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Network-based bioinformatics analyses on molecular pathways and pharmacological properties of oleuropein

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Abstract

Oleuropein, a valuable nutraceutical with powerful antioxidant, anticancer, anti-inflammatory, and cardioprotective properties, is the most abundant polyphenolic compound in olive leaf, fruits, and olive oil from the olive tree (*Olea europaea* L., Fam. Oleaceae). Biological activities and nutritional value of oleuropein have been previously identified; however, the molecular mechanisms of its action and pharmacological properties based on bioinformatics analyses have not been systematically revealed yet. In this work, network-based bioinformatics approached were implemented to evaluate targets of oleuropein in human genomes and proteomes, interacting genes of oleuropein and probable modulated pathways. The oleuropein was input into the ChEBI database, and the targets of its were predicted using DIGEP-Pred, and then, top interacting genes were identified by gene cards database. Afterward, STRING and KEGG enrichment database were used to construct a protein-protein interaction (PPI) network and molecular targeting pathway network, respectively. A total of 21 genes coding proteins, *i.e*., APP, BCHE, CAT, CCND1, CDKN1A, CREB1, CYP3A4, EPHX2, FASN, GPX1, MAPK1, MAPK14, MMP7, MMP9, NFKB1, NGF, OXA1L, PTGS2, PTGS2, SOD1, and VCAM1 affected by oleuropein were identified by gene set enrichment analysis. Further, multiple pathways including TNF signaling pathway, Kaposi sarcoma-associated herpesvirus infection, human cytomegalovirus infection, prostate cancer pathway, microRNAs in cancer, endocrine resistance, proteoglycans in cancer, and IL-17 signaling pathway were also determined to be regulated by oleuropein. Results indicated that oleuropein exhibits highly active pharmacological activity as a cholesterol antagonist, anti-inflammatory agent and hepatoprotective. This study provides the network-based scientific researches that will most likely be useful in screening biological, molecular and pharmacological properties of oleuropein for clinical application in the human diseases.

1. Introduction

Oleuropein is a secoiridoid type of phenolic compound that consists of three structural subunits, including a polyphenol (hydroxytyrosol), asecoiridoid (elenolic acid), and a glucose molecule. It abundantly presents in olive (*Olea europaea* Linn.), which commonly known as zaytoon and an essential component of Mediterranean diets. The content of oleuropein in olives varies according to the developmental stages and is divided in to three stages; first is growth phase in which accumulation of oleuropein occurs; second stage is green ripening stage in which a decrease in the oleuropein content occurs; and third is the black ripening stage where the oleuropein level is very low. In other words, the concentration of oleuropein decreases in many olive cultivars depending on the maturation of olives; however, it increases in the leaves of the olive trees during the same period, which may probably occur to protect against herbivores (Ahamad *et al.*, 2019; Sekeroglu *et al.*, 2020; Aggarwal *et al.*, 2021).

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Oleuropein, as well as olive oil, fruits and olive leaves, have gained a great interest in phytochemical and pharmacologic research, since oleuropein exerts beneficial biological effects such as antidiabetic, antiobesity, antioxidant, anticancer, antiatherogenic, hypolipidemic, antihypertensive, anti-inflammatory, cardioprotective, neuro-protective and hepatoprotective (Menendez *et al.*, 2008; Notarnicola *et al.*, 2011; Priore *et al.*, 2014; Hadrich *et al.*, 2016; Shi *et al.*, 2017; Das and Gezici, 2018; Sherif and Al-Gayyar, 2018; Zhang *et al.*, 2019).Thanks to wide range of biological and pharmacological properties of oleuropein, it has been used in traditional medicine from ancient times. It has also proven to reduce the risk of many diseases and conditions such as, oxidative stress, obesity, diabetes, cholesterol, cancer, hypertension, hepatitis B, cerebral hemorrhage, myocarditis, Alzheimer's, Parkinson, hemorrhagic, cystitis, hypothyroidism, and amyloid diseases (Corona *et al.*, 2007; Notarnicola, 2011; De Nicoló *et al.*, 2013; Carito *et al.*, 2015; Feng *et al.*, 2017; Castejan *et al.*, 2019; Zhang *et al.*, 2019; Gao *et al.*, 2020; Hsu *et al.*, 2021).

Although, studies have been conducted to reveal the biological activity of oleuropein, network-based molecular and pharmacological activities of oleuropein have not been proposed yet. Hence, we aimed to elucidate the probable interactions of oleuropein by gene-set enrichment and network pharmacology analyses to provide a new approach to uncover the therapeutic mechanisms of oleuropein

that will facilitate its future clinical applications in the treatment of diseases.

2. Materials and Methods

2.1 Chemical properties and targets

Chemical Entities of Biological Interest (ChEBI) database, a part of ELIXIR Core Data Resources, was used for dictionary of molecular entities and chemical properties of oleuropein (Hastings *et al.*, 2016). The targets of oleuropein were identified using DIGEP-Pred (Prediction of drug-induced changes of gene expression profile) based on structural formula of oleuropein (Lagunin *et al.*, 2013).

