INNOVARE JOURNAL OF MEDICAL SCIENCES

Vol 11, Issue 4, 2023

ISSN - 2321-4406

Research Article

# NETWORK PHARMACOLOGY AND MOLECULAR DOCKING-BASED PREDICTION OF PHARMACOLOGICAL PROPERTIES OF OSTHOLE

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***Received: 30 March 2023, Revised and Accepted: 20 June 2023***

**ABSTRACT**

**Objectives:** In this study, the term called network pharmacology (NP) process which was used to understand the underlying mechanism of the pharmacological properties of Osthole. NP is developed that is used to understand drug actions and interactions with multiple targets and it is also capable of completely articulating the complexity between diseases and medications. The research was carried out for the identification of diverse drug-target interactions using NP to discover novel medications for difficult conditions such as Parkinson's, Cancer, and Alzheimer's disease and many more. Osthole was used for prediction which could be used in the pharmaceutical background.

**Methods:** To understand the binding affinity of Osthole with the corresponding target proteins, it was analyzed. It was determined from the pathway by which diseases can be caused, such as cancer and Alzheimer's disease. A PyRx tool was used to carry out the molecular docking. For this research, structures of protein and phytocompounds were retrieved from UniProtKB and PubChem. Furthermore, along with the help of BIOVIA discovery studio software, the protein structure was analyzed and ADMET screening was done to evaluate the Osthole pharmacological properties.

**Results:** The ligands were retrieved for Osthole from PubChem, then target prediction was carried out where it showed 100 potential targets. The protein-protein network and interaction were done using the STRING database, in which it showed that these CREBBP, IDO1, and MAPK8 targets have maximum interactions followed by the Gene functional analysis, that is, go function and KEGG pathway. The molecular docking was carried out using PyRx in which 4U72 showed the best binding affinity to Osthole. Furthermore, visualization was done using BIOVIA Discovery Studio, which provided the 3D and 2D visualization.

**Conclusion:** According to the results obtained for molecular docking, these target proteins have pharmacological effects which can be considered as suggestions for the investigation of the pharmacological mechanism of Osthole.

**Keywords:** Osthole, Docking, Pharmacology, ADMET.

 DOI: http://dx.doi.org/10.22159/ijms.2023v11i4.47981. Journal homepage: https://innovareacademics.in/journals/index.php/ijms

## INTRODUCTION

Plants play an important role in both traditional and modern therapeutic systems as they are natural resources and also, they are present all throughout the world. Medicinal plants are the major sources of numerous valuable chemicals or drugs. Over 1300 medicinal plants are used in European countries, and out of them, 90% are from wild sources. In this world around 80% of population is still dependent on traditional or herbal medicines which are used for treatment of diseases [1,2].

Coumarins are widely distributed in high plants with more than 3560 compounds isolated. Even though many coumarin-containing plants have been utilized for inflammation-related diseases and conditions, there are not many coumarins that act as potent COX and LOX dual inhibitors. Osthole is also famously known as Osthol, which is a derivate of coumarin and also found in different medicinal plants. The study also says that it can be extracted or separated to obtained from plants. A lot of experiment recommended that it can exhibit multiple biological activities such as anti-inflammatory, neuroprotective, antimicrobial, and antitumor [3].

Osthole has a chemically known as 7-methoxy-8-(3-methyl-2-butenyl), which is natural coumarin that was first isolated from the *Cnidium* plant. High contents of Osthole are present in the mature fruit of *Cnidium monnieri* (Fructus Cnidii), which is commonly applied in the clinical practice of traditional Chinese medicine. It has a role as a metabolite. Osthole is also present in a wide range of other medicinal plants, including *Angelica*, *Archangelica*, *Citrus*, and *Clausena*. Osthole showed his existence during regulation of different pathways that modulate various apoptosis related to protein, protein kinase, cell cycle regulators, cytokines, transcriptional factors, and growth receptors. Osthole is mostly known for halt proliferation of cancerous cells which can arrest the cell cycle which also include the apoptosis [4,5]. In the present study we have conducted the pharmacological profiling of Osthole and identified its potential targets as depicted in Fig 1.

## METHODS

### Ligand retrieval: PubChem compound library

PubChem (https://pubchem.ncbi.nlm.nih.gov/) is managed by the National Institutes of Health, which is an open chemistry database. As it mostly consists of small molecules, along with larger molecules such as carbohydrates, lipids, nucleotides, and many more chemically modified macromolecules. The data present in PubChem is organized into three interlinked databases: Substance, Compound, and Bioassay. Structures that are mostly present in PubChem are drug-like compounds which satisfy Lipinski's rule of five. PubChem is widely known for its knowledge of biomedical research such as cheminformatics, chemical biology, drug discovery, and medical chemistry. PubChem has a CID and canonical SMILES for Osthole which was obtained from PubChem by passing the query into the query box (Fig. 2) [6,7].

