Egyptian Journal of Chemistry

http://ejchem.journals.ekb.eg/

Exploring The Potential Targets and Mechanisms of Vitexdoins Family Against Polycystic Ovary Syndrome Based on Network Pharmacology

Herlina Simanjuntak[a], Novarianti Marbun[b], Hariyadi Dharmawan Syahputra[c,*], Iksen Iksen[c,*], Nasri Nasri[c]

[a]Department of Midwifery, Sekolah Tinggi Ilmu Kesehatan Senior Medan, Medan 20141, Indonesia
[b]Faculty of Pharmacy, Institut Kesehatan Deli Husada, Deli Serdang 20355, Indonesia
[c]Department of Pharmacy, Sekolah Tinggi Ilmu Kesehatan Senior Medan, Medan 20141, Indonesia

Abstract

The effects of polycystic ovarian syndrome (PCOS) on women's health and happiness are substantial. Traditional medicines and natural products have gained popularity as potential anti-PCOS therapy due to their efficacy with fewer side effects. To properly map the molecular targets of natural products against a wide variety of illnesses, including PCOS, it has become obvious that network pharmacology investigations will be required. The purpose of this study was to use network pharmacology to better understand the pharmacological underpinnings of the action of the Vitexdoins family in the treatment of PCOS. Both the primary Vitexdoin family and its putative targets were retrieved from the PubChem and SwissTargetPrediction databases. The GeneCards and STRING databases were scoured for PCOS-related genes and known protein-protein interaction networks. Finally, Gene Ontology and Kyoto Encyclopedia of Genes and Genomes analyses were used to discover the mechanism of action of Vitexdoins by identifying important pathways and functions of these networks. The Vitexdoins family consists of 5 compounds, and these compounds matched 116 PCOS-related targets. The most important core targets of Vitexdoins against PCOS were further analyzed and found to be SRC, HSP90AA1, PIK3CA, EGFR, and STAT3. A total of 10 important pathways in PCOS and its treatment were found by pathway enrichment analysis. These included the pathway of EGFR tyrosine kinase inhibitors, endocrine resistance, PI3K-AKT, focal adhesion, and progesterone-mediated oocyte maturation. In conclusion, our network pharmacological study provides a theoretical framework for future investigations into the possible anti-PCOS effects of the Vitexdoins family.

Keywords: Network Pharmacology; PCOS; Vitexdoins

1. Introduction

Hyperandrogenism and chronic anovulation characterize polycystic ovarian syndrome (PCOS), one of the most common endocrine and metabolic illnesses in premenopausal women [1]. It is unknown what the precise cause of PCOS is. Insulin resistance, low-grade inflammation, genetics, and an excess of testosterone are some of the factors that might play a part [2]. These factors might interfere with ovulation, resulting in the ovary not developing regularly and not being released from the follicles where they form. Other factors that might play a role include heredity. Symptoms of PCOS, which affect anywhere from 5-20 percent of reproductive-age women, typically begin in early adolescence and include irregular menstrual cycles, acne, overweight, and infertility [3]. Surgery, long-term oral contraceptives, and ovulation stimulation are the mainstays of current PCOS treatment [4]. However, these methods have significant drawbacks, including high invasiveness, high recurrence rates, poor long-term effectiveness, and various adverse effects [5]. Because of this, it is imperative that an effective therapy for PCOS be developed. Alternative medicine from plants has a long history of usage in the treatment of PCOS and a low incidence of adverse effects and stable efficacy

which may therefore provide an effective treatment option [6].

Legundo (*Vitex negundo* L.) is one of the many types of plants that live in the tropics. It has been found to exhibit powerful pharmacological qualities such as anticancer, antioxidant, anti-inflammatory, anti-rheumatic, antibiotic, hepatoprotective, anticonvulsant, antidiabetic, snake venom neutralization, and anti-allergic activities [7-9]. Additionally, Legundo has been shown to help women as an anti-androgen and anti-inflammatory to prevent hirsutism-causing effects with acne and irregular periods [10]. Legundo consists of various types of chemical compounds, especially the Vitexdoin family including Vitexdoin A, Vitexdoin B, Vitexdoin C, Vitexdoin D, and Vitexdoin E. In this regard, this family of Vitexdoin may be effective in treating PCOS [11]. However, the effect of the Vitexdoin family on PCOS remains unknown.

In recent years, network pharmacology has been used to study how alternative medicines work to treat diseases [12]. Network pharmacology focuses on the multi-pathway regulation of signaling pathways, which fits with the characteristics of multi-component, multi-target, and multi-pathway [13]. Thus, based on network pharmacology, we aimed to explore the complex mechanism of action of the Vitexdoin family on PCOS. This study predicted the mechanisms of Vitexdoin family action on PCOS using network pharmacology. The evidence provided from the preliminary investigation by using the network pharmacology approach might indicate that the Vitexdoin family may be a promising new clinical treatment for PCOS.

