeca-9,11,13-trienoic acid, cis-9,trans-11,cis-13-Octadecatrienoic acid, eleostearic acid, (9E,11Z,13E)-9,11,13-Octadecatrienoic acid, (e,Z,e)-9,11,13-Octadecatrienoic acid, 9-trans,11-cis,13-trans-Octadecatrienoic acid, 9t,11C,13t-CLN, 9t,11C,13t-Conjugated linolenic acid, 9trans,11-cis,13trans-Octadecatrienoic acid, C18:3 N-5 trans, 7 cis, 9 trans, Octadeca-9t,11C,13t-trienoic acid, Octadeca-9t,11C,13t-triensaeure, t9,C11,t13-CLN, t9,C11,t13-CLnA, t9,C11,t13-Conjugated linolenic acid, t9,C11,t13-Linolenic acid, and 9t,11C,13t-Linolenic acid. The chemical and molecular information of PuA obtained from ChEBI and PubChem were summarized in Table 1.

The prediction targets of PuA were identified using the prediction of drug-induced changes in gene expression profile of proteins at the pharmacological activity (Pa)>0.7. The findings regarding the prediction of drug-induced changes for PuA were summarized in Table 2 in which Pa (probability to be active) means the chance that PuA, whereas Pi (probability to be inactive) means the chance that PuA belongs to the subclass of inactive compounds. As presented in Table2, PuA was found to have remarkable biological activities including anticancer, antiobesity, antileukemic, antioxidant, anti-inflammatory, and antineoplastic. In fact, cyclooxygenase 1 inhibitor, hormone antagonist, phosphatidylglycerophosphatase inhibitor, alkylacetylglycerophosphatase inhibitor, mucomembranous protector, hydroxyls scavenger, metal chelator, and lipid metabolism regulatorwere determined as the most significant properties of PuA (Table 2).

Table 1: Chemical and physicochemical properties of PuA

IUPAC name	(9Z,11E,13Z)-octadeca-9,11,13-trienoic acid
ID	ChEBI: 8638 / PubChem CID: 5281126
Chemical structure	
Name	Punicic acid
Synonyms	punicate,trichosanoicacid,(9Z,11E,13Z)-octadeca-9,11,13-trienoic acid,octadeca-9*c*,11*t*,13*c*-trienoic acid,cis-9,trans-11,cis-13-Octadecatrienoic acid, 9-*cis*,11-*trans*,13-*cis*-octadecatrienoic acid
Formula	$C_{18}H_{30}O_2$
Net charge	0
MW	278.429 g/mol
Monoisotopic mass	278.224 g/mol
Hdon	1
Hacc	2
Rbon	13
Melting point	44-45°C / 491.91°C at 760.00 mm Hg (est)
WS	0.024 mg/ml at 25°C
InChI	1S/C18H30O2/c1-2-3-4-5-6-7-8-9-10-11-12-13-14-15-16-17-18(19)20/h5-10H,2-4,11-17H2,1H3,(H,19,20)/b6-5-,8-7+,10-9-
SMILES	CCCC\C=C/C=C/C=C\CCCCCCCC(O)=O
Canonical SMILES	CCCCC=CC=CC=CCCCCCCCC(=O)O
Isomeric SMILES	CCCC/C=C\C=C\C=C/CCCCCCCC(=O)O
Top chemical roles	Acid, ligand, catalyst, reducing agent
Top biological roles	Antileukemic, anticancer, antiobesity, anti-inflammatory, antioxidant, and antineoplastic agent

WS = water solubility, Hacc = hydrogen bond acceptors, Hdon = hydrogen bond donors, MW = molecular weight, Rbon = rotatable bonds.

Table 2: Prediction of drug induced changes of gene expression profile for PuA at pharmacological activity

Pa	Pi	Activity
0,977	0,001	CYP2J substrate
0,970	0,001	CYP2J2 substrate
0,951	0,001	Phosphatidylglycerophosphatase inhibitor
0,950	0,002	Acylcarnitinehydrolase inhibitor
0,949	0,003	Mucomembranous protector
0,943	0,002	Alkylacetylglycerophosphatase inhibitor
0,942	0,003	Antieczematic
0,939	0,001	All-trans-retinyl-palmitatehydrolase inhibitor
0,940	0,003	Alkenylglycerophosphocholine hydrolase inhibitor
0,935	0,003	Acrocylindropepsin inhibitor
0,935	0,003	Chymosin inhibitor