### Target gene screening: Swiss target prediction

Swiss target prediction (http://www.swisstargetprediction.ch) is an online server which is used to predict the targets of bioactive molecules

which accurately depend on a combination of 2D and 3D similarity values, along with their known ligands. These predictions can occur in five different organisms, along with mapping by homology within and between various species, which allows to enable close paralogs and orthologs. This service is free of charge and it does not require you to login. It allows anyone to achieve reverse screening toward previously carefully prepared chemical libraries. The user-friendly graphical interface protects non-experts from procedural drawbacks and it also decreases the tedious technical efforts. The canonical smiles of Osthole were passed in Swiss target prediction where the species was Homo sapiens and the option of predicting a target was selected.

**Protein-protein interaction network construction: String database**

The STRING database (https://string-db.org/) is known to integrate all known and predicted associations between proteins, which include physical interactions, along with functional associations. As STRING has an aim for wide coverage where it can collect and score evidence from a number of sources, such as databases of interaction experiments, automated text mining of the scientific literature, and annotated pathways, systematic transfers of interaction evidence from one organism to another and computational interaction predictions from co-expression and from conserved genomic context. This entire database information of STRING is pre-computed and stored in a relational database where it also allows you to download separately. It also allows the users to log on and make their searches precise, and it offers online users to facilitate the inspection of the evidence supporting each protein-protein association. After the target prediction, there were 100 potential targets for Osthole. To obtain a protein-protein interaction network, a file of potential target was passed where Homo sapiens was selected as a species, along with this minimum interaction score was set at high confidence, that is, 0.900.

**Gene functional analysis: Go function and KEGG pathway**

ShinyGO (http://bioinformatics.sdstate.edu/go/) is a graphical and intuitive tool which is used for enrichment analysis. ShinyGO is a large annotation and pathway database which is based on several R/Bioconductor packages, and compiled from many sources. ShinyGO provides detailed analysis of gene lists, pathways, gene characteristics, protein interaction, along with graphical visualization of enrichment. This also consists of plant, animal, archaeal, and bacterial species which are represented in the extensive annotation database for ShinyGO, as it was taken from the Ensembl and STRING-Database. ShinyGO has unique features, which also shows query genes in pathway diagrams and PPI networks, which is based on API which gives access to STRING and KEGG, which is also used to visualize the overlaps in enriched pathways using interactive networks and hierarchical clustering that recognize the difference in GC content, gene type, length etc. As ShinyGo was used to annotate the interaction of Osthole with target proteins and understand their following role [8].

**Molecular docking and visualization**

Molecular docking is widely used to understand the interaction between a small molecule and a protein at the atomic level. That also allows us to understand the behavior of small molecules in the binding site of target proteins and hence, it was done using PyRx (https://pyrx.sourceforge.io/). The proteins were purified by BIOVIA and docked in PyRx software. PyRx usually works on Windows, Mac OS X, or Unix/Linux operating systems as it is open-source software. PyRx includes a docking wizard and is known for its easy-to-use interfaces, which makes it a valuable tool for Computer-Aided Drug Designing. Furthermore, it includes chemical spreadsheet-like functionality and which also has a powerful visualization engine that is almost important for structure-based drug design [9,10].

Discovery studios BIOVIA (https://www.3ds.com/products-services/biovia/products/molecular-modeling-simulation/biovia-discovery-studio/) were used for Visualization. BIOVIA Discovery Studio Visualizer is used as it is free, known for its feature-rich molecular modeling application for sharing, viewing, and analyzing proteins