2. Experimental
2.1. Identification of potential targets from the Vitexdoin family

We investigated the Vitexdoin family that had been described in the previous research, and after doing so, we chose five compounds for more investigation. It was decided to retrieve and export the canonical simplified molecular input line entry specification (SMILES) for five different compounds (Vitexdoin A, Vitexdoin B, Vitexdoin C, Vitexdoin D, and Vitexdoin E) from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/). We loaded the aforementioned data into the SwissTargetPrediction database (http://www.swisstargetprediction.ch/), which allowed us to search for the targets that correlate

to these compounds.

2.2. ADMET study of the Vitexdoin family
The prediction of physicochemical properties such as molecular weight (MW), log P, oral bioavailability, and pharmacokinetic and toxicity profiles (ADMET) were performed by inputting the canonical SMILES from each compound into the free access web server of and pkCSM tool (https://biosig.lab.uq.edu.au/pkcsm/).

2.3. PCOS targets identification
The targets for PCOS were taken from the GeneCards database, which may be found online at https://www.genecards.org/. During the course of our analysis, the human disease target databases were searched for targets related to PCOS using the keywords "PCOS" in conjunction with the species "*Homo sapiens*". The target genes associated with PCOS were obtained after the deletion of the duplicated genes; the common targets associated with PCOS. The prospective targets of bioactive substances (Vitexdoin family) were identified as Vitexdoins targets against PCOS.

2.4. Construction of Venn diagram and compounds-targets network
The intersection between the Vitexdoins and PCOS targets was established by using Venny 2.1, (https://bioinfogp.cnb.csic.es/tools/venny/). By using the 116 targets related to interception, the result was imported into Cytoscape version 3.10 (https://cytoscape.org/) to build the compounds-target network.

2.5. Construction of protein-protein network interaction
The STRING v_11.5 database (https://string-db.org/) was used to develop a PPI network by having 116 targets linked to Vitexdoins against PCOS uploaded to it. The parameters for the PPI network's construction were constrained in accordance with the "*Homo sapiens*" model, and the level of confidence associated with the interaction between the targets was increased to 0.900 in order to reflect the maximum possible level of certainty. The nodes of the network stood in for individual proteins, while the edges portrayed the various ways in which proteins might interact with one another. The results from the STRING database were then imported into Cytoscape

version 3.10, where it was used to build a PPI network for subsequent analysis. This was accomplished with the use of an additional CytoHubba plugin, which identified the five primary targets.

2.6. Investigation of Gene Ontology and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis

STRING database was used to perform functional annotation analysis and pathway enrichment analysis on proteins from the combined Vitexdoins and PCOS. This analysis included GO and KEGG pathway enrichment analysis. After obtaining the data from STRING, it was then uploaded into RStudio so that it could be used to generate the bubble plot by using R language tools. The GO enrichment study consists of a biological process (BP), a molecular function (MF), and a cellular component (CC), all of which display 10 items that are exceptionally rich within their respective categories. In a manner analogous to that of the GO analysis, the findings of the top 10 KEGG pathway enrichment were utilized to explain the possible molecular mechanism by which Vitexdoins are responsible for PCOS. All of the enrichment analyses in this study used a threshold of P less than 0.01 to determine whether or not the results were statistically significant.
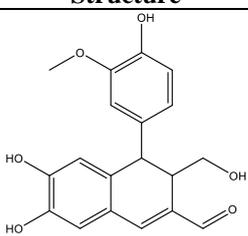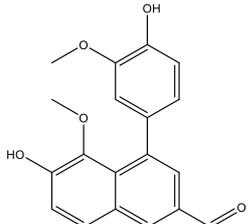
3. Results and Discussion
## 3.1. Screening of molecular properties and ADMET of Vitexdoins

The Vitexdoin family has five members that were found in the PubChem database. About 90% of innovative drug candidates do not make it through testing in the drug development process. This is typical because of concerns with toxicity, poor absorption or bioavailability or ineffectiveness [14]. In spite of the fact that the ADME properties of the drug are extremely important, conducting biological tests on each and every potential candidate drug is unfeasible due to the high costs and amount of time needed [15]. Because of this, in recent years the attention has switched to bioinformatics in order to identify the pharmacokinetic features of potential medications [16]. We established various factors as the criteria for screening the active compound in order to increase the likelihood of discovering fully active compounds. These metrics include molecular weight, log P, and oral bioavailability (OB). According to what can be seen in Table 1, all of the compounds that were chosen satisfied the requirements set forth by Lipinski for good drug candidates, which include having a molecular weight not exceeding 500, a log P value that is lower than 5 and an OB that is higher than 30%.