2.2 Gene set enrichment analysis

Gene cards, the human gene database, was used to determine probable interacting genes of oleuropein. Based on this database, top interacting genes were analyzed using unique gene cards identifiers (GC ids) and gene cards inferred cunctionality scores (GIFtS), provided by the gene loc Algorithm (Harel *et al.*, 2009; Fishilevich *et al.*, 2016).

2.3 Protein-protein interaction (PPI) analysis

STRING database was used to annotate the role of probable interacting genes and proteins associated with oleuropein. PPI network mapping was conducted on oleuropein 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).

2.4 KEGG pathway analysis

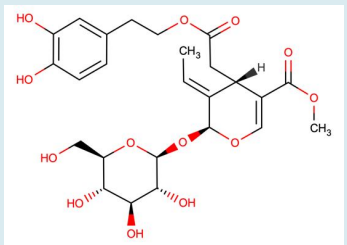
KEGG (kyoto encyclopedia of genes and genomes) is an integrated database of genes and genomes used for mapping pathways at molecular level. KEGG enrichment analysis was performed for construction the network regulated by oleuropein (Aoki-Kinoshita and Kanehisa, 2007; Kanehisa *et al.*, 2017).

3. Results

3.1 Chemical composition and molecular properties of oleuropein

Oleuropein ($C_{25}H_{32}O_{13}$) is a glycosylated secoiridoid that the most valuable phenolic compound found in the olive tree, *Olea europaea* L. This molecule is an ester of hydroxytyrosol and glucoside of elenolic acid with a molecular mass of 540.518 g/mol. The chemical structure and molecular identification of oleuropein are presented in the Table 1.

Table 1: Chemical structure and molecular properties of oleuropein

ID	ChEBI: 7747
Name	Oleuropein
Chemical structure	
Synonyms	dihydroxyphenyl)ethyl ester
Formula	$C_{25}H_{32}O_{13}$
Net charge	0
Average mass	540.518
Monoisotopic mass	540.18429
InChI	/m0/s1
SMILES	[H][C@]1(CC(=O)OCCC2=CC(O)=C(O)C=C2)\C(=C/C) [C@H](O[C@@H]2O[C@H](CO)[C@@H](O)[C@H](O) [C@H]2O)OC=C1C(=O)OC
Top chemical roles	Radical scavenger, antioxidant, antineoplastic agent
Top biological roles	Plant metabolite, antiinflammatory, antihypertensive, hepatoprrotective, cardiyoprotective, apoptosis inducer, NF-kappaB inhibitor

The targets of oleuropein were analyzed based on prediction of drug-induced changes of gene expression profile for proteins at the pharmacological activity (Pa) > 0.7. The findings were summarized in the Table 2. Pa (probability to be active) means the chance that oleuropein is belonging to the subclass of active compounds, whilst Pi (probability to be inactive) means the chance that oleuropein is belonging to the subclass of inactive compounds. According to the data presented in the table, oleuropein shows highly active pharmacological activity as a cholesterol antagonist, anti-inflammatory agent and hepatoprotectant.

Table 2: Prediction of drug-induced changes of gene expression profile for oleuropein

Pa	Pi	Activity
0,706	0,006	Chemopreventive
0,719	0,005	Lactase inhibitor
0,726	0,020	Anaphylatoxin receptor antagonist
0,739	0,006	Anti-thrombotic
0,754	0,006	Antihypercholesterolemic
0,788	0,005	Vasodilator
0,802	0,017	CYP2H substrate
0,810	0,016	Benzoate-CoA ligase inhibitor
0,832	0,004	Hepatoprotectant
0,869	0,016	CDP-glycerol glycerophosphotransferase inhibitor
0,899	0,004	Anti-inflammatory
0,933	0,002	Cholesterol antagonist

Table 3: Top interacting genes with oleuropein

Sl. No.	Symbol	Description	Category	GIFtS	GCid	Score
1	APP	Amyloid beta precursor protein	Protein Coding	50	GC21M025880	1.28
2	BCHE	Butyrylcholinesterase	Protein Coding	48	GC03M165772	0.21
3	CAT	Catalase	Protein Coding	48	GC11P034460	1.13
4	CCND1	Cyclin D1	Protein Coding	51	GC11P069641	1.44
5	CDKN1A	Cyclin dependent kinase inhibitor 1A	Protein Coding	48	GC06P061315	1.44
6	CREB1	CAMP responsive element binding protein 1	Protein Coding	48	GC02P207529	0.21
7	CYP3A4	Cytochrome P450 family 3 subfamily A member 4	Protein Coding	50	GC07M099759	0.21
8	EPHX2	Epoxide hydrolase 2	Protein Coding	47	GC08P027490	0.21
9	FASN	Fatty acid synthase	Protein Coding	48	GC17M082078	0.30
10	GPX1	Glutathione peroxidase 1	Protein Coding	46	GC03M049603	1.13
11	MAPK1	Mitogen-activated protein kinase 1	Protein Coding	51	GC22M021759	1.59
12	MAPK14	Mitogen-activated protein kinase 14	Protein Coding	50	GC06P061307	0.21
13	MMP7	Matrix metallopeptidase 7	Protein Coding	48	GC11M102425	1.52
14	MMP9	Matrix metallopeptidase 9	Protein Coding	52	GC20P046008	0.21
15	NFKB1	Nuclear factor kappa B subunit 1	Protein Coding	51	GC04P102501	1.44
16	NGF	Nerve growth factor	Protein Coding	49	GC01M115285	1.52
17	OXA1L	OXA1L mitochondrial inner membrane protein	Protein Coding	38	GC14P022766	0.21
18	PTGS1	Prostaglandin-endoperoxide synthase 1	Protein Coding	45	GC09P122370	1.13
19	PTGS2	Prostaglandin G/H synthase 2	Protein Coding	48	GC01M186640	0.21
20	SOD1	Superoxide dismutase 1	Protein Coding	50	GC21P031659	1.13
21	VCAM1	Vascular cell adhesion molecule 1	Protein Coding	44	GC01P100719	0.37