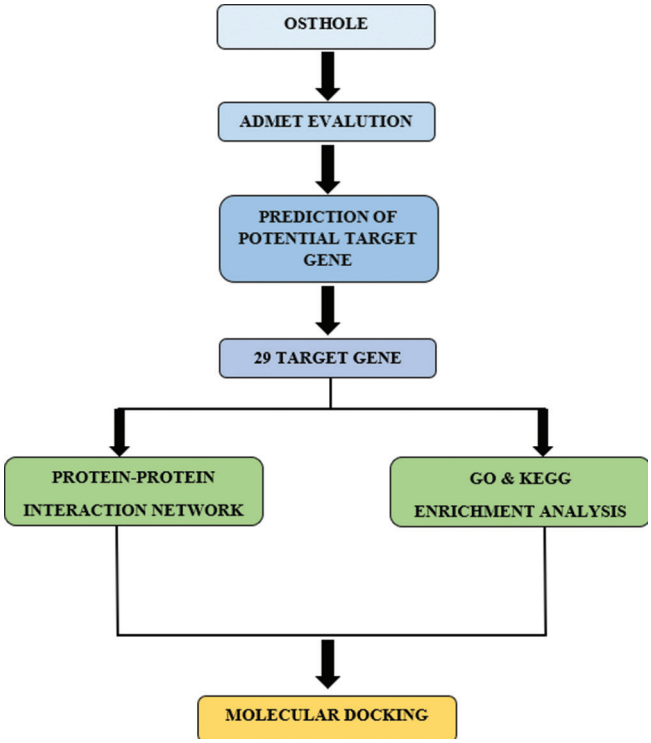


**Fig. 1: The overall flowchart of *in-silico* analysis of Osthole**

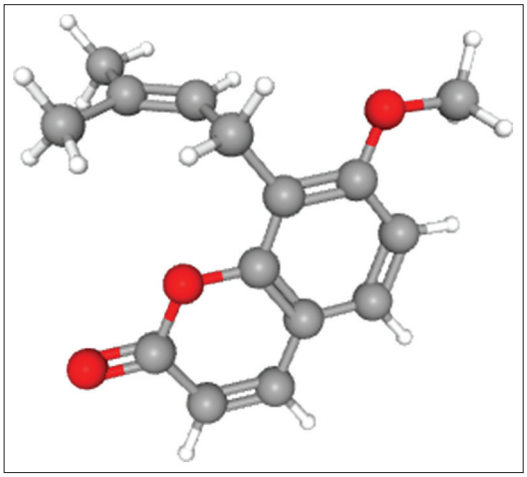

**Fig. 2: 3D-structure of Osthole**

and also understanding small molecule data. It was developed and distributed by Dassault Systems BIOVIA. Furthermore, it is easier for scientists to investigate and test hypotheses *in silico* before costly experimentation, which will reduce the time and expense to bring the product to the market. Discovery Studio combines the transcription of small compounds and macromolecules. The ligand which was docked using PyRx and purified proteins was loaded into BIOVIA where 2D and 3D structures were visualized and downloaded.

**ADMET screening: ADMET lab 2.0**

The undesirable pharmacokinetics, along with the toxic nature of candidate compounds, is the core reasons for the failure of drug development. Furthermore, it has been commonly known that absorption, distribution, metabolism, excretion, and toxicity should be evaluated. In ADMET evaluation models, that is, *in-silico,* these were established as a supplementary tool that guided the chemists in the form of designing and optimizing the leads. ADMETlab is known for ADMET evaluation of chemicals, which is based on a collected ADMET database

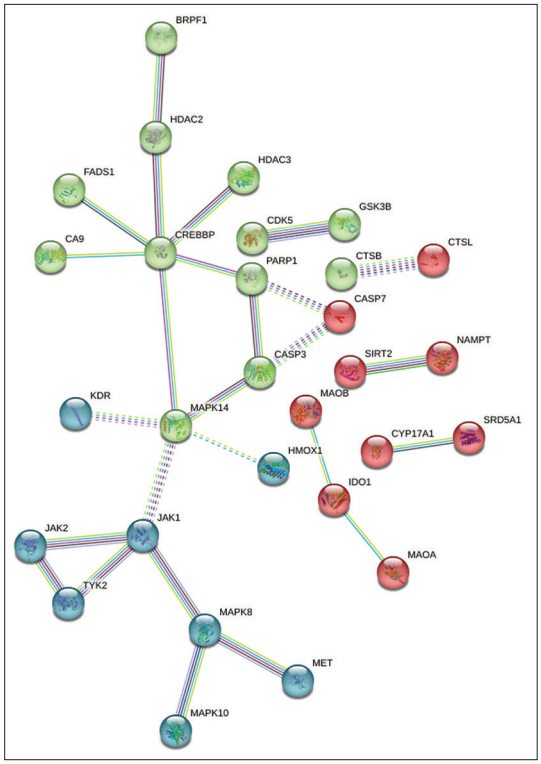


**Fig. 3: PPI network attained from KMEAN clustering**

**Table 1: The ligand name, canonical smiles, and pubchem ID of Osthole**

| Ligand name | Canonical smiles | PubChem CID |
|---|---|---|
| Osthole | CC(=CCC1=C (C=CC2=C1OC(=O) C=C2) OC) C | 10228 |

(http://admet.scbdd.com/). These studies evaluate the efficiency and biopharmaceutical features of drug candidates in parallel and have become standardized. The website of ADMETlab was released in 2018, which is armed with high-quality experimental data and tailored quantitative structure-property relationship models that allow the users to perform multiple drug-likeness analyses and the predictions of most ADMET-related properties. There are function modules on the platform which allows the users to perform different types of drug-likeness, ADMET endpoints prediction, and systematic evaluation, along with similarity search. This usually simplifies the drug discovery procedure by supporting primary drug-likeness evaluation, prioritization of chemical structures, and also rapid ADMET virtual screening or filtering. These modules are arranged in a user-friendly, freely available web interface. The SMILES which were retrieved for Osthole from PubChem were submitted to ADMETlab 2.0 for ADMET analysis where we can understand the pharmacological properties of Osthole [11,12].

