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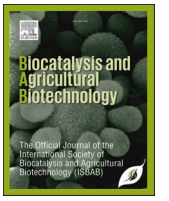

# A comparative study on chemical profiles and biological activities of different extracts of three *Verbascum* species from Turkey: *In vitro*, in silico and network pharmacological approaches

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ABSTRACT

The present study aimed to evaluate the antioxidant and enzyme inhibition properties of aerial part of *Verbascum cheiranthifolium* Boiss. *V. myriocarpum* Boiss. & Heldr. and *V. pyroliforme* (Boiss. & Heldr.) Kuntze as well as their chemical profiles. Results showed that MeOH and hydromethanol recovered the highest total polyphenolic content with highest content obtained from *V. cheiranthifolium* (27.61and 27.69 mg GAE/g respectively) and *V. myriocarpum* (24.24 and 25.34 GAE/g respectively). The estimated content of verbascoside was quite higher in *V. cheiranthifolium* (987.05–6780.07 mg/100 g) and *V. myriocarpum* (1506.47–6433.73 mg/100 g) than *V. pyroliforme* (49.54–121.97 mg/100 g). All extracts of *V. cheiranthifolium* (274.17–713.79 mg/100 g) and polar ones of *V. myriocarpum* (185.67–304.51 mg/100 g) accumulated higher chlorogenic acid content than *V. pyroliforme* extracts (36.33–112.56 mg/100 g). The latter species was dominated by luteolin-7-*O*-glucoside and apigenin-7-*O*-glucoside with highest content recorded from the hydromethanolic extract (639.70 and 196.14 mg/100 g respectively). Extracts of *V. cheiranthifolium* (46.15–48.74 mg TE/g) and *V. myriocarpum* (46.05–48.50 mg TE/g) exerted significantly higher scavenging activity than those from *V. pyroliforme* (12.77–34.64 mg TE/g). Highest significant anti-acetylcholinesterase activity was obtained by MeOH extract of *V. pyroliforme* (2.65 mg GALAE/g) and *V. myriocarpum* (2.57 mg GALAE/g). In conclusion, extracts from the three investigated *Verbascum* spp. can be a potential source of bioactive metabolites with interesting antioxidant and enzyme inhibition properties.

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## 1. Introduction

Natural bioactive components, especially those derived from plants, play a crucial role in healthcare to prevent and treat a wide range of human and animal diseases. They have received a lot of attention recently as new therapeutic options having significant curative effect and a lower adverse impact on human health (Atanasov et al., 2021). Antioxidant phytochemicals contained in plants like polyphenols (flavonoids and phenolic acids) can prevent and slow down the progression of various chronic diseases (Vona et al., 2021). Nowadays, enzyme inhibitors are considered as promising therapeutic targets in the treatment of many diseases, including diabetes mellitus, hyperpigmentation, and central nervous system-related diseases (Orhan, 2019). Therefore, it is important to continue searching for new therapies and lead molecules, especially from alternative natural sources.

*Verbascum* L. is belonging to the family Scrophulariaceae and comprise about 360 taxa worldwide. Many *Verbascum* species have been used for centuries in many countries, especially the Mediterranean region, as nutritional supplements and to cure several ailments in traditional medicine (Tatli and Akdemir, 2006). *Verbascum* plants are characteristize by the presence of iridoid and neolignan type glycosides, oleanan type terpenes, flavonoids, polysaccharides, saponins, steroids and alkaloids (Blanco-Salas et al., 2021; Küçük et al., 2016; M. Amin et al., 2020).

