Chemical-Protein and Protein-Protein Interaction Network of Vanillin from Clove: Functional and Pathway Analysis in *Homo sapiens*

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Posted Date: 27 March 2025

doi: 10.20944/preprints202503.2090.v1

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Article

Chemical-Protein and Protein-Protein Interaction Network of Vanillin from Clove: Functional and Pathway Analysis in *Homo sapiens*

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Abstract: Clove is an ancient spice with powerful medicinal characteristics that may treat a wide range of diseases while also controlling beneficial effects on the body. Its bioactive component, vanillin (4-hydroxy-3-methoxybenzaldehyde), has a beneficial effect on human health. This study aims to identify proteins that interact with vanillin in order to predict their possible impacts on the human body. We retrieved 99 protein sequences from the STITCH, STRING, UniProtKB, and NCBI databases and interacted with them, resulting in 49 sequences after the BLAST search. A functional annotation research revealed both positive and negative regulatory mechanisms important for the maintenance of biological processes, molecular activities, and cellular components in human health. Pathway analysis indicated that these compounds play significant roles in KEGG and Reactome-related pathways. Furthermore, the vanillin molecule possesses anticancer, antioxidant, antisickling, antimicrobial, and anti-inflammatory activities that suppress a variety of genetic diseases, neurological diseases, and cancers. Consequently, this research will aid in the identification of therapeutic-related protein interactions and signaling pathways, as well as the development of medications containing vanillin.

Keywords: clove; vanillin; functional analysis; pathway analysis

1. Introduction

A medium-sized tree (8–12 m) belonging to the Myrtaceae family, *Syzygium aromaticum* (synonym: Eugenia cariophylata) is a common phenolic plant, spice, and more specifically essential oils (EOs), which have a high content of bioactive compounds and are commonly known as clove(Cortés-Rojas, de Souza, et al., 2014). It is native to the Maluku Islands in east Indonesia and can be grown across the world, including Brazil in the state of Bahia, Madagascar (Ullah & Hamza, 2023). One of the most precious spices, cloves have been used for centuries as a food preservation agent, for therapeutic use, and as an essential oil (EO) in the food, cosmetics, pharmaceutical, biomedical, and sanitary sectors(Cav, Roda Rita, Amaury Taboada Rodriguez, 2021). One of the most efficient sources of phenolic compounds, comprising eugenol, vanillin, gallic acid, eugenol acetate, β-caryophyllene, and several others, contains cloves.

Vanillin (4-hydroxy-3-the methoxybenzaldehyde) is the most widespread phenolic compound isolated from cloves(Kamat et al., 2000). It has substantial antimutagenic and anticarcinogenic capabilities that alleviate the proliferation of malignancies in the colon that are triggered by multiple agents in rat models. Many researchers have been investigating vanillin's antimutagenic qualities during the past few decades(Akagi et al., 1995; Imanishi et al., 1990). Vanillin's antimutagenic role in mutagenesis generated by 4-nitroquinoline 1-oxide, furylfuramide, captan, or methylglyoxal in Escherichia coli was initially demonstrated back in 1986 (Tai et al., 2011). A diet containing considerable amounts of vanillin, which has anticancer and antioxidant properties, may lessen the development of cancer via free radicals (Tai et al., 2011). Human health will be adversely affected by high quantities of vanillin consumed from meals and beverages(Tai et al., 2011). Both in vitro and in

vivo, vanillin was said to be lowering X-ray or UV light-induced mutations in mammalian cells(Imanishi et al., 1990). Reactive oxygen species (ROS) scavenging, error-prone SOS repair inhibition, non-homologous DNA end-joining inhibition, and recA-dependent recombinational repair-enhancing protein have all been proposed as reasons for vanillin's antimutagenic properties(K. L. Ho et al., 2009). In order to cure sickle cell anemia (HBS), vanillin boasts anti-sickling properties(Arya et al., 2021). High-performance liquid chromatography was used to find that vanillin covalently comprises sickle hemoglobin (HBS) in red blood cell solutions(Abraham et al., 1991).Vanillin facilitated insulin glycation and amyloid aggregation for causing neurodegenerative disease and nephropathy, neuropathy, and retinopathy diabetes, and additionally it demonstrated cytoprotective and antioxidant actions against Advanced Glycation End Products (AGE) and Non-Enzymatic Glycation (NEG)(Awasthi & Saraswathi, 2016). Vanillin reveals neuroprotective properties in animals, including ischemia and Huntington's disease (HD)(Gupta & Sharma, 2014a). It also has a substantial impact on learning memory and locomotory motor coordination in rats with 3-nitropropionic acid (3NPA)-induced HD(Gupta & Sharma, 2014a). Vanillin shows natural wound healing features, and its chitosan hydrogel based on Schiff base and hydrogen connection between them, and additionally the chitosan-vanillin membrane, have been shown recently for promoting angiogenic stimulation, collagen deposition, and re-epithelialization in tissue engineering(Xu et al., 2018). Vanillin has applications in the cosmeceutical industry due to its aroma and antioxidant features, which have played an important part in skin renewal and healing processes(Taboonpong et al., 2017). Vanillin blocked the production of pro-inflammatory cytokines and UV-B-induced phosphorylation of ataxia telangiectasia mutants (ATM), serine-threonine kinase checkpoint kinase 2 (Chk2), tumor suppressor protein 53 (p53), and p38/mitogen-activated protein kinase (p38), leading to therapeutic benefits and antioxidant effects(Lee et al., 2014).