## RESULTS

### Retrieval of ligand

Osthole which is also known as osthol, which is a first derivative of coumarin obtained from the plant known as *Cnidium*. There are a number of studies on Osthole which have already shown that it exhibits various numbers of pharmacological and biological effects, which also include anti-inflammation, antitumor, immunomodulator, neuroprotection, and hepatitis suppressor. The structural modifications of Osthole are mostly in the lactone ring, 7-methoxyl, 8-isopentenyl, 3,4-double bond of coumarin, or simultaneous modification of multiple sites. Hence, for Osthole, canonical SMILES and PubChem ID were retrieved, along with their 3D structures (Table 1).

### Target prediction

Target Predication was carried out using Swiss target prediction in which canonical SMILES for Osthole was passed as a query where Homo sapiens was selected as a species and also stich was used for predicting the targets. After the query was submitted, predicted targets were obtained for Osthole. It showed 100 targets for Osthole as per the probability bars (Table 2). The probability of the protein being a target for that query molecule is decided, which is basically considered as bioactive in nature. The values, which are equal to 1, most probably indicate that the molecule which is used as a query is actually known as active in nature.

### Protein-protein network construction and interaction

The database named STRING [13,14] was used to understand the protein-protein network and interaction where 100 targets were loaded, which was obtained from Swiss target prediction [15,16]. In this interaction, the score was set at the highest confidence, 0.900. Furthermore, in advanced settings, hiding the disconnected nodes was applied to the display network. Furthermore, for PPI networks, it was clustered into a specified number using KMEAN clustering where it was set by default. After parameters were included, it showed that 29 targets were found (Fig. 3) in a group and by referring to the edges and nodes of interactions, these CREBBP, IDO1, and MAPK8 targets were found to have maximum interactions. Other targets such as BRPF1, MAOB, CASP3, and JAK1 showed minimum interactions then the above one mentioned.

### Network stats

- Number of nodes: 93
- Number of edges: 26
- Average node degree: 0.559
- Avg. local clustering coefficient: 0.235
- PPI enrichment p=0.0405.

### Go enhancement evaluation

To perform Go enhancement evaluation, a ShinyGo tool was used for these 29 identified targets from the STRING database which was loaded. The pathways were displayed for biological process, molecular functions, and disease alliance categories, which were selected on the basis of a p-value cutoff which was set to <0.05, as shown in Fig. 4. Target proteins seen under the biological process such as bicarbonate transport, one carbon metabolic process, anion transport, and protein phosphorylation. The target proteins involved in the disease alliance such as breast cancer, Alzheimer's disease, Parkinson's disease, asthma, and Type 2 diabetes millets. For molecular functions, some of the examples are carbonate dehydratase activity, zinc ion binding, transition metal ion binding, kinase activity, molecular transducer activity, protein kinase activity, and many more.

### KEGG enhancement evaluation

KEGG enhancement evaluation was carried out using this ShinyGO tool. There were 100 potential targets from which 29 targets were found after clustering and adjusting to the highest confidence, which was used in the KEGG pathway enhancement study. This study showed the pathways for these targets (Fig. 5). Some of the examples are nitrogen metabolism, cell cycle, measles, apoptosis, viral carcinogenesis, pathway in cancer, metabolic pathways, cellular senescence, FoxO signaling pathway, Hepatitis B, Epstein–Barr virus infection, morphine addiction, prolactin signaling pathway, progesterone-mediated oocyte maturation, lL-17 signaling pathway, neuroactive ligand-receptor interaction, etc.

### Molecular docking

2LXT (UniProtKB: Q92793), 4U72 (UniProtKB: P14902), and 4YR8 (UniProtKB: P45983) are these potential targets which showed maximum interaction whose crystal structure was downloaded in the PDB format. As mentioned earlier for Osthole, the 3D structure was retrieved from PubChem and docking was performed against three proteins which were exhibited as potential targets. The binding