0,935	0,003	Saccharopepsin inhibitor
0,930	0,003	G-protein-coupled receptorkinase inhibitor
0,930	0,003	Beta-adrenergic receptorkinase inhibitor
0,925	0,002	Carboxypeptidase Taq inhibitor
0,923	0,002	Dextranase inhibitor
0,923	0,003	Lipid metabolism regulator
0,921	0,003	GST A substrate
0,915	0,001	CYP4A11 substrate
0,916	0,003	Lipoproteinlipase inhibitor
0,911	0,001	Leukotriene-B4 20-monooxygenase inhibitor
0,912	0,003	Linoleatediolsynthase inhibitor
0,910	0,002	Macrophagecolony stimulating factor agonist
0,912	0,004	Prostaglandin-E2 9-reductase inhibitor
0,909	0,004	Sugar-phosphatase inhibitor
0,909	0,005	Polyporopepsin inhibitor
0,905	0,003	Pullulanase inhibitor
0,904	0,003	Phosphatidylcholine-retinol O-acyl transferase inhibitor
0,901	0,004	Pro-opiomelanocort in converting enzyme inhibitor
0,898	0,002	CYP4A substrate
0,898	0,003	Sarcosineoxidase inhibitor
0,900	0,005	Phobicdisorders treatment
0,899	0,005	Ubiquinol-cytochrome-c reductase inhibitor
0,892	0,003	Glucan endo-1,3-beta-D-glucosidase inhibitor
0,892	0,003	Poly(alpha-L-guluronate) lyase inhibitor
0,892	0,005	Sphinganinekinase inhibitor
0,887	0,001	BRAF expression inhibitor
0,887	0,003	Methylamine-glutamate N-methyl transferase inhibitor
0,886	0,002	Xylan endo-1,3-beta-xylosidase inhibitor
0,886	0,003	Exoribonuclease II inhibitor
0,884	0,002	Glutarate-semialdehydede hydrogenase inhibitor
0,884	0,003	Phosphatidatephosphatase inhibitor
0,882	0,002	Poly(beta-D-mannuronate) lyase inhibitor
0,880	0,001	Ethanolamine-phosphatecytidylyl transferase inhibitor
0,878	0,002	Prostaglandin-A1 DELTA-isomerase inhibitor
0,873	0,003	D-lactaldehydedehydrogenase inhibitor
0,870	0,001	N-(long-chain-acyl)ethanol aminedeacylase inhibitor
0,869	0,002	Phenylacetate-CoAligase inhibitor
0,862	0,001	Plasmanylethanol aminedesaturase inhibitor
0,861	0,001	Linoleateisomerase inhibitor
0,863	0,003	Gluconate 5-dehydrogenase inhibitor
0,863	0,003	Peptide-N4-(N-acetyl-beta-glucosaminyl)asparagineamidase inhibitor
0,860	0,004	Levanase inhibitor

0,860	0,004	Lysine 2,3-aminomutase inhibitor
0,858	0,003	Alcoholdehydrogenase (NADP+) inhibitor
0,853	0,001	CYP4A2 substrate
0,856	0,005	Fucosterol-epoxidelyase inhibitor
0,852	0,004	Vasoprotector
0,848	0,002	Alcohol O-acetyl transferase inhibitor
0,844	0,004	IgA-specificmetalloendopeptidase inhibitor
0,844	0,004	Oxidoreductase inhibitor
0,841	0,004	Acetylesterase inhibitor
0,838	0,004	L-glucuronatereductase inhibitor
0,835	0,001	15-Hydroxyprostaglandin-D dehydrogenase (NADP+) inhibitor
0,837	0,004	Fatty-acyl-CoAsynthase inhibitor
0,844	0,013	Methylenetetrahydrofolatereductase (NADPH) inhibitor
0,835	0,006	Arginine 2-monooxygenase inhibitor
0,832	0,003	Aspartate-ammonialigase inhibitor
0,831	0,003	Antimutagenic
0,829	0,002	Plateletaggregation stimulant
0,828	0,003	CYP2E1 inhibitor
0,831	0,007	Protein-disulfidereductase (glutathione) inhibitor
0,836	0,012	Benzoate-CoAligase inhibitor
0,824	0,002	Pectinlyase inhibitor
0,835	0,014	Chlordeconereductase inhibitor
0,837	0,017	Testosterone 17beta-dehydrogenase (NADP+) inhibitor
0,822	0,002	Protein-tyrosinesulfo transferase inhibitor
0,819	0,004	Glucan 1,4-alpha-maltotriohydrolase inhibitor
0,818	0,003	Alkenylglycerophosphoethanolamine hydrolase inhibitor
0,816	0,003	N-formylmethionyl-peptidase inhibitor
0,817	0,005	Dimethylargininase inhibitor
0,814	0,006	Superoxidedismutase inhibitor
0,804	0,002	Peroxidasesubstrate
0,804	0,004	Phenol O-methyl transferase inhibitor
0,811	0,013	Mucositis treatment
0,799	0,004	HMOX1 expression enhancer
0,797	0,005	Cutinase inhibitor
0,793	0,004	Eyeirritation, inactive
0,793	0,004	Rhamnulose-1-phosphate aldolase inhibitor
0,791	0,003	GABA aminotransferase inhibitor
0,791	0,004	Lactase inhibitor
0,815	0,029	Aspulvinonedimethylallyl transferase inhibitor
0,787	0,002	Phosphatidylinositoldiacylglycerol-lyase inhibitor
0,791	0,007	Cl-transportingATPase inhibitor
0,785	0,002	Uroporphyrinogendecarboxylase inhibitor