Bioinformatics has historically given important tools for evaluating these types of investigations. The Biochemical Interactions Indexing Engine and the Protein Interactions Database Search Engine have notable manuals on chemical-protein and protein-protein interaction systems, making it straightforward to figure out the adverse effects of Vanillin. STITCH is a web-based service that analyzes the interactions between chemicals and proteins inside particular organisms via testing and methodical scraping (Kuhn et al., 2014). STRING 12.0 is a web server for conserving and organizing protein-protein interactions, particularly biological and physical(Szklarczyk et al., 2023). Furthermore, Omicsbox has been utilized for analyzing genomic and proteomic data, discovering correlations between genetic sequences in biological functions, molecular activities, and cellular components, and identifying pathways utilizing resources such as KEGG and Reactome. Understanding whether human proteins interact with the naturally occurring substance vanillin, as well as showcasing the biological, cellular, and molecular functions of all of these proteins and the beneficial and adverse consequences of vanillin, are among the intended objectives of this study.

2. Methods and Materials

2.1. Network Retrieval

In the current study, Vanillin interacts with human (*Homo sapiens*) proteins compiled from the STITCH 5.0 (http://stitch.embl.de) internet server, as described by Ali et al. Vanillin attaches to human proteins and is then utilized for detecting protein-protein interactions. Use STRING to get all known human protein linkages from both direct (physical) and indirect (functional) sources.

2.2. Sequencing Assortment

Protein-protein interactions have been highlighted in the table, which was used to generate protein sequences by utilizing the FASTA and UniProt (https://www.uniprot.org/) databases. During the sequence collecting procedure, redundant sequences were discarded and all acquired protein sequences have been assembled in a single FASTA file for additional analysis.format retrieved from the NCBI protein database (https://www.ncbi.nlm.nih.gov/protein/).

2.3. Functional and Pathway Analysis

OmicsBox 3.2 is bioinformatics software used to evaluate NGS data from genomes, transcriptomes, and metagenomes. OmicsBox software has been employed to execute biological annotation and pathway investigation on FASTA protein sequences.

3. Results

3.1. Interaction Prediction

In the current research on both the beneficial and detrimental effects of clove vanillin on *Homo sapiens* have been explored using STITCH 5.0, a bioinformatics analysis tool that provides a possible network of 9 proteins (Figure 1). The function and interaction scores is illustrated in Table 1.

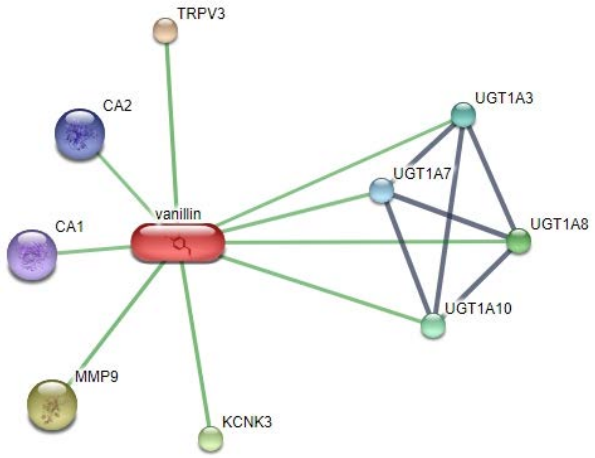

Figure 1. Using STITCH 5.0, interactions between vanillin and human proteins are predicted.

Table 1. List of proteins that interact with Vanillin identified using STITCH 5.0, along with their short descriptions.

PROTEIN CODE	ACCESSION NUMBER	INFORMATION	SCORE	AMINO ACID
CA1	ENSP00000430656	carbonic anhydrase I;	0.700	261
CA2	ENSP00000285379	carbonic anhydrase II;	0.700	260
UGT1A7	ENSP00000362525	UDP glucuronosyltransferase 1 family, polypeptide A7	0.737	530
UGT1A3	ENSP00000418532	UDP glucuronosyltransferase 1 family, polypeptide A7;	0.737	534
UGT1A10	ENSP00000343838	UDP glucuronosyltransferase 1 family, polypeptide A7;	0.739	530
UGT1A8	ENSP00000362549	UDP glucuronosyltransferase 1 family, polypeptide A7;	0.739	530
KCNK3	ENSP00000306275	potassium channel, subfamily K, member 3;	0.800	394
MMP9	ENSP00000361405	matrix metallopeptidase 9 (gelatinase B, 92kDa gelatinase, 92kDa type IV collagenase);	0.800	707
TRPV3	ENSP00000461518	transient receptor potential cation channel, subfamily V, member 3	0.818	790

Using the bioinformatics tools STRING 12.0, it was determined that each of the 9 proteins for Vanillin interacted with another 10 proteins, yielding a total of 99 proteins for Vanillin. Each protein's

 doi:10.20944/preprints202503.2090.v1

interaction network was graphically depicted (Figure 2), and their unique protein-protein interactions (PPI) were reported in Table 2.