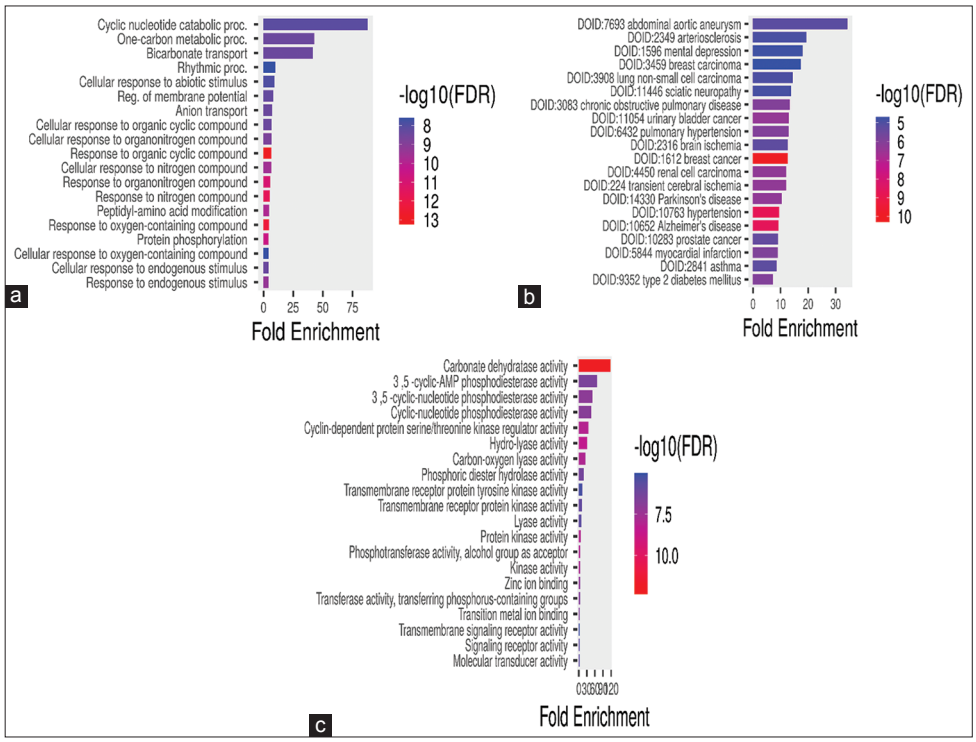


**Fig. 4: GO enrichment analysis of target genes. (a) Biological process (BP), (b) Disease alliance (DA), and (c) Molecular function (MF)**

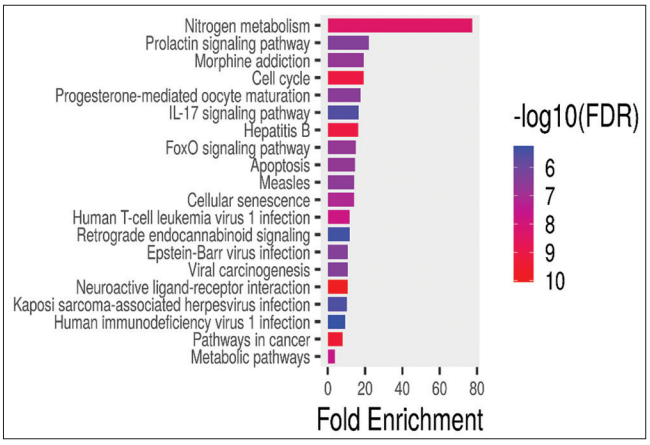


**Fig. 5: KEGG enhancement evaluation of target genes**

affinities of the proteins for Osthole are shown in Table 3. 4U72 showed the highest binding affinity.

**Visualization**

Visualization was carried out for docked proteins using BIOVIA Discovery Studio. In this, it provided detailed information about the interactions, bond distances, and amino acid residues which were obtained by 3D and 2D visualization (Figs. 6 and 7).

**ADMET analysis**

ADMET analysis was carried out which indicated that Osthole contains all drug-likeness properties which were confirmed through ADMET analysis.

**DISCUSSSION**

Network biology and polypharmacology approaches have gained a lot of appreciation recently as they play a significant role in omics data integration and multitarget drug development. The new approach of pharmacology has successfully addressed the two main factors in drug

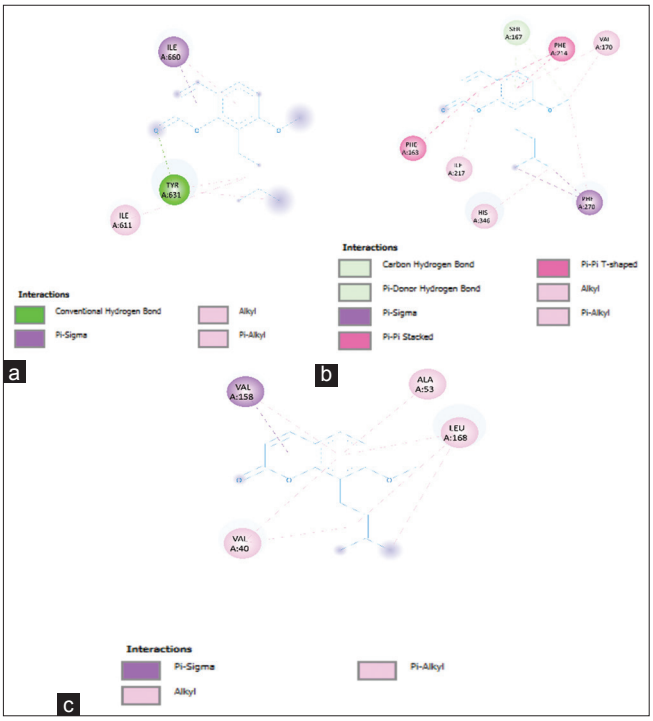


**Fig. 6: 2D visualization images of target proteins with RUTIN. (a) 2LXT, (b) 4U72, and (c) 4YR8**

development, that is efficacy and toxicity. However, the rational design of polypharmacology has faced a lot of difficulties for new methods which are used to validate target combinations and also it optimizes multiple structure-activity relationships that can maintain drug-like properties. The combination of these two approaches has created a novel paradigm which is known as network pharmacology (NP) which involves the effect of drugs on both the disease and the interactome level. NP has been developed in which it tries to understand drug actions