3.2 Top gene enrichment results

A total of twenty-one genes were identified as top interacting genes that are regulated by oleuropein in human. All the genes are protein coding genes and NGF, APP, CYP3A4, MAPK1, CCND1, NFKB1, SOD1, MAPK14, and MMP7 were found the most interacting genes, whereas OXAIL, VCAM1, PTGS, and GPX1 were defined as the least interacting genes with oleuropein. The list of top genes interacts with oleuropein was given in the Table 3.

3.3 Construction of protein-protein interaction (PPI) network

The relationship of a total of 21 proteins between each other were constructed from STRING database with FDR < 0.05 and p-value= 1.33e-13. According to the interaction network diagram, MAPK14, CREB1, NFKB1, NGF, MMP9, APP, and PTGS2 are located in the center of the network (Figure 1).

3.4 KEGG enrichment pathway analysis

The probably modulated pathways were determined regarding the KEGG pathway database. According to the KEGG analysis, 116 different pathways were identified corresponding to 21 protein targets. The pathways modulated by oleuropein were presented in the Table 4. As can be seen in the Table 4, several target proteins are simultaneously involved in one pathway, while one target protein is also present in many pathways. TNF signaling pathway, Kaposi sarcoma-associated herpesvirus infection, human cytomegalovirus

infection, hepatitis B, prostate cancer pathway, micro RNAs in cancer, human papillomavirus infection, endocrine resistance, proteoglycans in cancer, chemical carcinogens, pathways in neurodegeneration, hepatitis B, IL-17 signaling pathway and so forth were the top oleuropein-regulated pathways with the lowest false discovery rate (FDR<0.05), and screened in Figure 2.

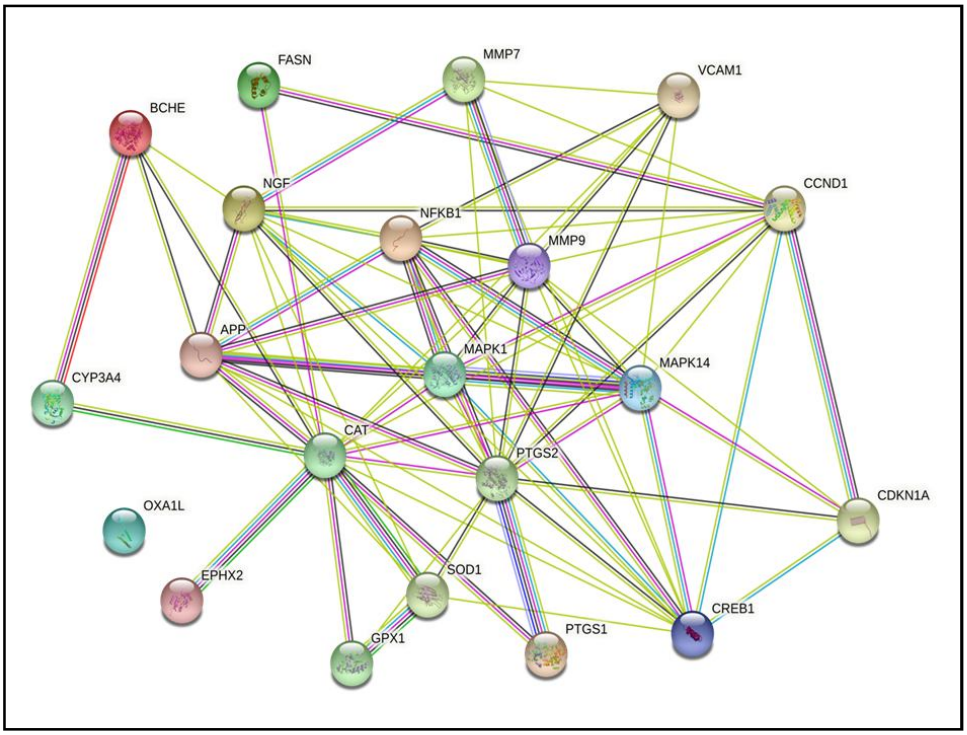


Figure 1: Protein-protein interaction of regulated proteins by oleuropein.