In Turkey there are 234 *Verbascum* species of which 196 are endemic growing in different soil habitats (Catara et al., 2016; Karavelioğulları et al., 2014; Küçük et al., 2016). Some of them are used in Turkish traditional medicine (Baytop, 1999). For example *V. cheiranthifolium* is used to cure different respiratory and skin disorders, abdominal pains, rheumatic pain, earache, hemorrhoids and diarrhea (Tatli and Akdemir, 2006). Despite the great number of *Verbascum* spp. grown in Turkey, few studies based on a literature review, exploring their phytochemistry and potential bioactivity were performed. Some examples; *V. mucronatum, V. bombyciferum* and *V. vacillans* are found to possess antibacterial, antioxidant and antimutagenic activities (HACIOĞLU et al., 2021). *V. lasianthum, V. latisepalum, V. mucronatum* and *V. salviifolum* showed anthelmintic activity (Kozan et al., 2011). *V. thapsus* (Turker and Camper, 2002) and *V. pseudonobile* (Ionkova and Alferman, 2000) had cytotoxic effect. *V. myriocarpum* (Saltan et al., 2011) and *V. cheiranthifolium* (Dalar and Konczak, 2012) possessed antioxidant activity. The latter species also exhibited cytotoxic (Küçük et al., 2016), anti-inflammatory (Dalar et al., 2014), antimicrobial (Kunduhoglu et al., 2011), insecticide (Khoshnoud et al., 2008a, 2008b) and anti-ulcerogenic (Gürbüz et al., 2005) activities. Chemically, the stems and flowers of *V. cheiranthifolium* were found to contain catalpol, 6-*O*-(E)-coumaroylaucubin, 6-*O*-[(E)-*p*-methoxycinnamoyl]aucubin, verbascoside and luteolin hexoside in addition to volatile compounds (Dalar et al., 2018; Eribekyan et al., 1989). Although many studies were performed in this latter species, the present study was aimed to evaluate in depth the antioxidant activity of aerial part of *V. cheiranthifolium* Boiss. in addition to two scarcely studied species; *V. myriocarpum* Boiss. & Heldr. and *V. pyroliforme* (Boiss. & Heldr.) Kuntze by examining their capacity to scavenge radicals, reducing ions and chelating metals as well as their chemical profiles. Moreover and as far as we know, there have been no comprehensive studies exploring the enzyme inhibition potential of these three species and this prompted us to determine their capacity to inhibit the acetylcholinesterase, butyrylcholinesterase, tyrosinase, α-amylase and α-glucosidase enzymes.

## 2. Materials and methods

### 2.1. Plant materials

In the summer of 2021, we collected aerial parts of the tested *Verbacum* species (*V. cheiranthifolium*: Yazır Location, 1020 m, Konya; *V. pyroliforme*: Eskil, Küngönü Location, 950 m, Aksaray; *V. myriocarpum*: Çatdere Location, Hadim, 1340 m, Konya) in Turkey. The plant specimens were identified by one of our co-authors, Dr. Evren Yildiztugay, and one specimen from the plants was deposited at the Selcuk University herbarium. Prior to extraction, the plant materials were carefully washed with tap and distilled water to eliminate any soil and contaminants. After being air-dried for 10 days (in shade at room temperature), the aerial parts were powdered.

### 2.2. Extraction of samples

To prepare extracts, we employed different solvents: ethyl acetate, methanol, methanol/water (70%) and water. The maceration method was used for the organic extracts, whereby 10 g of plant material was mixed with 200 ml of each solvent and left for 24 h at room temperature. Using Whatman 1 filter paper, the mixtures were then filtered, and the solvents were eliminated using a rotary-evaporator. The water extracts, on the other hand, were prepared by infusing 10 g of plant material in 200 mL of boiled water for 15 min, followed by filtration and lyophilization for 48 h. All extracts were kept at 4 °C until analysis.

### 2.3. Total quantification of phenolics and flavonoids

Determining the total content of bioactive compounds involved the determination of total phenols (TPC) and flavonoids (TFC), for which the procedures described in the paper (Uysal et al., 2017) were used. All experimental details are given in supplemental materials.

### 2.4. Assays for antioxidant and enzyme inhibition

The biological potential of the obtained extracts was determined by measuring their antioxidant and enzyme-inhibitory potential. The antioxidant potential was assessed using six *in vitro* tests (2,2-diphenyl-1-pic- rylhydrazyl (DPPH), 2,20 -azino-bis (3-ethylbenzothiazoline-6- sulfonic acid (ABTS), ferric reducing antioxidant power (FRAP), cupric ion reducing antioxidant capacity (CUPRAC), ferrous metal chelating (MCA) and phosphomolybdenum tests). All used procedures are given in detail in the work of Nedić et al. (2022). The assessment of extracts' inhibition of acetylcholinesterase (AChE), butyrylcholinesterase (BChE), tyrosinase (Tyr), α-glucosidase and α-amylase, was performed in accordance with the protocol described in the study by Nedić et al. (2022). All

experimental details are given in supplemental materials.