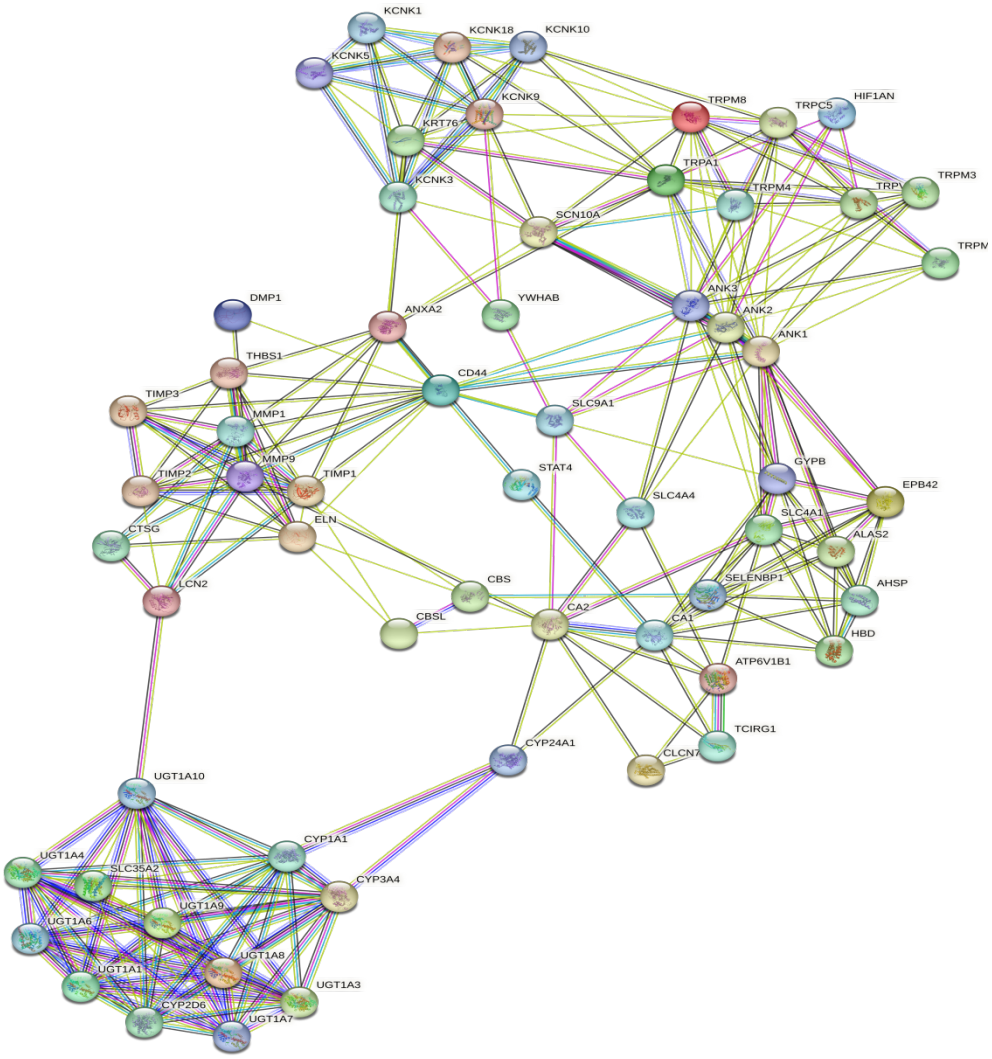

Figure 2. Protein-Protein interaction (PPI) of Vanillin identified via STRING 12.0.

Table 2. The interaction between vanillin and proteins has been identified using the STITCH and STRING technologies.

Stitch	CA1	CA2	UGT1A7	UGT1A3	UGTA10	UGT1A8	KCNK3	MMP9	TRPV3
String	AHSP	CA1	UGT1A6	UGT1A9	UGT1A1	UGT1A6	KCNK9	TIMP1	TRPM8
	ALAS2	ATP6V1B1	UGT1A1	UGT1A6	UGT1A8	UGT1A9	S100A100	TIMP2	HIF1AN
	HBD	CBS	UGT1A10	UGT1A1	UGT1A6	UGT1A3	KRT76	LCN2	ANK1
	CA2	CBSL	UGT1A9	UGT1A8	UGT1A9	UGT1A1	KCNK5	CD44	ANK3
	EPB42	TCIRG1	UGT1A4	UGT1A4	UGT1A4	UGT1A10	KCNK18	THBS1	ANK2
	SLC4A1	SLC9A1	UGT1A8	UGT1A10	UGT1A7	UGT1A4	KCNK1	TIMP3	TRPM3
	GYPB	SLC4A4	UGT1A3	UGT1A7	UGT1A3	UGT1A7	KCNK10	CTSG	TRPM4
	SELENBP1	SLC4A1	CYP3A4	CYP3A4	CYP3A4	CYP3A4	YWHAB	DMP1	TRPA1
	STAT4	CYP24A1	SLC35A2	CYP2D6	SLC35A2	SLC35A2	SCN10A	ELN	TRPC5
	CYP24A1	CLCN7	CYP1A1	SLC35A2	CYP1A1	CYP1A1	ANXA2	MMP1	TRPM7

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3.2. Protein Accession, Amino Acid Sequence Retrieval

Using STRING 12.0 for the PPI analysis, we identified 99 proteins from Vanillin. These proteins' accession numbers and amino acid lengths were compiled concurrently from the protein databases UniProtKB and NCBI. We used protein-protein blast to minimize duplication and gathered FASTA files for functional annotation analysis using these protein accession codes. Following a blast analysis in Table 3, we identified 49 proteins and conducted functional annotation.

Table 3. List of vanillin interacting proteins of STRING 12.0 after blast searching.