As observed in Table 2, when it comes to the pharmacokinetic characteristics that include aspects like absorption, distribution, metabolism, excretion, and toxicity, the selected compounds are worth noting that both compounds fall within the acceptable range of standard pharmacokinetic value. All the compounds have the potential to become the substrates for CYP2D6 and undergo metabolism in the liver. However, in toxicity, all the Vitexdoins family except Vitexdoin A might induce mutagenicity and therefore act as a carcinogen which can be seen in a positive value for AMES toxicity. In general, all Vitexdoins family don't have any toxicity heart, liver, and skin which are safe to be used as a novel drug candidate

Table 1. Vitexdoin family and their molecule properties

Compound	Structure	MW	Log P	Oral Bioavailability (%)
Vitexdoin A		342.3	2.14	55
Vitexdoin B		324.3	3.74	55

_____
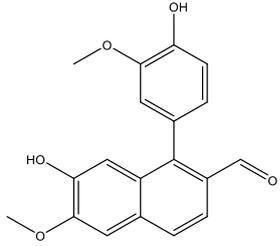
Vitexdoin C   324.3   3.75   55
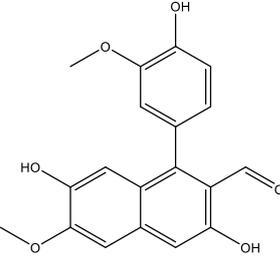
Vitexdoin D   340.3   3.45   55
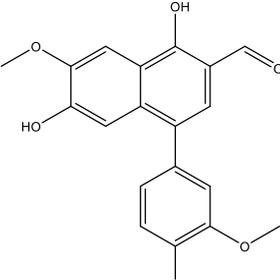
Vitexdoin E   340.3   3.45   55

Table 2. Vitexdoin family and their molecule properties

ADMET	Vitexdoin A	Vitexdoin B	Vitexdoin C	Vitexdoin D	Vitexdoin E
Intestinal absorption	69.222	94.176	94.928	91.996	94.101
BBB permeability	-1.1	-0.355	-0.273	-1.111	-1.109
CYP2D6 substrate	No	No	No	No	No
CYP3A4 substrate	Yes	Yes	Yes	Yes	Yes
Total Clearance	0.002	0.126	0.111	-0.001	-0.006
Renal OCT2 substrate	No	No	No	No	No
AMES toxicity	No	Yes	Yes	Yes	Yes
hERG I inhibitor	No	No	No	No	No
hERG II inhibitor	No	Yes	Yes	Yes	Yes
Hepatotoxicity	No	No	No	No	No
Skin sensitization	No	No	No	No	No

3.2. Identification of potential targets

Through their unique properties, natural products can be used to employ multi-targeted therapy techniques against a wide range of disorders [17,18]. By using the SwissTargetPrediction database which is an online database that uses reverse pharmacophore alignment to identify prospective drug targets by matching the query compound to a large internal pharmacophore model library [19] resulting in a total of 217 potential targets of 5 main families of Vitexdoins and 4795 PCOS targets were obtained from the GeneCards database were collected. As shown in the Venn diagram in Figure 1, a total of 116 potential anti-PCOS targets were obtained through a combined collection of common targets. These predicted targets were subjected to further investigation related to the protein-protein interaction network.

A total of data pairs of Vitexdoins family and PCOS target genes were prepared by using Cytoscape v 3.10 and the compounds-targets interaction network was constructed as shown in Figure 2. In this network, the yellow oval represents the Vitexdoins family, while the blue rectangle represents the target related to PCOS. To see the most important compound, the number of edges was used as the indicator. The more