### 2.5. HPLC analysis

Approximately 4 mg of sample was weighed into the Eppendorf tube and 1.5 mL of methanol was added, the extraction was performed on an ultrasonic bath for 15 min and using centrifuge for 10 min (8700 rpm). The supernatant was separated and re-extracted with 1.5 mL of methanol under the same conditions on ultrasound and centrifuge. Supernatants were collected and analyzed. Agilent HPLC system 1260 Infinity II (Agilent technology, Santa Clara, CA, USA) was used to identify/qualify the phenolic in the *Verbascum* extracts. The method used was previously described by Buljeta et al. (2021). All analytical details are also given in supplemental materials.

### 2.6. Molecular modeling

The prepared protein crystal structures of human AChE (PDB ID: 6O52)(Gerlits et al., 2019), BChE (PDB ID: 6EQP)(Rosenberry et al., 2017), and α-amylase (PDB ID: 1B2Y)(Maurus et al., 2008) were retrieved from our previous studies (Eltayeb et al., 2023; Fahmy et al., 2023). Also, the prepared model of human α-tyrosinase and α-glucosidase built using *Priestia megaterium* tyrosinase (PDB ID: 6QXD)(Ielo et al., 2019) and *Mus musculus* α-glucosidase (PDB ID: 7KBJ)(Karade et al., 2021) as templates were retrieved (Chiavaroli et al., 2023). The 3D structures of chlorogenic acid, verbascoside, apigenin, apigenin-glucoside, and luteolin-glucoside were downloaded from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/) and optimized using UCSF Chimera (Pettersen et al., 2004). MGLTools 1.5.6 program was used to merge all non-polar hydrogen atoms and to add gasteiger charges to all atoms. Finally, using AutoDock 4.2.6 (https://autodock.scripts.edu)(Morris et al., 2009), these ligands were docked to the catalytic channel of the target enzymes, adopting the previously applied docking protocol in ref. (Llorent-Martínez et al., 2022). The interaction between the studied ligands and the target enzymes was analyzed using Biovia DS Visualizer v4.5 (BIOVIA, San Diego, CA, USA).

### 2.7. Network pharmacology

#### 2.7.1. Target identification

The chemical structures of the 5 phenolic compounds in the form of canonical SMILES were retrieved from PubChem database (PubChem – accessed on May 3, 2023) (Kim et al., 2022). The canonical SMILES of the phenolic compounds were uploaded to the Swiss TargetPrediction web tool for target prediction (Swiss TargetPrediction – accessed on May 3, 2023) (Daina et al., 2019). The targets were filtered with the probability score greater than and equal to 0.1 and the filtered targets were taken for further analysis. The curation of diseases associated to the predicated targets DisGeNET was used (DisGeNET – accessed on May 3, 2023) (Piñero et al., 2021). The diseases were filtered based upon the gene-disease association score of greater than and equal to 0.7, this filter was used to get highly associated disease to the predicted targets. UniProt database was used for target name and gene symbol standardization. The plant compounds to diseases through target gene network was constructed using Cytoscape_v3.9.1 software and it was visualized in the hierarchical layout.

#### 2.7.2. Protein-protein interaction

The Protein-Protein Interaction network was constructed using STRING 11.5 database (STRING 11.5 – accessed on May 3, 2023) (Mering et al., 2003). The network was created using the minimal interaction threshold value to "high confidence >0.7" and it was analyzed. The thickness of the edges in the network represented the strength of the data. The network was clustered based upon the gene function using k means clustering option.

#### 2.7.3. Gene ontology and KEGG pathway enrichment analysis

The gene ontology and KEGG pathway analysis was performed using ShinyGo 0.77 (ShinyGo 0.77 – accessed on May 3, 2023) (Ge et al., 2020). The top 20 biological processes and pathways were selected based upon the fold enrichment. The top 20 biological processes, cellular components and molecular function of the genes were predicted and displayed in the form of lollipop plot (X-axis and Y-axis show the fold enrichment and full names of the processes, respectively, and the colour and size of each lollipop represent the gene count and –log10FDR, respectively). The biological pathways were represented in lollipop plot and hierarchical tree plot, and network was also obtained based upon the number genes involved in the pathway and percentage of overlapping genes (Kanehisa et al., 2021). Through this the bioactive compound's likely mechanism of action in diseases can be predicted.

#### 2.7.4. Physicochemical studies and ADME studies

The physicochemical and pharmacokinetic properties of the 5 phenolic compounds were determine by using Swiss ADME (SwissADME – accessed on May 3, 2023) (Daina et al., 2017). This helps to understand the important pharmacokinetic properties and significant physicochemical descriptors of the phenolic compounds.