SL No	Accession Number	Protein name	Amino acid	SL No	Accession Number	Protein name	Amino acid	SL No	Accession Number	Protein name	Amino acid
1	AAH27890.1	CA1	261	18	NP_659505.1		790	35	NP_002091.4	GYPB	91
2	CAG38763.1	CA2	260	19	NP_001305151.1	AHSP	102	36	CAG33133.1	SELENBP1	472
3	AAG30419.1	UGT1A7	530	20	NP_000023.2	ALAS2	587	37	NP_003142.1	STAT4	748
4	NP_061966.1	UDP1A3	534	21	NP_000510.1	HBD	147	38	NP_000773.2	CYP24A1	514
5	AAG30417.1	UGT1A10	530	22	AAH96094.1	EPB42	691	39	NP_066307.1	UGT1A9	530
6	NP_061949.3	UGT1A8	530	23	NP_061948.1	UGT1A10	530	40	NP_059488.2	CYP3A4	503
7	NP_002237.1	KCNK3	394	24	NP_009051.1	UGT1A3	534	41	NP_001306146.1	CD44	742
8	NP_004985.2	MMP9	707	25	NP_005651.1	SLC35A2	396	42	NP_000353.1	TIMP3	211
9	NP_001683.2	ATP6V1B1	513	26	NP_003237.2	THBS1	1170	43	NP_056932.2	KRT76	638
10	AAH10242.1	CBS	551	27	NP_001902.1	CTSG	255	44	NP_003731.1	KCNK5	499
11	XP_054180901.1	CBSL	551	28	NP_004398.1	DMP1	513	45	NP_862823.1	KCNK18	384
12	NP_006010.2	TCIRG1	830	29	NP_001265868.1	ELN	786	46	NP_002236.1	KCNK1	336
13	NP_003038.2	SLC9A1	815	30	NP_002412.1	MMP1	469	47	NP_612190.1	KCNK10	543
14	NP_001091954.1	SLC4A4	1079	31	NP_001269463.1	KCNK9	374	48	NP_003395.1	YWHAB	246
15	NP_001278.1	CLCN7	805	32	NP_002957.1	S100A10	97	49	AAH93056.1	ANXA2	339
16	NP_001063.2	UGT1A6	532	33	NP_006505.4	SCN10A	1956				
17	NP_000454.1	UGT1A1	533	34	NP_000333.1	SLC4A1	911				

3.3. Functional Annotation Analysis and Pathway Analysis after Blast Search

In the current research, functional annotation evaluation of vanillin reveals very effective biological processes, cellular components, and molecular activities in humans (Figure 3). Pathway analysis of vanillin was additionally identified using omics box tools, and the analysis indicates many sorts of signaling pathways in various species with their correct IDs and different kinds of databases, such as KEGG (Kyoto Encyclopedia of Genes and Genomes) and Reactome, as shown in Table 4.

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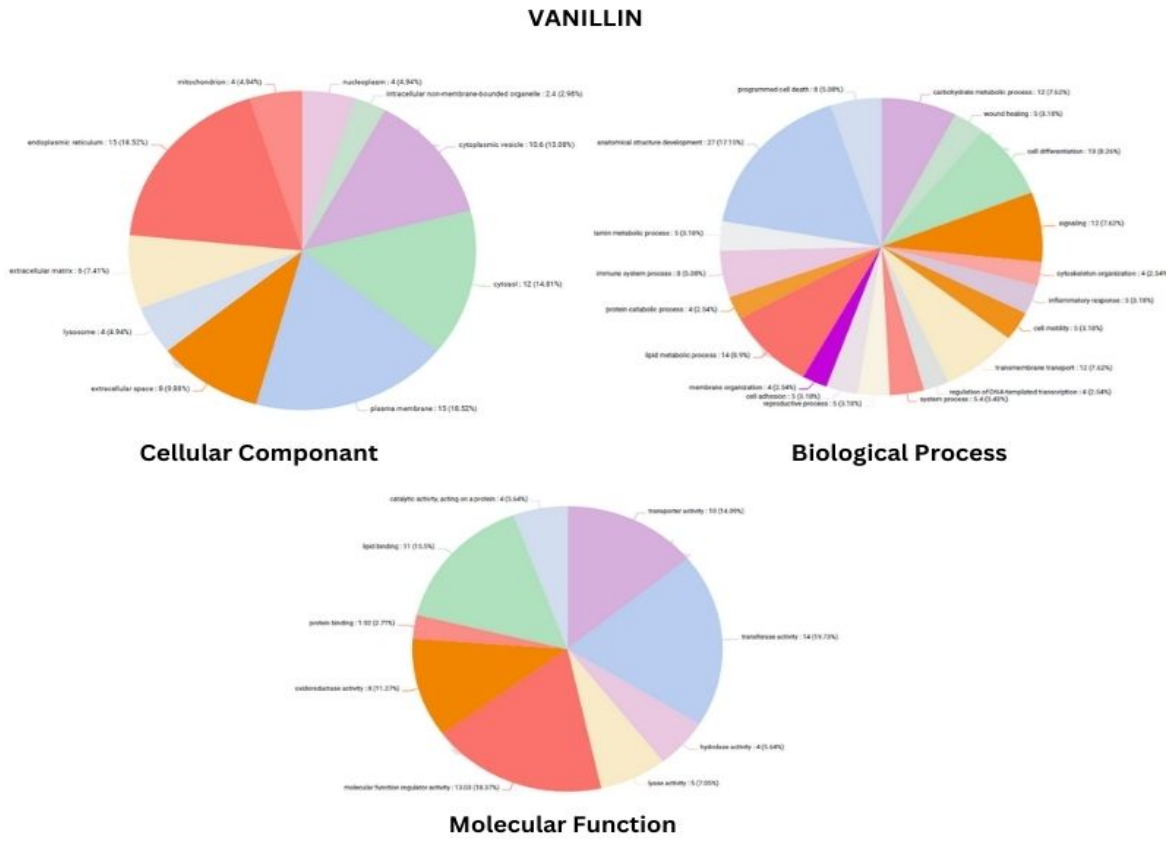