_____

edges connected to the compound indicate that the compound is more important in the network. According to our results, both Vitexdoin D and Vitexdoin C are linked to more than 100 target genes, which are considered to be the main active components from the Vitexdoins family against PCOS.
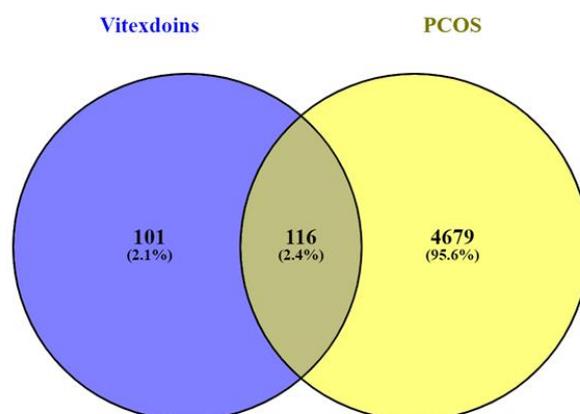
Fig. 1. Venn diagram of the potential anti-PCOS targets from the Vitexdoins family.
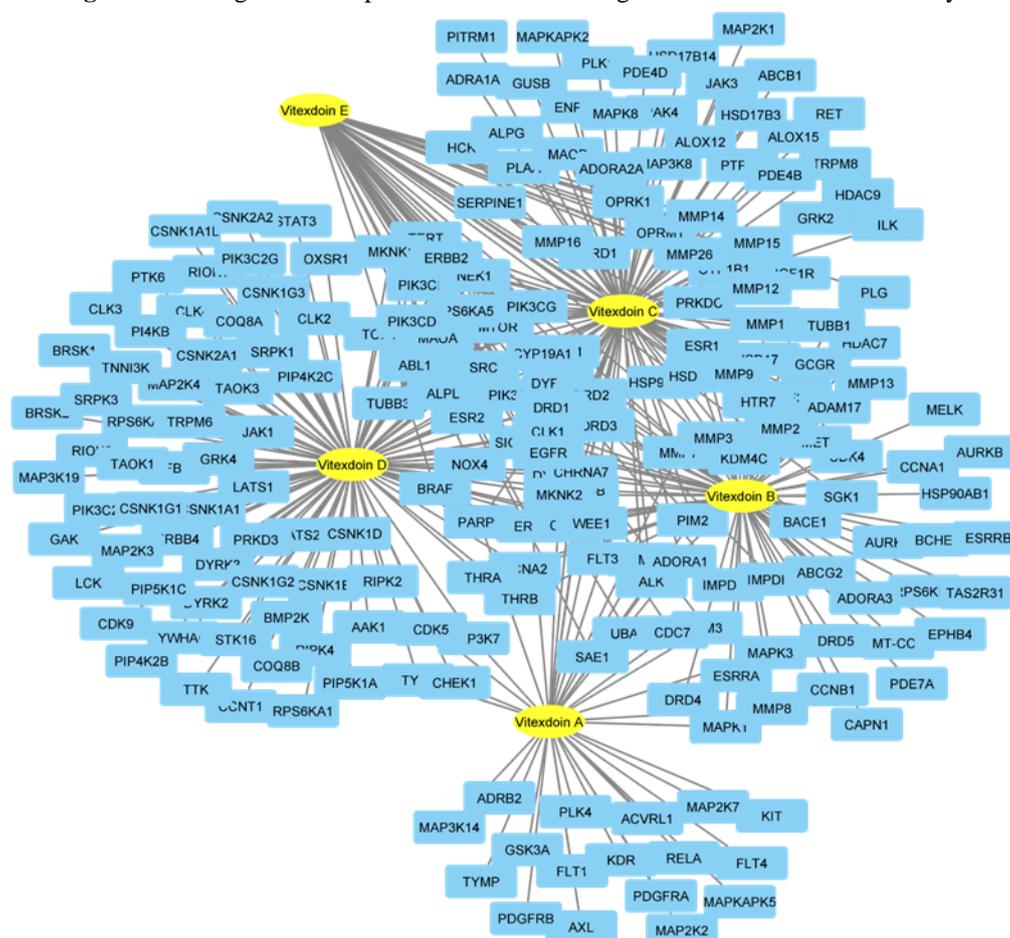
Fig. 2. The Compound-Target Network constructed by Cytoscape v.3.10.

3.3. Analysis of PPI and primary targets

The probable targets that were acquired from the Venn diagram were then imported into the online database that is part of the STRING database by selecting *Homo sapiens* as the species and setting the required score to 0.9. Following the completion of the analysis, the protein-protein interaction (PPI) network was recovered, and the results are presented in Figure

_____

_____

3A. The PPI network illustrates the interaction that occurs between the targets, with highly linked proteins in the network having a greater number of lines that interact with one another. 116 nodes and 227 edges make up the PPI, and its enrichment p-value is less than 1.0e-16. The primary cluster containing the primary proteins is shown in Figure 3B. This cluster is the most critical network in the PPI system, and it is one of the primary targets in the fight against PCOS.