0,787	0,003	Aminocarboxymuconate-semialdehydedecarboxylase inhibitor
0,787	0,004	Ecdysone 20-monooxygenase inhibitor
0,786	0,004	Reductant
0,795	0,014	Protein-glutamatemethylesterase inhibitor
0,785	0,008	NADPH-cytochrome-c2 reductase inhibitor
0,780	0,004	EndopeptidaseSo inhibitor
0,786	0,009	Fragilysin inhibitor
0,783	0,007	IgA-specific serine endopeptidase inhibitor
0,785	0,009	Fusarinine-C ornithinesterase inhibitor
0,777	0,003	Aminoacylase inhibitor
0,777	0,004	Anthranilate-CoAligase inhibitor
0,774	0,002	Flavin-containing mono oxygenase inhibitor
0,775	0,004	Leukopoiesis stimulant
0,786	0,015	Taurinedehydrogenase inhibitor
0,773	0,003	Gastrin inhibitor
0,774	0,004	Beta-carotene 15,15'-monooxygenase inhibitor
0,772	0,001	Guanidinobutyrase inhibitor
0,775	0,005	Electron-transferring-flavoproteindehydrogenase inhibitor
0,783	0,016	Glycosylphosphatidylinositolphospholipase D inhibitor
0,786	0,020	Gluconate 2-dehydrogenase (acceptor) inhibitor
0,774	0,007	Creatininase inhibitor
0,769	0,004	Preneoplasticconditions treatment
0,766	0,002	2,3,4,5-Tetrahydropyridine-2,6-dicarboxylate N-succinyl transferase inhibitor
0,770	0,006	CYP3A1 substrate
0,767	0,005	Lysostaphin inhibitor
0,770	0,008	Venombin AB inhibitor
0,776	0,014	Feruloylesterase inhibitor
0,766	0,005	Urethanase inhibitor
0,765	0,004	Allyl-alcoholde hydrogenase inhibitor
0,763	0,003	Hydroxylamineoxidase inhibitor
0,766	0,007	CYP2C8 substrate
0,761	0,004	Adenomatouspolyposis treatment
0,761	0,005	1,4-Lactonase inhibitor
0,758	0,002	Nitrilehydratase inhibitor
0,758	0,002	CYP2E1 inducer
0,755	0,001	Carboxy-cis,cis-muconatecyclase inhibitor
0,759	0,005	CYP2E1 substrate
0,756	0,003	Angiogenesis stimulant
0,758	0,005	NADH kinase inhibitor
0,758	0,005	Antisecretoric
0,757	0,006	Phosphatidylserinedecarboxylase inhibitor
0,756	0,005	Cholesterol antagonist

0,764	0,015	Glutamylendopeptidase II inhibitor
0,771	0,024	Antiseborrheic
0,750	0,003	Cytoprotectant
0,751	0,005	TNF expression inhibitor
0,749	0,003	Alaninetransaminase inhibitor
0,749	0,004	Aldehydedehydrogenase (NADP+) inhibitor
0,751	0,006	CYP2E substrate
0,747	0,003	Licheninase inhibitor
0,761	0,018	NADPH peroxidase inhibitor
0,746	0,004	Hydroxylaminereductase (NADH) inhibitor
0,745	0,002	Prostaglandin E1 antagonist
0,745	0,003	Dolichyl-phosphatase inhibitor
0,755	0,012	Ribulose-phosphate 3-epimerase inhibitor
0,745	0,003	Dolichyl-diphosphooligosaccharide-protein glycotransferase inhibitor
0,745	0,003	4-Hydroxybenzoate nonaprenyl transferase inhibitor
0,755	0,013	5-O-(4-coumaroyl)-D-quinate 3'-monooxygenase inhibitor
0,759	0,018	Membranepermeability inhibitor
0,752	0,012	Pseudolysin inhibitor
0,756	0,016	TP53 expression enhancer
0,745	0,007	Glutamine-phenylpyruvate transaminase inhibitor
0,740	0,004	Procollagen N-endopeptidase inhibitor
0,742	0,009	Polyamine-transportingATPase inhibitor
0,733	0,001	Leukotriene-C4 synthase inhibitor
0,735	0,003	1-Alkylglycerophosphocholine O-acetyl transferase inhibitor
0,736	0,005	Biotinidase inhibitor
0,733	0,004	CYP2C8 inhibitor
0,740	0,011	Arylsulfatesulfo transferase inhibitor
0,730	0,003	2-Oxoglutarate decarboxylase inhibitor
0,737	0,011	CYP2B6 substrate
0,731	0,005	Prenyl-diphosphatase inhibitor
0,730	0,004	2-Haloacid dehalogenase (configuration-inverting) inhibitor
0,729	0,003	Guanidinoacetase inhibitor
0,727	0,002	Creatinase inhibitor
0,727	0,004	4-Hydroxyglutamate transaminase inhibitor
0,727	0,004	Nitritereductase (NO-forming) inhibitor
0,728	0,007	Vasodilator, peripheral
0,723	0,004	Aspergillopepsin I inhibitor
0,721	0,003	Sclerosant
0,763	0,045	CYP2C12 substrate
0,720	0,002	Antiinflammatory, intestinal
0,720	0,003	Peptidoglycanglycosyl transferase inhibitor
0,732	0,016	Omptininhibitor