Figure 3. Functional annotation analysis of Vanillin showed the various biological processes (a), localization of cellular components (b) and molecular functions (c) of different proteins in *Homo sapiens.*

Table 4. KEGG and Reactome Pathways analysis of Vanillin interacted Proteins.

KEGG ID	PATHWAY	KEGG ID	PATHWAY	KEGG ID	PATHWAY
Ko04510	Focal adhesion	Ko05200	Pathways in cancer	Ko04360	Axon guidance
Ko00941	Flavonoid biosynthesis	Ko04350	TGF beta signaling pathway	Ko05171	Coronavirus disease-COVID19
Ko04115	P^{53} signaling pathway	Ko05160	Hepatitis C	Ko04080	Neuroactive ligand receptor interaction
Ko05204	Chemical carcinogenesis,DNA adducts	Ko05161	Hepatitis B	Ko00040	Pentose and glucuronate interconversions
Ko05322	Systemic lupus erythematosus	Ko04929	GnRH secretion	Ko05165	Human papillomavirus infection
Ko04750	Inflammatory mediator regulation of TRP channels	Ko04927	Cortisol synthesis and secretion	Ko00270	Cysteine and methionine metabolism
Ko05169	Epstein barr virus infection	Ko04928	Parathyroid hormone synthesis,secretion and action	Ko00791	Atrazine degradation
Ko05323	Rheumatoid arthritis	Ko04925	Aldosterone synthesis and secretion	Ko04624	Toll and Imd signaling pathway
Ko04630	JAK-STAT pathway	Ko00053	Ascorbate and aldarate metabilism	Ko00830	Retinol metabolism
Ko05202	Transcriptional misregulation in cancer	Ko04926	Relaxin signaling pathway	Ko04742	Taste transduction
Ko00944	Flavone and flavonol biosynthesis	Ko00450	Selenocompound metabolism	Ko04621	NOD like receptor signaling pathway
Ko04110	Cell cycle	Ko05219	Bladder cancer	Ko04066	HF-1signaling pathway
Ko01240	Biosynthesis of co factor	Ko05215	Prostrate cancer	Ko05152	tuberculosis
Ko05321	Inflammatory bowel disease	Ko04640	Hematopoietic cell lineage	Ko00380	Tryotophan metabolism
Ko04934	Cushing syndrome	Ko00232	Caffeine metabolism	Ko04919	Thyroid hormone signaling pathway
Ko04657	IL-17 signaling pathway	Ko04668	TNF signaling pathway	Ko00260	Glycine,serine and threonine metabolism