In order to gain a deeper and more comprehensive understanding of the network described above, a topological analysis was performed using a Cytoscape plugin called CytoHubba. This analysis displayed the top 5 proteins in terms of the number of degrees, as shown in Figure 3C. These proteins are the most important in the network, and they represent the main targets of the Vitexdoins family against PCOS.
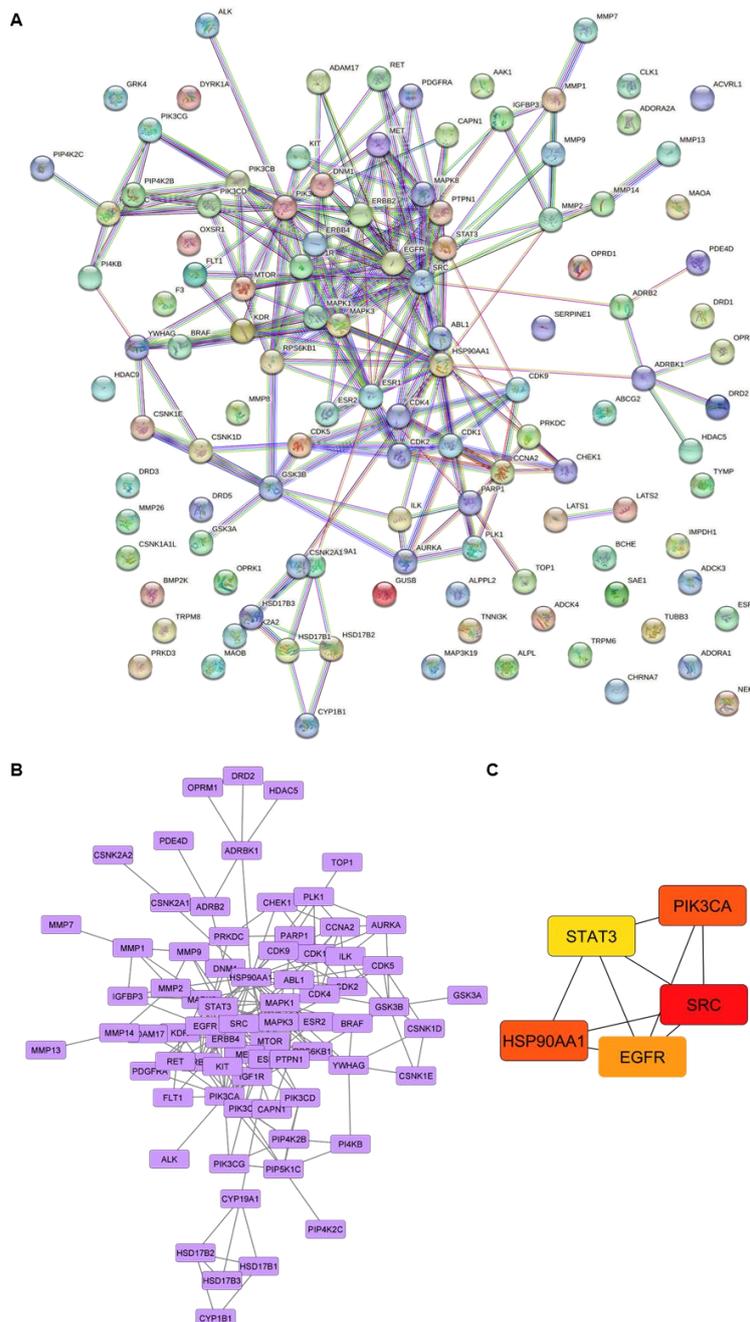
Fig. 3. PPI and hub genes of Vitexdoins family against PCOS. (A) Interaction between proteins from STRING. (B) Interaction between these genes as the main cluster (C) Genes with highest node degrees. Colors ranging from red to yellow indicate a higher to lower score of degree.

_____

Our results showed that the 5 core hub proteins identified as therapeutic targets were Proto-Oncogene c-Src (SCR), Heat Shock Protein 90 Alpha Family Class A Member 1 (HSP90AA1), Phosphatidylinositol-4,5-Bisphosphate 3-Kinase Catalytic Subunit Alpha (PIK3CA), Epidermal Growth Factor Receptor (EGFR), and Signal Transducer and Activator of Transcription 3 (STAT3). In humans, the SRC gene is responsible for encoding the SRC protein, which is a non-receptor tyrosine kinase protein [20]. It has been hypothesized that an increased level of SRC activity may be connected to acute inflammation, which may in turn promote other signals [21]. Inflammation is the primary cause of polycystic ovary syndrome (PCOS). The presence of an abundance of aggressive macrophages in unhealthy follicles is one factor that contributes to a state of proinflammatory ovarian activity that is produced by androgens [22]. PCOS patients have an increased serum HSP90, which has been proven to have a positive correlation with markers of oxidative stress and inflammation. HSP90AA1 is the primary component of heat shock proteins, and these proteins have been demonstrated to be responsible for the increased serum HSP in PCOS patients. It was found that dysfunctional polyps with acute to chronic inflammation were associated with overexpression of HSP90AA1[23]. PI3Ks are responsible for regulating a number of important events that take place throughout the inflammatory response to both tissue injury and infection [24]. Leukocytes have a high level of expression of PI3K, which plays a crucial function in the chemokine-mediated recruitment and activation of innate immune cells in sites of inflammation [25]. The activation of PI3K will result in the upregulation of its downstream molecule, which is called AKT [26]. This will result in an increase in the regulation of cell function, which will stimulate glucose metabolism, prevent the activation of an apoptotic cascade, promote cell survival, and increase proliferation, all of which were related to the formation of ovarian cysts [27]. In addition to the proteins that might influence the formation of PCOS, a number of studies have shown that the epidermal growth factor receptor (EGFR), which is responsible for the activity of epidermal growth factor, is involved in the inflammatory response in a number of illnesses that include inflammation, such as cancer, uncontrolled PCOS, kidney failure, and so on [28]. Through its promotion of pro-oncogenic inflammatory factors, STAT3 plays a part in multiple behaviors associated with inflammation and immunity. In patients with PCOS, this STAT3 pathway was engaged in the formation of follicles [29].