0,722	0,007	UGT1A9 substrate
0,720	0,005	Tprproteinase (Porphyromonasgingivalis) inhibitor
0,719	0,004	Protein-Npi-phosphohistidine-sugarphosphotransferase inhibitor
0,719	0,004	Cyclomaltodextrinase inhibitor
0,719	0,005	Coccolysin inhibitor
0,723	0,009	Radioprotector
0,716	0,002	CYP4B substrate
0,719	0,006	Trimethylamine-oxidealdolase inhibitor
0,722	0,009	Formaldehydetransketolase inhibitor
0,715	0,003	Plateletadhesion inhibitor
0,717	0,008	Methylumbelliferyl-acetatedeacetylase inhibitor
0,712	0,003	Catalase inhibitor
0,709	0,001	Cyclooxygenase 1 substrate
0,753	0,045	Membraneintegrityagonist
0,710	0,003	3-Oxoadipate enol-lactonase inhibitor
0,711	0,004	Galactolipase inhibitor
0,710	0,003	Carnosinesynthase inhibitor
0,712	0,006	3-Phytase inhibitor
0,707	0,002	Oxidizingagent
0,714	0,009	N-benzyloxycarbonylglycine hydrolase inhibitor
0,708	0,003	Phosphoenolpyruvate-protein phosphotransferase inhibitor
0,710	0,005	Shikimate O-hydroxycinnamoyl transferase inhibitor
0,717	0,013	2-Hydroxyquinoline 8-monooxygenase inhibitor
0,706	0,002	Thiosulfatesulfurtransferase inhibitor
0,705	0,002	(S)-2-hydroxy-acid oxidase inhibitor
0,706	0,004	Vitamin-K-epoxidereductase (warfarin-insensitive) inhibitor
0,706	0,004	Pyruvatedehydrogenase (lipoamide) inhibitor
0,705	0,003	Acyl-CoAhydrolase inhibitor
0,705	0,003	Palmitoyl-CoAhydrolase inhibitor
0,706	0,005	Arylmalonatedecarboxylase inhibitor
0,716	0,015	Peptidyl-dipeptidaseDcp inhibitor
0,702	0,002	Carnitine 3-dehydrogenase inhibitor
0,712	0,013	Phthalate 4,5-dioxygenase inhibitor
0,705	0,006	Phosphoinositide 5-phosphatase inhibitor
0,711	0,013	ProteasomeATPase inhibitor
0,702	0,004	Phosphatidylcholine-sterol O-acyltransferase inhibitor
0,701	0,004	2-Hydroxy-3-oxoadipate synthase inhibitor
0,703	0,008	Antithrombotic
0,713	0,018	Fibrinolytic
0,701	0,008	Antihypoxic
0,706	0,016	UDP-N-acetylglucosamine 4-epimerase inhibitor

3.2 Pharmacokinetic properties and drug-likeness analysis

The relevant drug-likeness scores obtained from SwissADME were detailed in Table 3. SwissADME predicted pharmacokinetics of the PuA, including topological polar surface Lipinski score, natural product score, gastrointestinal absorption, drug permeability, blood-brain barrier, plasma protein permeability, drug excretion long half-life, and clearance were shown in Table 3.

Table 3: Drug-likeness and chemical ADMET properties of PuA

DLS	-0.30
TPSA ($^{-2}$)	37.3 $^{-2}$
Lipinski	Yes
Bioavailability score	0.55
NPS	1.275
Glabsorpsion	High
Coco-2 Permeability	-4826
MDCK Permeability	4e-05
BBB permeant	Yes
PPB	99.26%
$T_{1/2}$	0.768
CL	4.689
MolLogP	6.39
MolLogS	-5.61 (in Log(moles/l)) 0.68 (in mg/l)
MolLogD	3.043

DLS = drug-likeness score, TPSA = topological polar surface area, NPS = natural product score, GI = gastrointestinal absorption,MDCK = mardin-darby canine kidney, BBB = blood-brain barrier,PPD = plasma protein permeability,$T_{1/2}$ = excretion long half-life, CL = clearance.

As shown in Figure 1, PuAis predicted to possess a good drug-likeness activity with a score of -0.30, as well as good brain barrier permeability (BBB score = 4.40) and gastrointestinal adsorption. In addition, LogP, an octaol-water partition coeffic ient and one of the important components of Lipinski's Rule of 5, was determined as 6.39 which means PuA can be an oral drug. *In silico* drug-likeness possibilities of PuAare given in Figure 1.