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Ko04658	Th1 and Th2 cell differentiation	Ko04024	cAMP signaling pathway	Ko00140	Steroid hormone biosynthesis
Ko04810	Regulation of actin cytoskeleton	Ko04145	Phagosome	Ko04915	Estrogen signaling pathway
Ko04015	Rap1 signaling pathway	Ko00910	Nitrogen metabolism	ko04913	Ovarian steroidogenesis
Ko04013	MAPK signaling pathway-fly	Ko04142	Lysosome	Ko05207	Chemical carcinogenesis-receptor activation
Ko03320	PRAP signaling pathway	Ko05110	Vibrio cholera infection	Ko04911	Insulin secretion
Ko04371	Apelin signaling pathway	Ko04260	Cardiac muscle contraction	Ko05208	Chemical carcinogenesis – reactive oxygen species
Ko00190	Oxidative phosphorylation	Ko04261	Adrenergic signaling in cardiomyocytes	Ko01522	Endrocrine resistance
Ko00071	Fatty acid degradation	Ko00100	Steroid biosynthesis	Ko04512	ECM -receptor interaction
Ko00590	Arachidonic acid metabolism	Ko00980	Metabolism of xenobiotics by cytochrome P450	Ko05205	Proteoglycans in cancer
Ko00073	Cutin,suberine and wax biosynthesis	Ko00860	Porphyrin metabolism	Ko05206	microRNAs in cancer
Ko00591	Linoleic acid metabolism	Ko00982	Drug metabolism-cytochrome P450	Ko05203	Viral carcinogenesis
Ko00195	photosynthesis	Ko00983	Drug metabolism –other enzymes	Ko04114	Oocyte meiosis
Ko05120	Epithelial cell signaling in Helicobacter pylori infection	Ko00363	Bisphenol degradation	Ko05144	malaria
Ko04670	Leukocyte transendothelial migration	Ko04613	Neutrophil extracellular trap formation	Ko05143	African trypanosomiasis
Ko04150	mTOR signaling pathway	Ko04217	necroptosis	Ko05131	Shigellosis
Ko04151	PI3K-Akt signaling pathway	Ko04976	Bile secreation	Ko05132	Salmonella infection
Ko05418	Fluid shear stress and atherosclerosis	Ko04614	Renin angiotensin system	Ko04978	Mineral absorption
Ko04390	Hippo signaling pathway	Ko04218	Cellular senescence	Ko04971	Gastric acid secretion
Ko04391	Hippo signaling pathway-fly	Ko04974	Protein digestion and absroption	Ko04964	Proximal tubule bicarbonate reclamation
Ko05417	Lipid and atherosclerosis	Ko04972	Pancreatic secretion	Ko05415	Diabetic cardimyopathy
Reactome id	**Description**	**Reactome id**	**Description**	**Reactome id**	**Description**
R-HSA-6805567	keratinization	R-HSA-5576892	Phase 0 rapid depolarisation	R-BTA-450513	Tristetraprolin binds and destabilizes mRNA
R-HSA-9020958	Interleukin-21signaling	R-HSA-8984722	Interleukin-20 family signaling	R-HSA-727802	Transport of nucleotide sugars
R-HSA-8957275	Post-translational protein phosphorylation	R-HSA-425986	Sodium exchange	R-BTA-111447	Activation of BAD and translocation to mitrochondria
R-HSA-5579016	Defective UGT1A4 causes hyperbilurubinemia	R-HSA-5579002	Defective UGT1A1 causes hyperbilirubinemia	R-HSA-381426	Regulation of insulin-like Growth Factor transport and uptake by insulin
R-HSA-1592389	Activation of matrix Metalloproteinases	R-BTA-392517	Rap 1 signaling	R-HSA-1614603	Cysteine formation from homocysteine
R-HSA-9027307	Biosynthesis of maresin-like SPMs	R-HSA-3000178	ECM proteoglycans	R-HSA-77387	Insulin receptor recycling
R-HSA-2022377	Metabolism of Angiotensinogen to Angiotensins	R-HSA-8854691	Interleukin-20 family signaling	R-HSA-1222556	ROS and RNS production in phagocytes
R-BTA-5628897	TP53 regulates metabolic genes	R-HSA-5576886	Phase 4- resting membrane potential	R-BTA-75035	Chk1/Chk2 mediatyed inactivation of Cyclin B:cdk1 complex
R-HSA-1475029	Reversible hydration of carbon dioxide	R-BTA-5675221	Negative regulation of MAPK activation	R-HSA-189493	Heme degredation
R-HSA-447043	Neurofascin interactions	R-HSA-983712	Ion channel transport	R-HSA-8950505	Gene and protein expression by JAK-STAT signaling after interleukin-12
R-HSA-1299308	Tandem of pore domain in a weak inwardly rectifying K+ channels(TWIK)	R-BTA-5674135	MAP2K and MAPK activation	R-HSA-211916	Vitamins
R-HSA-447041	CHL1 interactions	R-HSA-418890	Role of second messengers in netrin-1 signaling	R-HSA-2672351	Stimuli-sensing channels

R-HSA-9020933	Interleukin-23 signaling	R-HSA-3295583	TRP channels	Plant Reactom ID	Description
R-HSA-1566948	Elastic-fibre formation	R-HSA-9660826	Purinergic signaling in leishmaniasis infection	R-AHA-1119523	Tetrahydrofolate biosynthesis II
R-HSA-1299316	TWIK-related acid –sensitive K+(TASK)	R-HSA-1237044	Erythrocyte take up carbon dioxide and release oxygen	R-ACH-1119415	Leucopelargonidin and leucocyanidin biosynthesis
R-HSA-6785807	Interleukin-4 and interleukin-13 signaling	R-HSA-877300	Interferon gramma signaling	R-ACH-1119322	Leucodelphinidin biosynthesis
R-HSA-425381	Bicarbonate transporters	R-HSA-9754706	Atorvastatin ADME	R-ACH-9609573	Tricin biosynthesis
R-BTA-5673000	RAF activation	R-HSA-448706	Interleukin-1 processing	R-AHA-1119477	Starch biosynthesis
R-BTA-450385	BRF1 binds and destabilizes mRNA	R-HSA-2408508	Metabolism of ingested SeMet,Sec,MeSec into H2Se	R-QLO-5367729	Strigolactone biosynthesis
R-HSA-9623433	NR1H2 and NR1H3 regulate gene expression to control bile acid homeostate	R-SSC-75205	Dissolution of fibrin clot	R-AHA-1119265	Tetra ghydrofolate biosynthesis I
R-HSA-983231	Factors involved in megakaryocyte development and platelet production	R-HSA-5423646	Aflatoxin activation and detoxification	R-AHA-1119465	Sucrose biosynthesis
R-HSA-2160916	Hyaluronan uptake and degredation	R-HSA-917977	Transferrin endocytosis and recycling	R-HSA-1234174	Cellular responses to hypoxia
R-HSA-9757110	Prednisone ADME	R-HSA-9027307	Biosynthesis of maresin-like SPMs	R-HSA-189451	Heme biosynthesis
R-HSA-6798695	Neutrophil degranulation	R-HSA-2672351	Stimuli-sensing channels	R-HSA-9717207	Sensory perception of sweet,bitter and umami taste
R-BTA-166208	mTORC1- mediated signaling	R-HSA-211916	Vitamins	R-HSA-114608	Platelate degranulation
R-BTA-5625740	RHO GTPases activate PKNs	R-HSA-1299344	TRESK	R-HSA-5619072	Defective SLC35A2 causes congential disorder of glycosylation 2M
R-HSA-1299344	TWIK-related spinal cord K+ channel	R-HSA-9639288	Amino acids regulate mTORC1	R-HSA-196791	Vitamin D metabolism
R-HSA-9639288	Amino acids regulate mTORC1	R-HSA-9635465	Suppression of apoptosis	R-HSA-1234174	Cellular responses to hypoxia
R-HSA-9635465	Suppression of apoptosis	R-HSA-1989781	PPARA activates gene expressions	R-HSA-189451	Heme biosynthesis
R-HSA-1989781	PPARA activates gene expression	R-HSA-447038	NrCAM interactions	R-HSA-9717207	Sensory perception of sweet,bitter and umami taste
R-HSA-447038	NrCAM interactions	R-BTA-170968	Frs2-mediated activation	R-BTA-114608	Platelate degranulation
R-HSA-170968	Frs2-mediated activation	R-HSA-202733	Cell surface interactions at the vascular wall	R-HSA-5619072	Defective SLC35A2 causes congential disorder of glycosylation 2M
R-HSA-202733	Cell surface interactions at the vascular wall	R-HSA-156588	glucuronidation	R-HSA-6803157	Antimicrobial peptides
R-HSA-156588	glucuronidation	R-BTA-2028269	Signaling by Hippo	R-HSA-6807878	COPI-mediated anterograde transport
R-HSA-2028269	Signaling by Hippo	R-HSA-6783783	Interleukin-10 signaling	R-HSA-6809371	Formation of the cornifide envelope
R-HSA-6783783	Interleukin-10 signaling	R-HSA-445095	Interaction between L1 and Ankyrins	R-HSA-9753281	Paracetamol ADME
R-HSA-1592389	Activation of Matrix MEtalloproteinases	R-HSA-9749641	Asprine ADME	R-HSA-8936459	RUNX1 regulates genes involved in megakaryocyte differentiation
R-HSA-211981	xenobiotics	R-HSA-5619050	Defective SLC4A1 causes hereditary spherocytosis type 4	R-HSA-1299503	TWIK related potassium channel
R-HSA-1247673	Erythrocytes take up oxygen and release carbon dioxide	R-HSA-5619054	Defective SLC4A4 causes renal tubular acidosis,poximal	R-HSA-6803157	Antimicrobial peptides
R-HSA-5579010	Defective CYP24A1 causes HCAI	R-BTA-96144399	Regulation of localization of FOXO transcription factors		