**Table 2: Potential genes targeted by Osthole**

| Gene | UniProt ID | Description |
|---|---|---|
| XPO1 | O14980 | Exportin-1 |
| PDE4D | Q08499 | Phosphodiesterase 4D |
| SRD5A1 | P18405 | Steroid 5-alpha-reductase 1 |
| CA9 | Q16790 | Carbonic anhydrase IX |
| CA7 | P43166 | Carbonic anhydrase VII |
| CA12 | O43570 | Carbonic anhydrase XII |
| CA13 | Q8N1Q1 | Carbonic anhydrase XIII |
| CA1 | P00915 | Carbonic anhydrase I |
| HRH2 | P25021 | Histamine H2 receptor |
| ADRA1D | P25100 | Alpha-1d adrenergic receptor |
| ADORA2A | P29274 | Adenosine A2a receptor |
| ESR2 | Q92731 | Estrogen receptor beta |
| NAMPT | P43490 | Nicotinamide phosphoribosyltransferase |
| TAAR1 | Q96RJ0 | Trace amine-associated receptor 1 (by homology) |
| MET | P08581 | Hepatocyte growth factor receptor |
| MAOB | P27338 | Monoamine oxidase B |
| TYK2 | P29597 | Tyrosine-protein kinase TYK2 |
| PDE7A | Q13946 | Phosphodiesterase 7A |
| MAOA | P21397 | Monoamine oxidase A |
| GRM2 | Q14416 | Metabotropic glutamate receptor 2 (by homology) |
| PDE9A | O76083 | Phosphodiesterase 9A |
| GRM5 | P41594 | Metabotropic glutamate receptor 5 (by homology) |
| PABPC1 | P11940 | Polyadenylate-binding protein 1 |
| GABRB3 GABRG2 GABRA5 | P28472 P18507 P31644 | GABA-A receptor; alpha-5/beta-3/gamma-2 |
| JAK2 | O60674 | Tyrosine-protein kinase JAK2 |
| KDR | P35968 | Vascular endothelial growth factor receptor 2 |
| P2RX7 | Q99572 | P2X purinoceptor 7 |
| KCNN4 | O15554 | Intermediate conductance calcium-activated potassium channel protein 4 |
| TNKS2 | Q9H2K2 | Tankyrase-2 |
| TRPV1 | Q8NER1 | Vanilloid receptor |
| CCNE2 CDK2 CCNE1 | O96020 P24941 P24864 | Cyclin-dependent kinase 2/cyclin E |
| CCNB3 CDK1 CCNB1 CCNB2 | Q8WWL7 P06493 P14635 O95067 | Cyclin-dependent kinase 1/cyclin B |
| MST1R | Q04912 | Macrophage-stimulating protein receptor |
| PDE10A | Q9Y233 | Phosphodiesterase 10A |
| RGS4 | P49798 | Regulator of G-protein signaling 4 |
| EPHX1 | P07099 | Epoxide hydrolase 1 |
| KCNE1 KCNQ1 | P15382 P51787 | Voltage-gated potassium channel, IKs; KCNQ1(Kv7.1)/KCNE1(MinK) |
| PDE4B | Q07343 | Phosphodiesterase 4B |
| HMOX1 | P09601 | Heme oxygenase 1 |
| ACE | P12821 | Angiotensin-converting enzyme (by homology) |
| CA3 | P07451 | Carbonic anhydrase III |
| CA14 | Q9ULX7 | Carbonic anhydrase XIV |
| CA5B | Q9Y2D0 | Carbonic anhydrase VB |
| GSK3B | P49841 | Glycogen synthase kinase-3 beta |
| PDE2A | O00408 | Phosphodiesterase 2A |
| CNR2 | P34972 | Cannabinoid receptor 2 |
| CDK7 CCNH | P50613 P51946 | Cyclin-dependent kinase 7/cyclin H |
| GRM4 | Q14833 | Metabotropic glutamate receptor 4 |
| PARP1 | P09874 | Poly [ADP-ribose] polymerase-1 |
| PRMT3 | O60678 | Protein arginine N-methyltransferase 3 |
| IDO1 | P14902 | Indoleamine 2,3-dioxygenase |
| MMP13 | P45452 | Matrix metalloproteinase 13 |
| MMP1 | P03956 | Matrix metalloproteinase 1 |
| TUBB1 | Q9H4B7 | Tubulin beta-1 chain |
| MAPK8 | P45983 | c-Jun N-terminal kinase 1 |
| SLC8B1 | Q6J4K2 | Sodium/potassium/calcium exchanger 6, mitochondrial |
| PARP2 | Q9UGN5 | Poly [ADP-ribose] polymerase 2 |
| SIRT2 | Q8IXJ6 | NAD-dependent deacetylase sirtuin 2 |
| CHEK1 | O14757 | Serine/threonine-protein kinase Chk1 |
| CYP17A1 | P05093 | Cytochrome P450 17A1 |
| MAPK10 | P53779 | c-Jun N-terminal kinase 3 |
| NAT1 | P18440 | Arylamine N-acetyltransferase 1 |
| CREBBP | Q92793 | CREB-binding protein/p53 |
| KDM4C | Q9H3R0 | Lysine-specific demethylase 4C |
| TBXAS1 | P24557 | Thromboxane-A synthase |
| THRB | P10828 | Thyroid hormone receptor beta-1 |
| CCNE1 CDK2 | P24864 P24941 | Cyclin-dependent kinase 2/cyclin E1 |
| IMPDH2 | P12268 | Inosine-5'-monophosphate dehydrogenase 2 |
| FNTA FNTB | P49354 P49356 | Protein farnesyltransferase |