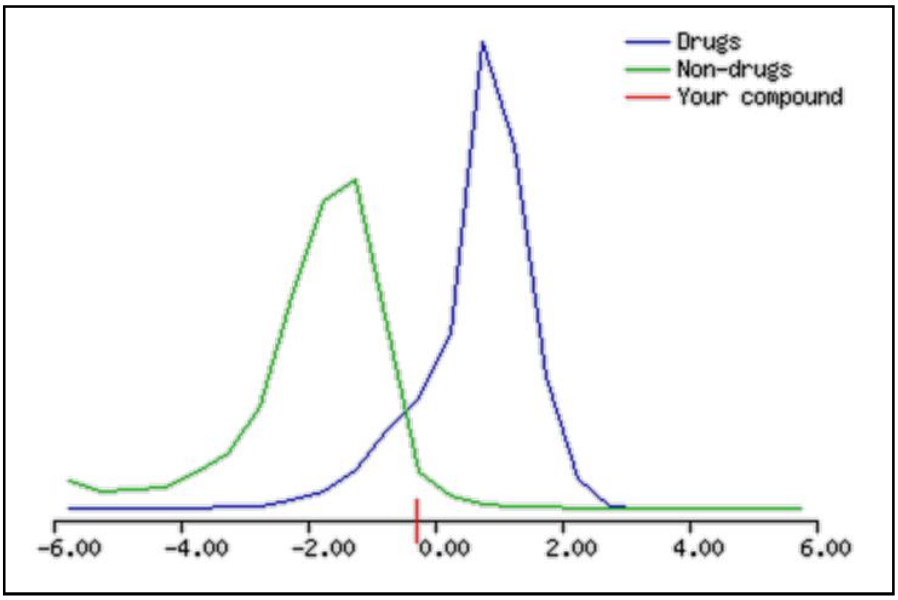


Figure 1: *In silico* drug-likeness model of PuA.

In silico toxicological parameters of PuA were evaluated using ProTox-II software, and the results are presented in Table 4. Oral toxicity prediction results were determined as LD_{50} (lethal dose) values in 3200 mg/kg body weight and the predicted toxicity class of PuA was 5 with 79.13% average similarity and %69.26 prediction accuracy. According to the globally harmonized system of classification of labeling of chemicals, the results showed that PuA has no observable toxicity, including carcinogenicity, cytotoxicity, hepatotoxicity, immunotoxicity, and mutagenicity (Table 4).

Table 4: Oral toxicity prediction results for PuA

Classification	Target	Prediction	Probability
Organ toxicity	Hepatotoxicity	Inactive	0.59
Toxicity end points	Carcinogenicity	Inactive	0.65
Toxicity end points	Immunotoxicity	Inactive	0.98
Toxicity end points	Mutagenicity	Inactive	0.93
Toxicity end points	Cytotoxicity	Inactive	0.70
Tox21-Nuclear receptor signaling pathways	Aryl hydrocarbon receptor (AhR)	Inactive	0.99
Tox21-Nuclear receptor signaling pathways	Androgen receptor (AR)	Inactive	1.0
Tox21-Nuclear receptor signaling pathways	Androgen receptor ligand binding domain (AR-LBD)	Inactive	1.0
Tox21-Nuclear receptor signaling pathways	Aromatase	Inactive	1.0
Tox21-Nuclear receptor signaling pathways	Estrogen receptor alpha (ER)	Inactive	0.99
Tox21-Nuclear receptor signaling pathways	Estrogen receptor ligand binding domain (ER-LBD)	Inactive	1.0
Tox21-Nuclear receptor signaling pathways	Peroxisome proliferator activated receptor gamma (PPAR-Gamma)	Active	0.59
Tox21-Stress response pathways	Nuclear factor (erythroid-derived 2)-like 2/antioxidant responsive element (nrf2/ARE)	Active	0.67
Tox21-Stress response pathways	Heat shock factor response element (HSE)	Active	0.67
Tox21-Stress response pathways	Mitochondrial membrane potential (MMP)	Inactive	0.99
Tox21-Stress response pathways	Phosphoprotein (Tumor Suppressor) p53	Inactive	0.99
Tox21-Stress response pathways	ATPase family AAA domain-containing protein 5 (ATAD5)	Inactive	1.0

3.3 Enrichment analysis of protein-based prediction

The gene targets of PuA were collected using Gene Cards, DisGeNET, Pharm GKB, and SMILES into Swiss target prediction. Based on the prediction results, a total of thirty-six genes were identified as the intersection targets. The detailed information and target class of the protein targets of PuA was given in Table 5.

Table 5: Protein based prediction results for PuA

Description	Symbol	UniProt ID	Protein class
ATP-Binding cassette sub-family G Member 1	ABCG1	P45844	transporter
Acetylcholine receptor subunit epsilon	ACHE	Q04844	transporter and hydrolase
Catalase	CAT	P04040	oxidoreductase and peroxidase
C-C Motif chemokine ligand2	CCL2	P13500	cytokine
Monocyte differentiation antigen CD14	CD14	P08571	transporter and receptor
CD83 antigen	CD83	Q01151	receptor
T-Lymphocyte activation antigen CD86	CD86	P42081	receptor
Collagen type I alpha-1 chain	COL1A1	P02452	metal-binding
Glyceraldehyde-3-phosphate dehydrogenase	GAPDH	P04406	oxidoreductase and transferase
Growthhormone 1 (somatotropin)	GH1	P01241	hormone
Glutathione disulfide reductase, mitochondrial	GSR	P00390	oxidoreductase