Vanillin regulates anatomical structure development, lipid metabolism, transmembrane transport, inflammatory response, signaling and carbohydrate metabolism, transferase activity,

transporter activity, lipid-binding molecular function, and regulatory activity (Figure 3). The analysis of pathways also reveals several types of signaling pathways, including the P53 signaling pathway, JAK-STAT pathway, relaxin signaling pathway, mTOR signaling pathway, PPAR signaling pathway, IL-17 signaling pathway, MAPK pathway, thyroid hormone signaling pathway, cAMP signaling pathway, and Hippo pathway. The pathway assessment depicts a variety of diseases, most notably coronavirus disease (COVID-19), tuberculosis, hepatitis B, hepatitis C, diabetic cardiomyopathy, malaria, and Cushing syndrome; it is also responsible for several types of cancer, including microRNA cancer, proteoglycan cancer, prostate cancer, bladder cancer, and transcriptional misregulation.

Vanillin and its related proteins are often found in cellular components, including the endoplasmic reticulum, extracellular space, cytosol, plasma membrane, and mitochondria (Figure 3). These proteins additionally serve a crucial function in many different types of metabolism, particularly ascorbate and aldarate metabolism, selenocompound metabolism, vitamin D metabolism, cysteine and methionine metabolism, retinol metabolism, tryptophan metabolism, glycine, serine metabolism, arachidonic acid metabolism, linoleic metabolism, nitrogen metabolism, porphyrine metabolism, drug metabolism, and the ingestion of SetMet, Sec, and Msec into H2Se met (Table 4).

4. Discussion

Clove (*Syzygium aromaticum*) is a spice plant with chemotherapeutical properties, including vanillin, which has constructive impacts on the human physique (Cortés-Rojas, De Souza, et al., 2014). Vanillin is observed in numerous species and has bioenergetic interactions. It is a flavor and aromatic compound and modulates metabolic and signaling pathways .

Bioinformatics research makes it easier to find a wide range of proteins and their activities, as well as functionally annotate and analyze these proteins. These kinds of proteins have been linked to a variety of illnesses, including metabolic, neurological, and genetic disorders, as well as several forms of cancer. Vanillin is absorbed via many modes of exposure and eliminated in the urine, with a tiny amount excreted as unmetabolized vanillin (K. Ho et al., 2011).

Vanillin was found to interact with 49 functional proteins that are linked to human health. Vanillin has anti-inflammatory, antibacterial, antibiotic potential, antifungal, and antiviral properties, among other things (*Vanillin: A Review on the Therapeutic Prospects of a Popular Flavouring Molecule | Advances in Traditional Medicine*, n.d.) (*Antibacterial Mechanism of Vanillin against Escherichia Coli O157: H7: Heliyon*, n.d.) (*Vanilla Modulates the Activity of Antibiotics and Inhibits Efflux Pumps in Drug-Resistant Pseudomonas Aeruginosa | Biologia*, n.d.) (*Full Article: Antifungal Activity of Vanilla Juice and Vanillin against Alternaria Alternata*, n.d.) . Vanillin was additionally shown to have anticlastogenic, antimutagenic, and anticancer effects, making it a potential nutraceutical molecule (Mourtzinos et al., 2009).According to the study that we conducted, vanillin acts on the cell cycle, causes apoptosis, and prevents cancer. Ho et al. found that the compound inhibited G2/M and G0/G1 cell cycle stages in 5-bromo-2-deoxyuridine-labeling cell proliferation and caused apoptosis in HT-29 cancer cells (IC50 value of 400 μg/ml), indicating potential for colorectal cancer prevention (K. Ho et al., 2009).