### 2.8. Data analysis

All data were given as mean ± standard deviation of three parallel experiments. Statistical analysis was performed by analysis of variance (ANOVA). A post hoc test (Turkey) was done when the differences shown by data were significant ($p < 0.05$). R v.4.1.2 statistical program was used for all analysis included the principal component analysis and hierarchical clustering heatmaps.

**Table 1**
Total phenolic and flavonoid contents of *V. cheiranthifolium, V. myriocarpum* and *V. pyroliforme* extracts.

| Species | Solvents | TPC (mg GAE/g) | TFC (mg RE/g) |
|---|---|---|---|
| *Verbascum cheiranthifolium* | Ethyl acetate | $19.81 \pm 1.10^{c}$ | $26.72 \pm 0.64^{b}$ |
| | MeOH | $27.61 \pm 0.62^{a}$ | $30.99 \pm 0.86^{a}$ |
| | MeOH/Water | $27.69 \pm 0.98^{a}$ | $13.25 \pm 0.23^{d}$ |
| | Water | $19.69 \pm 0.31^{c}$ | $4.20 \pm 0.10^{f}$ |
| *Verbascum myriocarpum* | Ethyl acetate | $19.84 \pm 0.30^{c}$ | $15.31 \pm 0.18^{c}$ |
| | MeOH | $24.24 \pm 0.18^{b}$ | $13.79 \pm 0.28^{d}$ |
| | MeOH/Water | $25.34 \pm 0.93^{b}$ | $2.88 \pm 0.11^{g}$ |
| | Water | $19.05 \pm 0.17^{c}$ | $1.56 \pm 0.10^{h}$ |
| *Verbascum pyroliforme* | Ethyl acetate | $14.86 \pm 0.88^{d}$ | $3.15 \pm 0.48^{fg}$ |
| | MeOH | $19.15 \pm 0.18^{c}$ | $14.08 \pm 0.16^{d}$ |
| | MeOH/Water | $19.50 \pm 0.29^{c}$ | $11.03 \pm 0.20^{e}$ |
| | Water | $15.01 \pm 0.20^{d}$ | $10.07 \pm 0.08^{e}$ |

*Values are reported as mean ± SD of three parallel measurements. GAE: Gallic acid equivalent; RE: Rutin equivalent. Different letters in the same column indicate significant differences in the extracts ($p < 0.05$).

**Table 2**
Concentration (mg/100 g) of individual polyphenols in *V. cheiranthifolium, V. myriocarpum* and *V. pyroliforme* extractsdetermined using HPLC analysis.

| Species | Extracts | Chlorogenic acid | Verbascoside | Apigenin | Apigenin-7-*O*-glucoside | Luteolin-7-*O*-glucoside |
|---|---|---|---|---|---|---|
| *Verbascum cheiranthifolium* | Ethyl acetate | $274.17 \pm 4.72^{e}$ | $6780.07 \pm 0.91^{a}$ | $24.92 \pm 0.36^{c}$ | $9.85 \pm 0.11^{e}$ | $229.01 \pm 2.71^{b}$ |
| | MeOH | $713.79 \pm 16.18^{a}$ | $5287.95 \pm 35.55^{c}$ | $60.48 \pm 0.67^{a}$ | $12.88 \pm 0.08^{de}$ | $183.78 \pm 3.93^{c}$ |
| | MeOH/Water | $599.66 \pm 5.97^{b}$ | $1668.43 \pm 35.67^{f}$ | $55.72 \pm 0.50^{b}$ | $10.79 \pm 0.12^{e}$ | $150.29 \pm 4.49^{d}$ |
| | Water | $489.42 \pm 13.42^{c}$ | $987.05 \pm 23.85^{h}$ | $10.77 \pm 0.04^{g}$ | n.d. | $50.89 \pm 1.49^{h}$ |
| *Verbascum myriocarpum* | Ethyl acetate | $29.54 \pm 0.42^{i}$ | $5017.05 \pm 17.78^{d}$ | $13.48 \pm 0.32^{f}$ | $36.89 \pm 1.00^{c}$ | $22.67 \pm 0.71^{i}$ |
| | MeOH | $276.39 \pm 11.04^{e}$ | $6433.73 \pm 23.43^{b}$ | n.d. | $19.40 \pm 0.02^{d}$ | $61.07 \pm 0.90^{g}$ |
| | MeOH/Water | $304.51 \pm 4.19^{d}$ | $3579.30 \pm 38.33^{e}$ | $12.52 \pm 0.30^{f}$ | $11.43 \pm 0.41^{e}$ | $18.76 \pm 0.41^{ij}$ |
| | Water | $185.67 \pm 0.26^{f}$ | $1506.47 \pm 13.81^{g}$ | $10.63 \pm 0.17^{g}$ | $9.58 \pm 0.31^{e}$ | $23.54 \pm 0.26^{j}$ |
| *Verbascum pyroliforme* | Ethyl acetate | $41.98 \pm 0.37^{hi}$ | $49.54 \pm 0.65^{j}$ | $14.73 \pm 0.21^{e}$ | $43.49 \pm 0.81^{bc}$ | $13.25 \pm 0.24^{j}$ |
| | MeOH | $112.56 \pm 1.72^{g}$ | $121.97 \pm 1.77^{i}$ | $15.12 \pm 0.26^{e}$ | $193.28 \pm 2.66^{a}$ | $114.13 \pm 3.04^{f}$ |
| | MeOH/Water | $62.82 \pm 1.72^{h}$ | $96.10 \pm 2.48^{ij}$ | $19.80 \pm 0.52^{d}$ | $196.14 \pm 7.49^{a}$ | $639.70 \pm 3.24^{a}$ |
| | Water | $36.33 \pm 1.29^{i}$ | $59.63 \pm 0.65^{ij}$ | n.d. | $45.63 \pm 1.94^{b}$ | $123.10 \pm 3.28$ |