Hemoglobin subunit alpha	HBA1	P69905	transporter
DNA-Binding protein inhibitor ID-1	ID1	P41134	repressor
Interleukin-6	IL6	P05231	cytokine
Involucrin	IVL	P07476	transglutaminase and keratinocyte
E3 Ubiquitin-protein ligase MDM2	MDM2	Q00987	transferase
Matrix metalloproteinase 2	MMP2	P08253	protease
Nuclear receptor coactivator 2	NCOA2	Q15596	activator
Serum paraoxonase/arylesterase 1	PON1	P27169	hydrolase
Peroxisome proliferator-activated receptor alpha	PPARA	Q07869	nuclear receptor
Peroxisome proliferator-activated receptor delta	PPARD	Q03181	nuclear receptor
Peroxisome proliferator-activated receptor Gamma	PPARG	P37231	nuclear receptor
Protein kinase C alpha	PRKCA	P17252	kinase and transferase
Prostaglandin G/H synthase 1	PTGS1	P23219	oxidoreductase and peroxidase
Prostaglandin G/H synthase 2	PTGS2	P35354	oxidoreductase and peroxidase
RacFamily small GTPase 1	RAC1	P63000	hydrolase
Retinoic acid receptor alpha	RARA	P10276	nuclear receptor
P-Selectin	SELP	P16109	metal-binding
Solute carrier family 2, facilitated glucose transporter member 4	SLC2A4	P14672	transporter
Superoxide dismutase 1	SOD1	P00441	oxidoreductase
Serine-threonine kinase receptor-associated protein	STRAP	Q9Y3F4	kinase
Transferrin receptor protein 1	TFRC	P02786	receptor
TIMP Metalloproteinase inhibitor 1	TIMP1	P01033	protease
DNATopoisomeraseII alpha	TOP2A	P11388	isomerase
Vitamin D3 receptor	VDR	P11473	nuclea rreceptor
Vimentin	VIM	P08670	structural protein, cytoskeleton

3.4 Results of protein-protein interaction network

The relationship of a total of 36 proteins between each other was constructed from STRING database with PPI enrichment p-value< 1.0e-16 (FDR<0.05) and a confidence score < 0.4.This enrichment value means that these proteins have more interactions among themselves than what would be expected for a random set of proteins of the same size and degree distribution drawn from the genome (Figure 2).

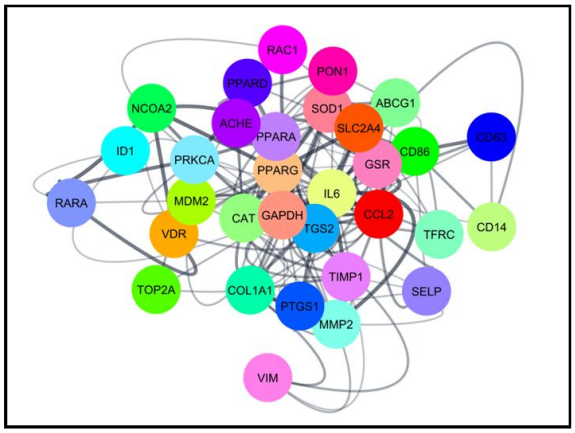


Figure 2: Protein-protein interaction networks of PuA.

As can be seen in the Figure 2, a higher degree value node represented significant targets of punicic acid. While PTGS2 (prostaglandin G/H synthase 2), IL6 (interleukin-6), PPARG (peroxisome proliferator-activated receptor gamma), GSR (glutathionedisulfide reductase), PPARA (peroxisome proliferator-activated receptor alpha), PPARG (peroxisomeproliferator-activated receptor gamma), CAT (catalase), SLC2A4 (solute carrier family 2, facilitated glucose transporter member4), CCL2 (C-C motif chemokine ligand2), and GAPDH (glyceraldehyde-3-phosphate dehydrogenase) were selected as core targets in the PPI network, it was determined that proteins such as GH1 (growth hormone1), HBA1 (hemoglobin subunit alpha), IVL (involucrin), and STRAP (serine-threoninekinase receptor-associated protein) have not interacted with other proteins in the network.

3.5 Results of KEGG enrichment pathway

According to the KEGG enrichment pathway analyses, a total of 129 distinct pathways were identified as the probably modulated pathways by PuA. A network corresponding to 36 protein targets is schematized in Figure 4, summarizing the correlations between the major pathways listed in the enrichment network.