*(Contd...)*

**Table 2:** ***(Continued)***

| Gene | UniProt ID | Description |
|---|---|---|
| PGGT1B FNTA | P53609 P49354 | Geranylgeranyl transferase type I |
| CASP3 | P42574 | Caspase-3 |
| CASP7 | P55210 | Caspase-7 |
| NQO2 | P16083 | Quinone reductase 2 |
| CDK5 | Q00535 | Cyclin-dependent kinase 5/CDK5 activator 1 |
| CDK5R1 CDK5 | Q15078 Q00535 | Cyclin-dependent kinase 5/CDK5 activator 1 |
| EPHX2 | P34913 | Epoxide hydratase |
| LIPE | Q05469 | Hormone sensitive lipase |
| BRD4 | O60885 | Bromodomain-containing protein 4 |
| BRD2 | P25440 | Bromodomain-containing protein 2 |
| BRDT | Q58F21 | Bromodomain testis-specific protein |
| BRD3 | Q15059 | Bromodomain-containing protein 3 |
| JAK1 | P23458 | Tyrosine-protein kinase JAK1 |
| MCHR1 | Q99705 | Melanin-concentrating hormone receptor 1 |
| MGLL | Q99685 | Monoglyceride lipase |
| CSF1R | P07333 | Macrophage colony stimulating factor receptor |
| CTSC | P53634 | Dipeptidyl peptidase I |
| CTSS | P25774 | Cathepsin S |
| BDKRB2 | P30411 | Bradykinin B2 receptor |
| CTSL | P07711 | Cathepsin L |
| CTSB | P07858 | Cathepsin (B and K) |
| EPHB3 | P54753 | Ephrin type-B receptor 3 |
| TSPO | P30536 | Translocator protein (by homology) |
| MAPK14 | Q16539 | MAP kinase p38 alpha |
| TGFBR1 | P36897 | TGF-beta receptor type I |
| QPCT | Q16769 | Glutaminyl-peptide cyclotransferase |
| FADS1 | O60427 | Fatty acid desaturase 1 |
| PIP4K2C | Q8TBX8 | Phosphatidylinositol-5-phosphate 4-kinase type-2 gamma |
| HDAC3 | O15379 | Histone deacetylase 3 |
| HDAC2 | Q92769 | Histone deacetylase 2 |
| BRPF1 | P55201 | Peregrin |

**Table 3: Binding affinity of target proteins toward Osthole**

| Ligand | Binding affinity | | |
|---|---|---|---|
| | 2LXT | 4U72 | 4YR8 |
| Osthole | −6.1 | −7.8 | −6.9 |