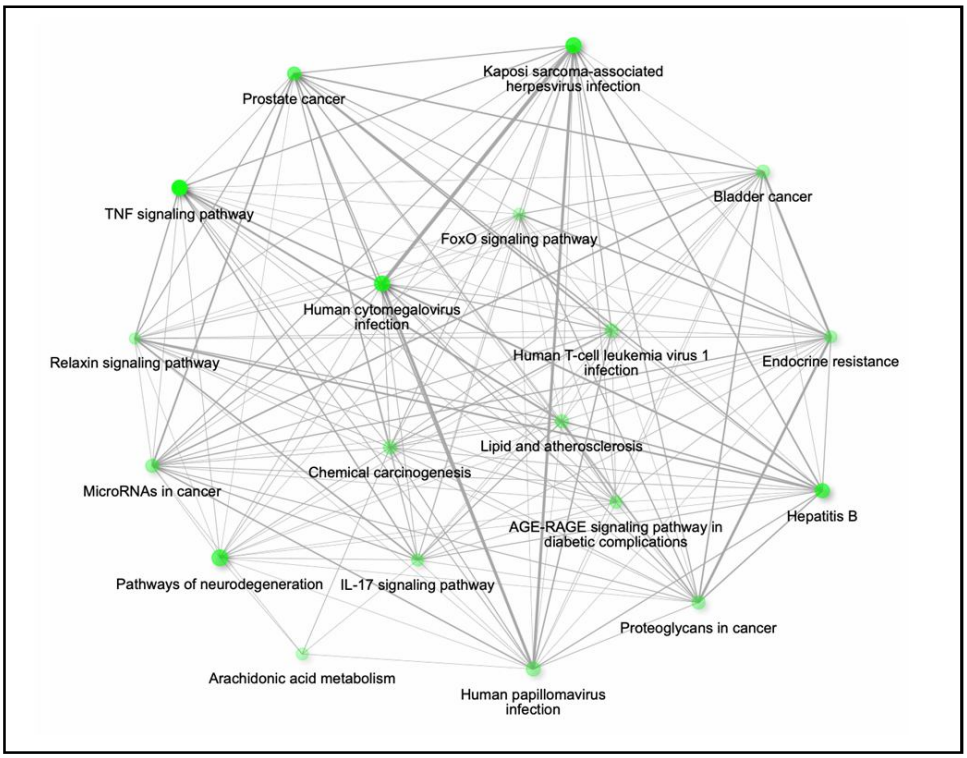


Figure 2: Top related pathways construction according to KEGG enrichment from STRING database.

Table 4: KEGG enrichment analysis of modulated proteins

#term ID	Term description	Observed gene count	Back ground gene count	Strength	False discovery rate	Matching proteins in the network (labels)
hsa04668	TNF signaling pathway	8	112	1.82	8.43e-09	MAPK1, NFKB1, MAPK14, VCAM1, PTGS2, MMP7, MMP9, CREB1
hsa05167	Kaposi sarcoma-associated herpesvirus infection	8	194	1.56	1.32e-07	CDKN1A, CREB1, MAPK14, FAS, NFKB1, MAPK1, PTGS2, CCND1
hsa05163	Human cytomegalovirus infection	8	224	1.5	1.86e-07	MAPK1, NFKB1, CCND1, MAPK14, PTGS2, CDKN1A, CREB1, FAS
hsa05161	Hepatitis B	7	161	1.57	1.04e-06	CDKN1A, CREB1, MAPK14, FAS, MMP9, NFKB1, MAPK1
hsa05215	Prostate cancer	6	97	1.79	1.32e-07	MAPK1, NFKB1, CCND1, MMP9, CDKN1A, CREB1
hsa05206	MicroRNAs in cancer	6	161	1.56	1.04e-06	MAPK1, NFKB1, CCND1, PTGS2, MMP9, CDKN1A
hsa04657	IL-17 signaling pathway	5	92	1.73	2.55e-06	MAPK1, NFKB1, MAPK14, PTGS2, MMP9
hsa01522	Endocrine resistance	5	95	1.71	2.60e-06	MAPK1, CCND1, MAPK14, MMP9, CDKN1A
hsa04933	AGE-RAGE signaling pathway in diabetic complications	5	98	1.7	2.68e-06	MAPK1, NFKB1, CCND1, MAPK14, VCAM1
hsa05166	Human T-cell leukemia virus 1 infection	6	211	1.44	2.68e-06	MAPK1, NFKB1, CCND1, MMP7, CDKN1A, CREB1
hsa05219	Bladder cancer	4	41	1.98	4.73e-06	MAPK1, CCND1, MMP9, CDKN1A
hsa04068	FoxO signaling pathway	5	127	1.59	6.97e-06	MAPK1, CCND1, MAPK14, CAT, CDKN1A
hsa04926	Relaxin signaling pathway	5	128	1.58	6.97e-06	MAPK1, NFKB1, MAPK14, MMP9, CREB1
hsa04218	Cellular senescence	5	150	1.51	1.33e-05	MAPK1, NFKB1, CCND1, MAPK14, CDKN1A
hsa05165	Human papillomavirus infection	6	325	1.26	2.14e-05	MAPK1, NFKB1, CCND1, PTGS2, CDKN1A, CREB1
hsa04917	Prolactin signaling pathway	4	69	1.75	2.33e-05	MAPK1, NFKB1, CCND1, MAPK14
hsa05140	Leishmaniasis	4	70	1.75	2.33e-05	MAPK1, NFKB1, MAPK14, PTGS2
hsa04151	PI3K-Akt signaling pathway	6	350	1.22	2.57e-05	MAPK1, NFKB1, CCND1, NGF, CDKN1A, CREB1
hsa05203	Viral carcinogenesis	5	182	1.43	2.57e-05	MAPK1, NFKB1, CCND1, CDKN1A, CREB1