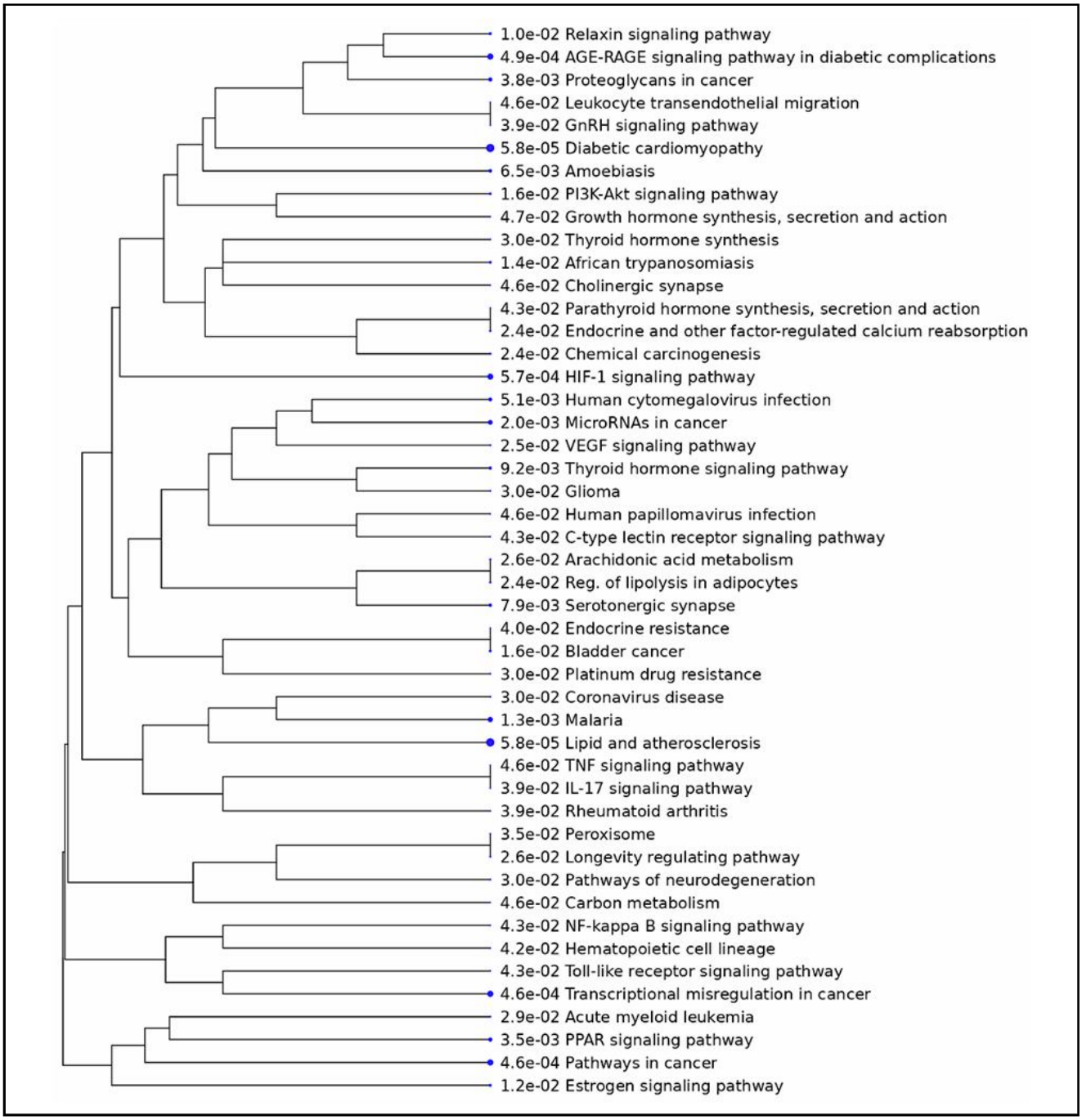


Figure 4: Network of top related signaling pathways construction with KEGG enrichment.

As presented in Figure 4, several target proteins are simultaneously involved in one pathway, while one target protein is also present in many pathways. Pathways in cancer, (FDR=0.00018), hypoxia-inducible factor 1 (HIF-1) signaling pathway (FDR=3.90e-05), transcriptional misregulation in cancer (FDR=0.00019), advanced glycation endproducts-receptor for advanced glycation end products (AGE-RAGE) signaling in diabetic complications (FDR=0.00020), fork head box O (FOXO) signaling pathway (FDR=0.00053), microRNAs in cancer (FDR=0.0013), human cytomegalo virus infection (FDR=0.0041), PI3K-Akt signaling pathway (FDR=0.0166), amoebiasis (FDR=0.0031), thyroid hormone signaling pathway (FDR=0.0047), peroxisome proliferator-activated receptors (PPAR) signaling pathway (FDR=0.0139), and interleukin-17 (IL-17) signaling pathway (FDR=0.0176) were detected as the top pathways associated with PuA-regulated proteins with the lowest false discovery rate (FDR<0.05) (Figure 4). Furthermore, nine of these proteins, including MMP2, RARA, MDM2, NCOA3, PPARG, PPARD, PTGS2, IL6, and PRKCA were found to involve in pathways in cancer, while the other groups of proteins including TIMP1, GAPDH, NCOA3, TFRC, IL6, PRKCA were found to involve in hypoxia-inducible factor 1 (HIF-1) signaling pathway.

4. Discussion

Punicic acid, a long-chain conjugated linolenic acid found naturally in pomegranate seed oil, bitter gourd seed oil, and snake gourd seed oil, has been shown to have anti-inflammatory and antioxidant properties. In recent years, numerous *in vivo* and *in vitro* studies have focused on health benefits of PuA and its potential role in the prevention and treatment of several diseases, including obesity, diabetes, metabolic syndrome, cardiovascular disease, cancer, and neurodegenerative disorders. Research has also suggested that PuA may play a role in modulating immune function and improving cognitive function. Additionally, studies have demonstrated that PuA may be beneficial for skin health by helping to reduce wrinkles and fine lines. The potential therapeutic effects of PuA are still being explored; however, these promising results suggest that it could have wide-ranging applications as a functional food ingredient or dietary supplement in the future (Aruna *et al*., 2016; Shabbir *et al*., 2017; Franczyk - Zarów *et al*., 2023).