Vanillin's anti-inflammatory and antioxidant qualities aid in the treatment of hypoxic-ischemic brain damage and Alzheimer's disease(Albrecht et al., 2019) (Iannuzzi et al., 2023). It's a neurodevelopmental and neurodegenerative condition. It occurs as a result of a shortage of oxygen and a restriction of blood flow in the brain, triggering anaerobic metabolism (Albrecht et al., 2019). Vanillin's antioxidant properties, the hippo signaling pathway, and other interleukin signaling pathways all contribute to the prevention of some types of deficits. According to Lan and colleagues' research on the rat model, vanillin plays a critical part during these diseases; hippo signaling pathways and interleukin pathways help to prevent those diseases because vanillin promotes early neurofunction development, mitigates histo-morphological damage, and protects neuronal damage in the cortex and hippocampal CA1 and CA3 regions after HIBD in neonatal rats (Lan et al., 2019).

Vanillin modulates a wide range of metabolic activities and signaling pathways in the human body. This molecule has an effect on cell signaling, differentiation, anatomical structure creation, programmed cell death, the inflammatory response, glucose and lipid metabolism, nerve control, and other processes. Furthermore, these bioactive compounds have a direct relationship with the cell's subcellular organelles. Vanillin has been seen to interact with cell membranes, nucleoplasm, endoplasmic reticulum, cytosol, mitochondria, and the extracellular space. According to Gupta and Sharma's rat model experiments, vanillin ameliorated 3-nitropropionic acid-induced defective mitochondrial enzyme complexes (I, II, and IV) and prevented Huntington's disease (Gupta & Sharma, 2014b). Vanillin also protects the mitochondria, and its protective mechanism assists to activate the antiapoptotic pathway via the mitochondria(*Neurosupportive Role of Vanillin, a Natural Phenolic Compound, on Rotenone Induced Neurotoxicity in SH-SY5Y Neuroblastoma Cells - Dhanalakshmi - 2015 - Evidence-Based Complementary and Alternative Medicine - Wiley Online Library*, n.d.) . Moreover, Sheetal D Ullal and Yogesh Belagali demonstrated that vanillin serves a key part in lipid metabolism, and that when co-administered with HFD, vanillin can lower total cholesterol, triglyceride, and VLDL-C levels compared to the HFD control and atorvastatin groups, as well as contribute to reducing those parameters via free radical scavenging activity (Belagali et al., 2013).

Vanillin and its derivatives have medicinal qualities that treat a number of human ailments. Amor et al. (2014) found that vanillin works on a variety of neurodegenerative diseases, including Parkinson's disease, traumatic brain and spinal cord injury, stroke, multiple sclerosis, neuropsychiatric disorders, and amyotrophic lateral sclerosis, as well as neuropsychiatric disorders and genetic disorders caused by progressive neuron dysfunction in the central nervous system (Amor et al., 2014). Industrially manufactured vanillin is utilized as a flavoring ingredient in a wide range of goods, including fragrances. It is a source of dopa, leading to the precursors of the neurotransmitters dopamine, norepinephrine, and epinephrine (Martău et al., 2021). Vanillin is projected to play a major part in pharmaceuticals and biomedicine due to its derivatives activity, which employs different chemicals for diverse reasons and develops innovative composites that are employed in a variety of illnesses, disorders, and other applications. Many chemicals have already been successfully tested, including metal particles, metal oxides, nanoparticles, phenolic compounds, plant extracts, biopolymers, pharmaceuticals, and so on. Even if the synthesized compounds are low in toxicity and biocompatible, the possibilities for combinations are endless (Kafali et al., 2024). Our study solely looked at the potential adverse outcomes of vanillin; we need molecular drug design and animal experiments to determine the advantageous and detrimental impacts of these compounds.

5. Conclusions

Our findings show that several proteins interact with vanillin and have potential therapeutic effects (biological, molecular, and cellular), as well as other types of pathway activity that are directly or indirectly influenced by the human body. This bioinformatics technique precisely identified various possible activities and pathways (apoptosis, hippo signaling pathways, interleukin pathway) that mitigate cancer, inflammation, and neurodegenerative illness. Apoptosis is a critical function that regulates the cell cycle and prevents colorectal cancer; hippo signaling pathways protect neurons and prevent hypoxic-ischemic brain damage. Notably, these medications are utilized to treat a wide range of illnesses. Vanillin inhibits infectious diseases such as malaria, hepatitis B, and hepatitis C. However, another research revealed that vanillin is widely used in the pharmaceutical business due to its antibacterial properties and wound healing activity. The limitation of this study is that it needs to declare those proteins, and additionally, in vitro and in vivo studies are needed to illustrate the rest. The downside of this investigation involves the fact that it must reveal those proteins, and more in vitro and in vivo investigations are required to demonstrate the rest.

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