hsa05212	Pancreatic cancer	4	73	1.73	2.57e-05	MAPK1, NFKB1, CCND1, CDKN1A
hsa05220	Chronic myeloid leukemia	4	75	1.72	2.57e-05	MAPK1, NFKB1, CCND1, CDKN1A
hsa05205	Proteoglycans in cancer	5	196	1.4	3.06e-05	MAPK1, CCND1, MAPK14, MMP9, CDKN1A
hsa05222	Small cell lung cancer	4	92	1.63	4.88e-05	NFKB1, CCND1,PTGS2,CDKN1A
hsa04625	C-type lectin receptor signaling pathway	4	102	1.58	6.95e-05	MAPK1, NFKB1, MAPK14, PTGS2
hsa04722	Neurotrophin signaling pathway	4	114	1.54	0.00010	MAPK1,NFKB1,MAPK14,NGF
hsa04380	Osteoclast differentiation	4	122	1.51	0.00013	MAPK1,NFKB1,MAPK14,CREB1
hsa05216	Thyroid cancer	3	36	1.91	0.00014	MAPK1,CCND1,CDKN1A
hsa05418	Fluid shear stress and atherosclerosis	4	130	1.48	0.00015	NFKB1,MAPK14,VCAM1,MMP9
hsa05200	Pathways in cancer	6	517	1.06	0.00016	MAPK1, NFKB1, CCND1, PTGS2, MMP9, CDKN1A
hsa04921	Oxytocin signaling pathway	4	149	1.42	0.00024	MAPK1,CCND1,PTGS2,CDKN1A
hsa04934	Cushing syndrome	4	153	1.41	0.00026	MAPK1,CCND1,CDKN1A,CREB1
hsa05160	Hepatitis C	4	156	1.4	0.00027	MAPK1,NFKB1,CCND1,CDKN1A
hsa05152	Tuberculosis	4	168	1.37	0.00034	MAPK1,NFKB1,MAPK14,CREB1
hsa04370	VEGF signaling pathway	3	57	1.71	0.00040	MAPK1,MAPK14,PTGS2
hsa05213	Endometrial cancer	3	57	1.71	0.00040	MAPK1,CCND1,CDKN1A
hsa00590	Arachidonic acid metabolism	3	61	1.68	0.00046	PTGS2,GPX1,EPHX2
hsa05169	Epstein-Barr virus infection	4	193	1.31	0.00052	NFKB1, CCND1, MAPK14, CDKN1A
hsa05221	Acute myeloid leukemia	3	66	1.65	0.00055	MAPK1,NFKB1,CCND1
hsa05223	Non-small cell lung cancer	3	68	1.64	0.00058	MAPK1,CCND1,CDKN1A
hsa05214	Glioma	3	72	1.61	0.00067	MAPK1,CCND1,CDKN1A
hsa05218	Melanoma	3	72	1.61	0.00067	MAPK1,CCND1,CDKN1A
hsa05133	Pertussis	3	74	1.6	0.00069	MAPK1, NFKB1, MAPK14
hsa04146	Peroxisome	3	79	1.57	0.00081	CAT, SOD1, EPHX2
hsa05210	Colorectal cancer	3	82	1.55	0.00088	MAPK1, CCND1, CDKN1A
hsa04211	Longevity regulating pathway	3	87	1.53	0.0010	NFKB1, CAT, CREB1
hsa04658	Th1 and Th2 cell differentiation	3	87	1.53	0.0010	MAPK1, NFKB1, MAPK14
hsa05235	PD-L1 expression and PD-1 checkpoint pathway in cancer	3	88	1.52	0.0010	MAPK1, NFKB1, MAPK14
hsa05020	Prion disease	4	265	1.17	0.0013	MAPK1, MAPK14, SOD1, CREB1
hsa04064	NF-kappa B signaling pathway	3	101	1.46	0.0014	NFKB1, VCAM1, PTGS2
hsa04066	HIF-1 signaling pathway	3	106	1.44	0.0014	MAPK1, NFKB1, CDKN1A
hsa04620	Toll-like receptor signaling pathway	3	101	1.46	0.0014	MAPK1, NFKB1, MAPK14
hsa04659	Th17 cell differentiation	3	101	1.46	0.0014	MAPK1, NFKB1, MAPK14
hsa04660	T cell receptor signaling pathway	3	101	1.46	0.0014	MAPK1, NFKB1, MAPK14
hsa04928	Parathyroid hormone synthesis, secretion and action	3	103	1.45	0.0014	MAPK1, CDKN1A, CREB1
hsa05142	Chagas disease	3	99	1.47	0.0014	MAPK1, NFKB1, MAPK14
hsa05145	Toxoplasmosis	3	105	1.45	0.0014	MAPK1, NFKB1, MAPK14
hsa04010	MAPK signaling pathway	4	288	1.13	0.0015	MAPK1, NFKB1, MAPK14, NGF