3.4 GO and KEGG analysis

Next, using the STRING database to enrich GO and KEGG pathways, the biological functions and pathways of Vitexdoins family targets against PCOS were identified. Biological process, molecular function, cellular component, and KEGG with the highest corrected p-values are analyzed by using RStudio. According to the degree of significance, the top ten associated functions in biological processes that participated in the targets included protein phosphorylation, phosphorylation, phosphate-containing compound metabolic process, regulation of signaling, regulation of cell communication, metabolic process, regulation of signal transduction, organic substance metabolic process, peptidyl-amino acid modification, and organonitrogen compound metabolic process (Figure 4A). For enrichment analysis of molecular function, these targets were involved in kinase activity, phosphotransferase activity, alcohol group as acceptor, protein kinase activity, adenyl ribonucleotide binding, ATP binding, nucleotide binding, protein serine/threonine kinase activity, purine ribonucleotide binding, purine ribonucleoside triphosphate binding, and small molecule binding (Figure 4B). For the cellular component, targets were mainly found in the dendrite, cytoplasm, somatodendritic compartment, cell junction, synapse, cell projection, neuron projection, plasma membrane-bounded cell projection, axon, and cytosol (Figure 4C). KEGG pathway enrichment analysis showed that the top 10 pathways in which the identified targets participated are shown (Figure 5A). The pathways relevant to cancer pathogenesis were EGFR tyrosine kinase inhibitor resistance, endocrine resistance, pathways in cancer, ErbB signaling pathway, proteoglycans in cancer, breast cancer, prostate cancer, PI3K-Akt signaling pathway, focal adhesion, and progesterone-mediated oocyte maturation. An extensive literature review revealed that these signaling pathways are either directly or indirectly linked to PCOS, suggesting that they may be strongly associated with the mechanism of anti-PCOS impact of the Vitexdoins family. The KEGG pathway also enriched the pathway related to EGFR and several downstream, suggesting that the Vitexdoins family

_____

may play an anti-lung cancer role by suppressing
EGFR in PCOS (Figure 5B).
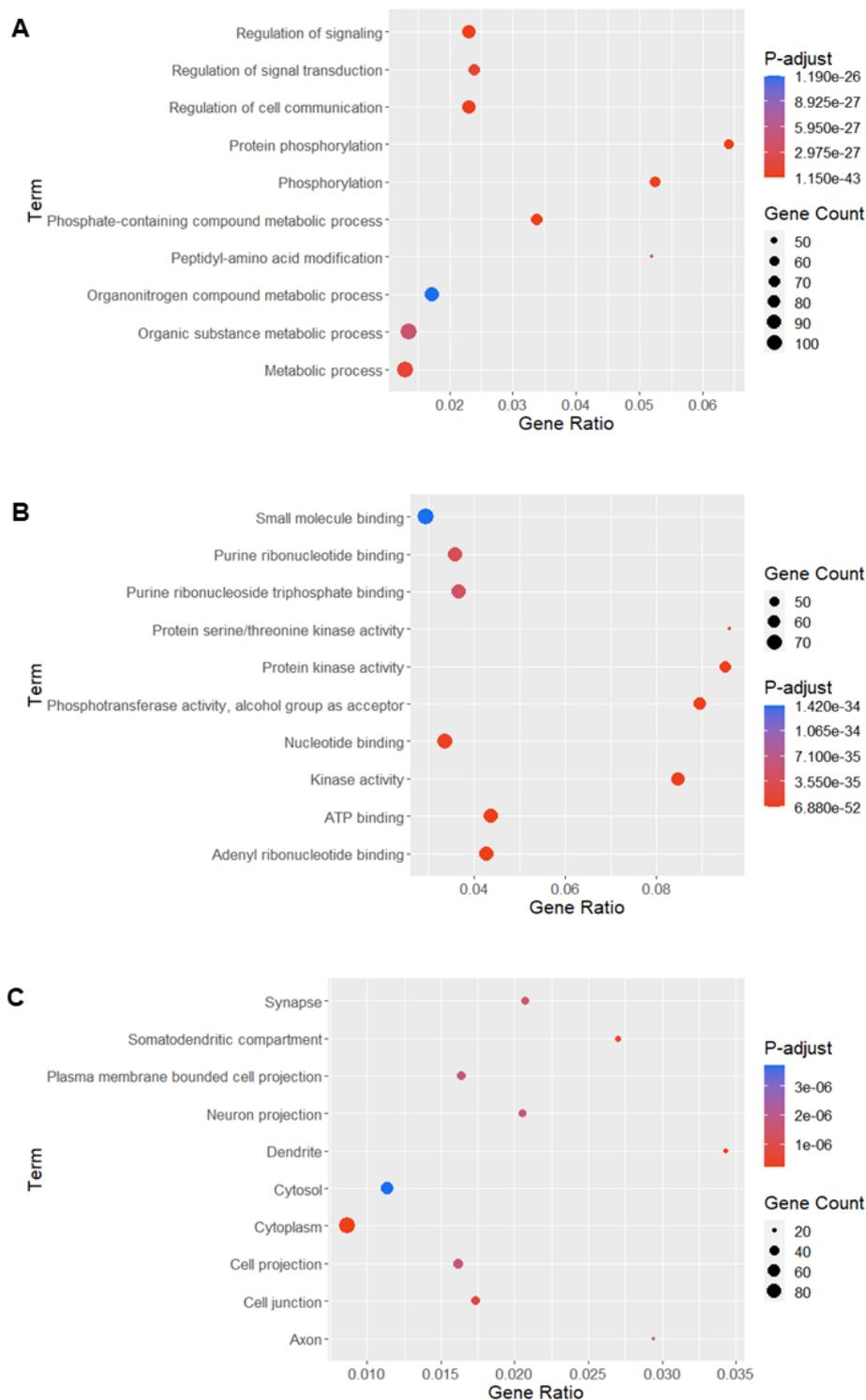
Fig. 4. Bubble plot of GO functional enrichment analysis. (A) Biological process. (B) Molecular function. (C) Cellular component.