In this study, the pharmacokinetic properties and toxicity of PuA were revealed using electronic databases. PuA compliance with Lipinski rule of 5, and with a higher LD_{50} value. Based on the literature survey, there is limited research available on the *in vivo* and *in vitro* toxicity of PuA. There have been several studies conducted on the *in vivo* and *in vitro* toxicity of PuA. *In vitro* studies have shown that punicic acid has anti-inflammatory and antioxidant properties, making it a potential therapeutic agent for various diseases. However, in vivo studies have reported mixed results regarding its toxicity. Some animal studies have suggested that high doses of PuA may cause damage to the liver and kidneys, while others have found no adverse effects even at high doses (Meerts *at el*., 2009; Bassaganya Riera *et al*., 2011; Holic *et al*., 2018). Studies have shown that PuA, a fatty acid found in pomegranate seed oil, exhibits low toxicity in both *in vivo* and *in vitro* studies. In an *in vivo* study conducted on rats, it was observed that even at high doses of PuA, there were no adverse effects on the animals' body weight or organ function. Similarly, *in vitro* studies on human cells have shown that PuA does not cause any significant cytotoxicity or genotoxicity. In fact, some studies suggest that PuA may even have potential health benefits due to its anti-inflammatory and antioxidant properties (Boroushaki *et al*., 2016; Mota Ferreira *et al*., 2016; Paul and Radhakrishnan, 2020). More research is needed to fully understand the potential benefits and risks of punicic acid, but so far, the evidence suggests that it may hold promise as a natural remedy for certain health conditions.

PPI analyses were conducted to determine top target proteins regulated by PuA and PTGS2, IL6, PPARG, GSR, PPARA, PPARG, CAT, SLC2A4, CCL2, and GAPDH were selected as core targets in the PPI network. In addition, target signaling pathways modulated by PuA were detected using KEGG enrichment and a total of 129 different signaling pathways was identified as possible pathways in this research. PuA is a bioactive compound and has been shown to have various health benefits by *in vivo* and *in vitro* studies. . In agreement with the findings from this network-based research, previous reports have also explored the molecular signaling pathways involved in the biological effects of PuA, including the inhibition of NF-kB, PI3K/Akt, and MAPK pathways. *In vitro* studies have further elucidated the mechanisms of PuA action, showing that it can induce apoptosis and cell cycle arrest in cancer cells (Mete *et al*., 2019; Franczyk-Zarów *et al*., 2023). In addition, research has shown that punicacid can modulate various molecular signaling pathways involved in the prevention of neurode generation, including reducing inflammation and oxidative stress *via* peroxisome proliferator-activated receptor (PPAR)s and high-densitylipoprotein (HDL) associated paraoxonase 1 (PON1), as well as promoting lipid metabolism and syna pticplasticity through calpains and glucose metabolism with glucose transporter type 4 (GLUT4). PuA has also been found to have anti-apoptotic effects, which can help prevent the death of neurons. Further more, studies have demonstrated that PuA can improve cognitive function in animal models of neurode generative diseases, such as Alzheimer's and Parkinson's disease (Shabbir *et al.,* 2017; Guerra-Vázquez *et al*., 2022).

The investigation of pharmaceutical properties and the drug-likeness score of PuA using bioinformatics tools is an important step towards understanding the potential of this compound as a drug candidate. The use of bioinformatics tools such as molecular docking, ADMET predictions, and drug-likeness score calculations helped to assess the feasibility of PuA as a drug molecule. The results of this investigation showed that PuA has good drug-likeness properties, including good solubility, low toxicity, and high bioavailability. Additionally, the network-based pharmacological studies revealed that PuA has a strong binding affinity towards certain target proteins, indicating its potential as a therapeutic agent. Overall, this study highlights the importance of using bioinformatics tools to evaluate the pharmaceutical properties of natural compounds like PuA and provides valuable insights into the potential use of this molecule in drug development. In other words, one way to assess the potential therapeutic benefits of PuA is through the use of drug signature score analysis. This method measures the similarity between the gene expression profiles of cells treated with PuA and those of cells treated with known drugs with established pharmacological properties. Studies have shown that PuA has a high drug signature score for agents that have antioxidant and anti-inflammatory properties, suggesting that it may have similar effects. However, more research is needed to fully understand the potential benefits and risks associated with the use of PuA and potential clinical applications.

5. Conclusion

In conclusion, the investigation of pharmaceutical properties and the drug-likeness score of PuA using bioinformatics tools has provided valuable insights into its potential as a drug candidate. The results of the study suggest that PuA has favorable pharmacokinetic properties and a high drug-likeness score, indicating that it may have good bioavailability, permeability, and metabolic stability in the body. Furthermore, the findings suggest that PuA may have potential therapeutic applications in the treatment of various diseases, including cancer, inflammation, and metabolic disorders. Overall, this study highlights the importance of using bioinformatics tools to identify potential drug candidates and underscores the need for further research to fully explore the pharmacological potential of PuA.

Conflict of interest

The authors declare no conflicts of interest relevant to this article.

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