hsa04670	Leukocyte transendothelial migration	3	109	1.43	0.0015	MAPK14, VCAM1, MMP9
hsa04726	Serotonergic synapse	3	108	1.43	0.0015	MAPK1, APP, PTGS2
hsa04071	Sphingolipid signaling pathway	3	116	1.4	0.0017	MAPK1, NFKB1, MAPK14
hsa04935	Growth hormone synthesis, secretion and action	3	118	1.4	0.0018	MAPK1, MAPK14, CREB1
hsa04152	AMPK signaling pathway	3	120	1.39	0.0019	CCND1, FASN, CREB1
hsa05135	Yersinia infection	3	125	1.37	0.0021	MAPK1, NFKB1, MAPK14
hsa04210	Apoptosis	3	132	1.35	0.0024	MAPK1, NFKB1, NGF
hsa04915	Estrogen signaling pathway	3	133	1.34	0.0024	MAPK1, MMP9, CREB1
hsa05014	Amyotrophic lateral sclerosis	4	352	1.05	0.0028	MAPK14, CAT, SOD1, GPX1
hsa04261	Adrenergic signaling in cardiomyocytes	3	147	1.3	0.0029	MAPK1, MAPK14, CREB1
hsa04723	Retrograde endocannabinoid signaling	3	145	1.31	0.0029	MAPK1, MAPK14, PTGS2
hsa05010	Alzheimer disease	4	355	1.04	0.0029	MAPK1, NFKB1, APP, PTGS2
hsa05224	Breast cancer	3	145	1.31	0.0029	MAPK1, CCND1, CDKN1A
hsa05226	Gastric cancer	3	144	1.31	0.0029	MAPK1, CCND1, CDKN1A
hsa05225	Hepatocellular carcinoma	3	160	1.26	0.0037	MAPK1, CCND1, CDKN1A
hsa05202	Transcriptional misregulation in cancer	3	171	1.23	0.0044	NFKB1, MMP9, CDKN1A
hsa04621	NOD-like receptor signaling pathway	3	174	1.23	0.0045	MAPK1, NFKB1, MAPK14
hsa05130	Pathogenic Escherichia coli infection	3	187	1.2	0.0055	MAPK1, NFKB1, MAPK14
hsa04015	Rap1 signaling pathway	3	202	1.16	0.0067	MAPK1, MAPK14, NGF
hsa05170	Human immunodeficiency virus 1 infection	3	204	1.16	0.0068	MAPK1, NFKB1, MAPK14
hsa04024	cAMP signaling pathway	3	208	1.15	0.0071	MAPK1, NFKB1, CREB1
hsa05030	Cocaine addiction	2	49	1.6	0.0071	NFKB1, CREB1
hsa05132	Salmonella infection	3	209	1.15	0.0071	MAPK1, NFKB1, MAPK14
hsa05131	Shigellosis	3	218	1.13	0.0079	MAPK1, NFKB1, MAPK14
hsa04014	Ras signaling pathway	3	226	1.11	0.0086	MAPK1, NFKB1, NGF
hsa04213	Longevity regulating pathway - multiple species	2	61	1.51	0.0102	CAT, SOD1
hsa04664	Fc epsilon RI signaling pathway	2	66	1.47	0.0117	MAPK1, MAPK14
hsa05211	Renal cell carcinoma	2	66	1.47	0.0117	MAPK1, CDKN1A
hsa05120	Epithelial cell signaling in Helicobacter pylori infection	2	67	1.47	0.0118	NFKB1, MAPK14
hsa01524	Platinum drug resistance	2	70	1.45	0.0127	MAPK1, CDKN1A
hsa04622	RIG-I-like receptor signaling pathway	2	70	1.45	0.0127	NFKB1, MAPK14
hsa01100	Metabolic pathways	6	1447	0.61	0.0131	CAT, FASN, CYP3A4, PTGS2, GPX1, EPHX2
hsa04115	p53 signaling pathway	2	72	1.43	0.0131	CCND1, CDKN1A
hsa04918	Thyroid hormone synthesis	2	74	1.42	0.0135	CREB1, GPX1
hsa05204	Chemical carcinogenesis	2	75	1.42	0.0137	CYP3A4, PTGS2
hsa04662	B cell receptor signaling pathway	2	78	1.4	0.0146	MAPK1, NFKB1
hsa04012	ErbB signaling pathway	2	83	1.37	0.0162	MAPK1, CDKN1A
hsa05016	Huntington disease	3	298	0.99	0.0162	SOD1, CREB1, GPX1
hsa04912	GnRH signaling pathway	2	89	1.34	0.0182	MAPK1, MAPK14