_____

Fig 5. KEGG pathway enrichment analysis of Vitexdoin family against PCOS. (A) Bubble plot of KEGG pathway. (B) The potential pathway related to EGFR for anti-PCOS effects of Vitexdoins family.

4. Conclusions

This study is the first to apply the network pharmacology approach to study the potential targets and key action mechanisms of the Vitexdoins family against PCOS. The results showed that 5 main families of Vitexdoins had potential anti-PCOS activity, involving 116 target genes related to PCOS. Our results showed that SRC, HSP90AA1, PIK3CA, EGFR, and STAT3 are the hub genes of the Vitexdoins family in the treatment of PCOS. The mechanism of the Vitexdoins family against PCOS is related to several pathways, and the key mechanism of the Vitexdoins family against PCOS may be related to the EGFR pathway. These network pharmacological predictions were verified in subsequent cell experiments. This study provides scientific evidence supporting the use of the Vitexdoins family for PCOS and suggests a new potential strategy for the treatment of PCOS from the perspective of network pharmacology which provides a scientific basis for further elucidating the effect of the Vitexdoins family in PCOS.

_____

Egypt. J. Chem. **66,** No. SI 13 (2023)

_____

5. Conflicts of interest

The authors declare that they have no conflicts of interest.

6. References

[1] Louwers YV, Laven JSE. Characteristics of polycystic ovary syndrome throughout life. *Ther Adv Reprod Health*. 2020;14:2633494120911038. doi:10.1177/2633494120911038.

[2] Azziz R. Polycystic ovary syndrome. *Obstet Gynecol*.2018;132(2):321-336. doi:10.1097/AOG.0000000000002698.

[3] Sharma S, Mahajan N. Polycystic ovarian syndrome and menopause in forty plus women. *J Midlife Health*. 2021;12(1):3-7. doi:10.4103/jmh.jmh_8_21.

[4] Hoeger KM, Dokras A, Piltonen T. Update on PCOS: consequences, challenges, and guiding treatment. *J Clin Endocrinol Metab*. 2021;106(3):e1071-e1083. doi:10.1210/clinem/dgaa839.

[5] Domecq JP, Prutsky G, Mullan RJ, et al. Adverse effects of the common treatments for polycystic ovary syndrome: a systematic review and meta-analysis. *J Clin Endocrinol Metab*. 2013;98(12):4646-4654. doi:10.1210/jc.2013-2374.

[6] Arentz S, Abbott JA, Smith CA, Bensoussan A. Herbal medicine for the management of polycystic ovary syndrome (PCOS) and associated oligo/amenorrhoea and hyperandrogenism; a review of the laboratory evidence for effects with corroborative clinical findings. *BMC Complement Altern Med*. 2014;14:511. doi:10.1186/1472-6882-14-511.

[7] Gill BS, Mehra R, Navgeet, Kumar S. Vitex negundo and its medicinal value. *Mol Biol Rep*. 2018;45(6):2925-2934. doi:10.1007/s11033-018-4421-3.

[8] Zheng CJ, Li HQ, Ren SC, et al. Phytochemical and Pharmacological Profile of Vitex negundo. *Phytother Res*. 2015;29(5):633-647. doi:10.1002/ptr.5303.

[9] Zheng CJ, Zhang XW, Han T, et al. Anti-inflammatory and anti-osteoporotic lignans from Vitex negundo seeds. *Fitoterapia*. 2014;93:31-38. doi:10.1016/j.fitote.2013.12.006.

[10] Kakadia N, Patel P, Deshpande S, Shah G. Effect of *Vitex negundo* L. seeds in letrozole induced polycystic ovarian syndrome. *J Tradit Complement Med*. 2018;9(4):336-345. doi:10.1016/j.jtcme.2018.03.001

[11] Zheng CJ, Huang BK, Han T, et al. Nitric oxide scavenging lignans from Vitex negundo seeds. *J Nat Prod*. 2009;72(9):1627-1630. doi:10.1021/np900320e

[12] Iksen I, Witayateeraporn W, Wirojwongchai T, et al. Identifying molecular targets of Aspiletrein-derived steroidal saponins in lung cancer using network pharmacology and molecular docking-based assessments. *Sci Rep*. 2023;13(1):1545. doi:10.1038/s41598-023-28821-8

[13] Iksen I, Sinsook S, Wattanathamsan O, Buaban K, Chamni S, Pongrakhananon V. Target Identification of 22-(4-pyridinecarbonyl) jorunnamycin A, a tetrahydroisoquinoline derivative from the sponge *Xestospongia* sp., in mediating non-small-cell lung cancer cell apoptosis. *Molecules*. 2022;27(24):8948. doi:10.3390/molecules27248948.