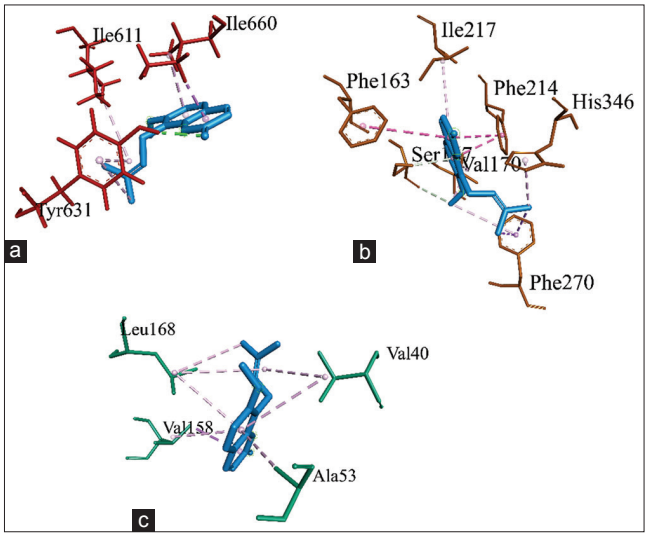


**Fig. 7: 3D visualization images of target proteins with Osthole. (a) 2LXT, (b) 4U72, and (c) 4YR**

and interactions with multiple targets. It also uses computational power to systematically catalogue molecular interactions of a drug molecule in a living cell. NP has been considered as an important tool for understanding the underlying complex relationships between botanical formulas and the whole body. Furthermore, it discovers new drug leads and targets and to repurpose existing drug molecules for various therapeutic conditions which allow, to be unbiased during the investigation of potential target spaces. However, this requires some kind of guidance which will help to select the right type of targets and new scaffolds for the drug molecules. As traditional knowledge plays a significant role during this process of formation of discovery and repurposing existing drugs [17]. This can be achieved by combining advances in systems biology and NP, which might be possible to rationally design the next generation of promiscuous drugs. NP analysis not only unlocks the options for new therapeutics, but it also helps to improve the safety and efficacy of existing medications [18,19].

Osthole is used for clinically absorbing as a component of medicinal plants and herbs, which is known for many pharmacological and biological activities. According to the new sources related to Osthole and its analogues from other sources, the pharmacological roles of these compounds have shown versatility and Osthole has also been selected for development as a hepatoprotectant, as well as an antipruritic [20,21]. Apparently, Osthole has shown an important role to play in brain function, liver health, and vasodilation, and also for more comprehensive and wider applications such as antihepatitic, anti-oxidation, anti-tumor, and cardiovascular agents, and immunity-strengthening. Due to these broad characteristics and applications, Osthole can be a well-developed lead compound for various disease therapies depending on the modifications and optimizations [22-26].

Go evaluations were analyzed into three sections: Biological process, molecular function, and disease alliance. The biological process showed cyclic nucleotide catabolic process which is a chemical reaction and pathways that result into breakdown of a cyclic nucleotide which can lead to hematological malignancies, epithelial tumors, ancer, for one carbon metabolic process which mediated by the folate cofactor, supports multiple physiological processes this can cause neural tube defects, hematopoiesis, or it can also lead to shuttling in liver and kidney

and bicarbonate transport is not freely permeable to membranes although bicarbonate must be moved across membranes, as part of $CO_2$ metabolism and to regulate cell pH this can cause cystic fibrosis, immune disorders, tumorigenesis, kidney diseases, etc. The molecular function presented carbonate dehydratase activity which reversibly catalyzes the hydration of carbon dioxide to HCO3− and H+ ions that can lead to glaucoma, idiopathic intracranial hypertension, altitude sickness, for 3' 5'-cyclic-amp phosphodiesterase activity that regulate the intracellular levels of cAMP and cGMP which can lead to cardiovascular system, fertility, immunity, and cancer. The disease alliance analysis showed arteriosclerosis, renal cell carcinoma, Alzheimer's, hypertension, breast cancer, urinary bladder cancer, Parkinson's disease, myocardial infarction, breast carcinoma, etc. The KEGG analysis showed Nitrogen metabolism one of the processes of plant physiology and also one of the important parts of global chemical cycle which can lead to lung adenocarcinoma, prolactin signaling pathway is activated by prolactin-PRLR interaction is the JAK/STAT pathway that can cause tumorigenesis, reproductive abnormalities, and diabetes, Cell cycle is a series of events that takes place in a cell as it grows and divides which may lead to Glaucoma and other retinal disorders, lL-17 signaling pathway initiates signaling through Act1-induced K63-linked ubiquitylation of TRAF6 which can cause diseases such as psoriasis, rheumatoid arthritis (RA), multiple sclerosis, scleroderma, and apoptosis are known to be a form of planned cell death that occurs in multicellular organisms which can lead to Alzheimer's disease, Huntington's disease, and Parkinson's disease [27].

## CONCLUSION

Osthole was used for NP and molecular docking which determined the vast range of pharmacological effects. As the investigations took place to see whether Osthole's mechanism of action could be used to be changed to form high-potential anticancer, Alzheimer, and tumor-related medications. This showed the different viewpoint on Osthole research which can lead to the development and therapeutic usage.

## ACKNOWLEDGMENT

I would like to acknowledge BioNome in Bengaluru, India, to provide their guidance and assist throughout the research studies. I would also like to thank Ms. Susha Dinesh for her assistance with the project.

## AUTHORS CONTRIBUTION

All authors contributed equally to the manuscript.

## CONFLICTS OF INTERESTS

Nil.

## AUTHORS FUNDING

Nil.

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