hsa04713	Circadian entrainment	2	92	1.33	0.0192	MAPK1, CREB1
hsa04750	Inflammatory mediator regulation of TRP channels	2	94	1.32	0.0198	MAPK14, NGF
hsa04914	Progesterone-mediated oocyte maturation	2	94	1.32	0.0198	MAPK1, MAPK14
hsa04916	Melanogenesis	2	95	1.31	0.0198	MAPK1, CREB1
hsa04931	Insulin resistance	2	107	1.26	0.0245	NFKB1, CREB1
hsa04725	Cholinergic synapse	2	110	1.25	0.0256	MAPK1, CREB1
hsa04919	Thyroid hormone signaling pathway	2	119	1.22	0.0295	MAPK1, CCND1
hsa04110	Cell cycle	2	120	1.21	0.0297	CCND1, CDKN1A
hsa04114	Oocyte meiosis	2	120	1.21	0.0297	MAPK1, MAPK14
hsa04611	Platelet activation	2	122	1.21	0.0300	MAPK1, MAPK14
hsa04728	Dopaminergic synapse	2	128	1.18	0.0326	MAPK14, CREB1
hsa04371	Apelin signaling pathway	2	131	1.17	0.0337	MAPK1, CCND1
hsa04910	Insulin signaling pathway	2	133	1.17	0.0344	MAPK1, FASN
hsa05162	Measles	2	138	1.15	0.0366	NFKB1, CCND1
hsa04550	Signaling pathways regulating pluripotency of stem cells	2	140	1.15	0.0372	MAPK1, MAPK14
hsa05034	Alcoholism	2	144	1.13	0.0389	MAPK1, CREB1
hsa04310	Wnt signaling pathway	2	154	1.1	0.0438	CCND1, MMP7
hsa04630	JAK-STAT signaling pathway	2	160	1.09	0.0467	CCND1, CDKN1A
hsa04022	cGMP-PKG signaling pathway	2	162	1.08	0.0473	MAPK1, CREB1
hsa05164	Influenza A	2	165	1.07	0.0486	MAPK1, NFKB1

2. Discussion

Network-based approaches is an emerging discipline to elucidate potential molecular mechanisms and pharmacological properties of natural compounds (Feng *et al.*, 2017; Yang *et al.*, 2020). Hence, target genes, proteins, and molecular pathways modulated by oleuropein were identified in this research, and twenty-one genes were determined as top interacting genes which are regulated by oleuropein in human genome. Oleuropein causes an increase in some of these genes, while it causes a decrease in some of them. For instance, oleuropein increases the activity of CAT genes, which encodes catalase, a key enzyme related to oxidative stress and immune system. Further, CAT protein co-treated with SOD1 protein inhibits the reaction of superoxide, resulting in increased response to oxidative stress and cell death. Therefore, oleuropein may behave as a powerful antioxidant, immune stimulator and cancer preventive agent (Shi *et al.*, 2017; Modi *et al.*, 2019; Guo *et al.*, 2020). NGF is another important gene that encodes a secreted protein with nerve growth stimulating activity and its expression level is induced by oleuropein. The expression level of NGF, associated with neuropathy, hereditary sensory and corneal ulcer, is induced by oleuropein (De Nicoló *et al.*, 2013; Carito *et al.*, 2015).

On the contrary, oleuropein suppresses activity of MAPK1 and MAPK14 proteins, and also promotes the reaction of TNF protein which are results in increased apoptotic process, as well as decreased cell proliferation (Feng *et al.*, 2017; Castejon *et al.*, 2019). It can be clearly conducted that oleuropein play significant roles as a valuable anticancer agent and inflammatory mediator. Likewise, NFKB1, a pleiotropic transcription factor, is the endpoint of a series of signal transduction events such as inflammation, immunity, differentiation, cell growth, tumorigenesis and apoptosis (Aggarwal *et al.*, 2021). In addition, oleuropein reduces the levels of FASN genes, encoding a multifunctional protein involved in the anabolic conversion of dietary carbohydrates to fat in mammals. Increased expression of this protein is found to be associated with fatty liver disease and non-alcoholic fatty liver disease, which supports the hypocholesterolemic effect of oleuropein as a cholesterol antagonist (Priore *et al.*, 2014; Hadrich *et al.*, 2016). Besides hypocholesterolemic effect, oleuropein have been shown to induce anticancer effects by suppressing FASN protein expression (Menendez *et al.*, 2008; Notarnicola *et al.*, 2011). The expression of CREB1 is significantly reduced by oleuropein, that contribute to decrease the expressions of genes associated with thermogenesis, mitochondrial biogenesis, and oxidative phosphorylation. Inhibition of CREB activity resulted in inhibition of cell proliferation (Corona *et al.*, 2007; Gao *et al.*, 2020).

Similarly, expression level of MMP-7 and MMP-9 are downregulated by oleuropein that resulted in increased cellular response to reactive oxygen species, and decreased in cell migration. These MMP proteins are involved in the breakdown of extracellular matrix in normal physiological processes, such as embryonic development, reproduction, and tissue remodeling, as well as in disease processes, such as arthritis and metastasis (Feng *et al.*, 2017; Sherif and Al-Gayyar, 2018). VCAM1 is another protein that the expression levels are downregulated by oleuropein, which inhibits the reaction of

lipopolysaccharides, tetradecanoylphorbol acetate, and tumor necrosis factor α (TNF-α) protein results in increased expression of VCAM1 protein. Oleuropein suppresses the endothelial adhesion molecule expression, thus revealing the notable antioxidant, anticancer and anti-inflammatory capacities of oleuropein (Turner *et al.*, 2005; Dell'Agli *et al.*, 2006).