[14] Guan L, Yang H, Cai Y, et al. ADMET-score - a comprehensive scoring function for evaluation of chemical drug-likeness. *Medchemcomm*. 2018;10(1):148-157. doi:10.1039/c8md00472b

[15] Gombar VK, Silver IS, Zhao Z. Role of ADME characteristics in drug discovery and their in silico evaluation: in silico screening of chemicals for their metabolic stability. *Curr Top Med Chem*. 2003;3(11):1205-1225. doi:10.2174/1568026033452014.

[16] Dingemanse J, Krause A. Impact of pharmacokinetic-pharmacodynamic modelling in early clinical drug development. *Eur J Pharm Sci*. 2017;109S:S53-S58. doi:10.1016/j.ejps.2017.05.042

[17] Islamie R, Iksen I, Buana BC, Gurning K, Syahputra HD, Winata HS. (2022). Construction of network pharmacology-based approach and potential mechanism from major components of *Coriander sativum* L. against COVID-19. *Pharmacia*, 69(3), 689-697. doi: 10.3897/pharmacia.69.e84388.

[18] Iksen I, Buana BC. (2022). Identification of potential COVID-19 targets and pathways derivate from various phenolic compounds from chives (*Allium schoenoprasum*) by using network pharmacology approach. *Media Pharmaceutica Indonesiana*, 4(2), 157-167. doi: 10.24123/mpi.v4i2.5272.

[19] Gfeller D, Grosdidier A, Wirth M, Daina A, Michielin O, Zoete V. SwissTargetPrediction: a web server for target prediction of bioactive small molecules. *Nucleic Acids Res*. 2014;42(Web Server issue):W32-W38. doi:10.1093/nar/gku293.

[20] Roskoski R Jr. Src protein-tyrosine kinase structure, mechanism, and small molecule inhibitors. *Pharmacol Res*. 2015;94:9-25. doi:10.1016/j.phrs.2015.01.003.

[21] Byeon SE, Yi YS, Oh J, Yoo BC, Hong S, Cho JY. The role of Src kinase in macrophage-mediated inflammatory responses. *Mediators Inflamm*. 2012;2012:512926. doi:10.1155/2012/512926.

[22] Aboeldalyl S, James C, Seyam E, Ibrahim EM, Shawki HE, Amer S. The role of chronic inflammation in polycystic ovarian syndrome-a systematic review and meta-analysis. *Int J Mol Sci*. 2021;22(5):2734. doi:10.3390/ijms22052734.

[23] Park E, Cockrem JF, Han KH, Kim DH, Jung MH, Chu JP. Stress-induced activation of ovarian heat shock protein 90 in a rat model of polycystic ovary syndrome. *J Obstet Gynaecol Res*. 2012;38(2):396-407. doi:10.1111/j.1447-0756.2011.01705.x.

[24] Hawkins PT, Stephens LR. PI3K signalling in inflammation. *Biochim Biophys Acta*. 2015;1851(6):882-897. doi:10.1016/j.bbalip.2014.12.006.

[25] Fung-Leung WP. Phosphoinositide 3-kinase delta (PI3Kδ) in leukocyte signaling and function. *Cell*

Signal. 2011;23(4):603-608. doi:10.1016/j.cellsig.2010.10.002.

[26] Iksen, Pothongsrisit S, Pongrakhananon V. Targeting the PI3K/AKT/mTOR signaling pathway in lung cancer: an update regarding potential drugs and natural products. *Molecules.* 2021;26(13):4100. doi:10.3390/molecules26134100.

[27] Li T, Mo H, Chen W, et al. Role of the PI3K-Akt Signaling Pathway in the Pathogenesis of Polycystic Ovary Syndrome. *Reprod Sci.* 2017;24(5):646-655. doi:10.1177/1933719116667606.

[28] Rayego-Mateos S, Rodrigues-Diez R, Morgado-Pascual JL, et al. Role of Epidermal Growth Factor Receptor (EGFR) and Its Ligands in Kidney Inflammation and Damage. *Mediators Inflamm.* 2018;2018:8739473. doi:10.1155/2018/8739473.

[29] Wang H, Feng X, Wang T, et al. Role and mechanism of the p-JAK2/p-STAT3 signaling pathway in follicular development in PCOS rats. *Gen Comp Endocrinol.* 2023;330:114138. doi:10.1016/j.ygcen.2022.114138.