Based on the PPI network analyses, MAPK14, CREB1, NFKB1, NGF, MMP9, APP, and PTGS2 are determined as the core proteins. Among them, MAPK14 (mitogen-activated protein kinase), also known as CSBP, CSBP1, CSBP2, CSPB1, EXIP, Mxi2, PRKM14, PRKM15, RK, SAPK2A, p38, p38ALPHA, is serin-threonine kinase protein, which is a significant component of the MAP kinase signal transduction pathway evoked by extracellular stimuli such as proinflammatory cytokines or physical stress leading to direct activation of transcription factors. CREB1 is a basic leucine zipper protein involved in different cellular processes including the synchronization of circadian rhythmicity and the differentiation of adipose cells. NFKB1 and NGF play roles as transcription factor, of which NFKB1 is involved in NF-kappa-B signaling pathway and stimulus related to many biological processes including inflammation, immunity, differentiation, cell growth, tumorigenesis and apoptosis, whilst NGF is involved in nerves system and act important roles for the development and maintenance of the sympathetic and sensory nervous systems. PTGS2, also named as cyclooxygenase-2, converts arachidonate to prostaglandin H2 that is responsible for production of inflammatory prostaglandins, which is closely related to increased cell adhesion, phenotypic changes, resistance to apoptosis and tumor angiogenesis in cancer. Finally, MMP9, a metalloproteinase protein located in extracellular matrix, is associated with pathways in inflammation, cancer and neurodegeneration (Sherif and Al-Gayyar, 2018; Cuyàs *et al.*, 2019; Ganeshpurkar *et al.*, 2019; Zhang *et al.*, 2019; Gao *et al.*, 2020).

In addition, target pathways modulated by oleuropein were identified in the presented research. It is well-acknowledged that multiple signaling pathways interact with each other in the metabolic processes normally occurred in living organisms. According to the KEGG pathway enrichment, most of the genes modulated by oleuropein are closely related to oxidative stress, immune system and neurodegeneration, as well as cancer. Among the top oleuropein-regulated pathways, MAPK1, NFKB1, MAPK14, VCAM1, PTGS2, MMP7, MMP9, CREB1 genes are involved in TNF signaling pathway, which are related to regulate wide range of intracellular signal pathways including apoptos is and cell survival as well as inflammation and immunity. The genes of MAPK1, NFKB1, CCND1, CDKN1A, CREB1, MAPK14, VCAM1, PTGS2, MMP7, and MMP9, whose expressions are regulated by oleuropein, are involved in cancer-related signaling pathways including, apoptosis, cell cycle, cell death, cellular proliferation, and metastasis. Consistent with the findings from this bioinformatics-based study, previous reports show that dietary oleuropein suppresses cancer-related pathways such as Janus kinase/signal transducer and activator of transcription (JAK/STAT), nuclear transcription factor-kappa B (NF-βB), mitogen-activated protein kinases (MAPKs), and inflammasome nucleotide-binding domain, leucine-rich repeats-containing family, pyrin domain-containing-3 (NLRP3) signaling pathways (Ratan *et al.*, 2017; Castejon *et al.*, 2019; Hsu *et al.*, 2021; Mounika and Hymavathi, 2021). On the other hand, oleuropein has also participated in fatty acid synthesis pathways that demonstrated by previous studies (Priore *et al.*, 2014; Hadrich *et al.*, 2016). As revealed in previous studies, oleuropein has been demonstrated to be an important pharmaceutical resource for drug targets, in agreement with these findings from network-based molecular and pharmacological analyzes.

3. Conclusion

Oleuropein is a promising phenolic compound that predominantly obtained from *O. europaea* (olive tree), and proven to have significant biological activities in the human body. TNF signaling pathway, micro RNAs in cancer, IL-17 signaling pathway, Kaposi sarcoma-associated herpesvirus infection, human cytomegalovirus infection, hepatitis B, prostate cancer pathway, endocrine resistance, proteoglycans in cancer, and neurodegenerative signaling pathways involved in cell proliferation, oxidative stress, metastasis, apoptosis, neurodegeneration, fatty acid synthesis, and catabolic processes were defined as the top pathways regulated by oleuropein. Taken together, MAPK1, NFKB1, MAPK14, VCAM1, NGF, PTGS2, MMP7, MMP9, and CREB1 are main core proteins involved in top the signaling pathways, and these proteins and targets may be the key points of the therapeutic potentials of oleuropein. Based on our network pharmacological analysis, oleuropein may exert a wide range of pharmacological effect *via* multiple targets, pathways, and biological processes, thereby regulating the metabolism. Further studies are required to verify the clinical efficacy of oleuropein and its mechanisms of action.

Conflict of interest

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

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