n.d. – not detected. Different letters in the same column indicate significant differences in the extracts ($p < 0.05$).

## 3. Results and discussion

### 3.1. Total polyphenolic (TPC) and flavonoids (TFC) contents

The TPC and TFC were determined for the different extracts from the aerial parts of the investigated *Verbascum* spp. Results are presented in Table 1. The highest amount of TPC in the three investigated *Verbascum* spp. was significantly ($p < 0.05$) recovered from the methanol and methanol/water extracts with maximum content obtained from *V. cheiranthifolium* (27.61and 27.69 mg GAE/g respectively) and *V. myriocarpum* (24.24 and 25.34 GAE/g respectively). All other extracts from these two species had the same TPC ($p > 0.05$) while all extracts of *V. pyroliforme* showed the least TPC (14.86–19.50 GAE/g). The TPC in different extracts from aerial parts of *V. cheiranthifolium,* obtained in the present study, was far behind that of the flowers and stems extracts using acidified ethanol as extraction solvent as reported by Dalar et al. (2018). The TFC varied according to species where the methanolic (30.99 mg RE/g) and ethyl acetate (26.72 mg RE/g) extracts respectively of *V. cheiranthifolium* had remarkably ($p < 0.05$) the highest TFC. Methanolic extracts of *V. myriocarpum* (13.79 mg RE/g) and *V. pyroliforme* (14.08 mg RE/g) had also considerable TFC. But it was noted that extraction solvents varied in their capacity to recover the TFC in the different species. For example, although the ethyl acetate extract recorded the highest TFC in *V. myriocarpum* it recovered the least amount of TFC in *V. pyroliforme.* Furthermore, the water as a solvent extracted from *V. pyroliforme* nearly the same TFC ($p > 0.05$) as that by the methanol/water but showed the least TFC in the other two *Verbascum* spp. It was noted that polarity of extraction solvents influences the extraction efficiency of phenolic compounds and, generally, highly hydroxylated aglycone forms of phenolic compounds are soluble in water, alcohols (ethanol, methanol), and their mixtures, while less polar and highly methoxylated aglycone forms are extracted into less polar solvents (ethyl acetate, acetone, chloroform) (Dorta et al., 2012; González-Montelongo et al., 2010). Furthermore, Garcia-Salas et al. (2010) reported that, beside the type of extraction solvent, the chemical composition and physical characteristics of the plant materials are among the most effective factors in the extraction process of phenolic constituents.

**Table 3**
Antioxidant ability of *V. cheiranthifolium, V. myriocarpum* and *V. pyroliforme* extracts.

| Plant species | Extracts | DPPH (mg TE/g) | ABTS (mg TE/g) | CUPRAC (mg TE/g) | FRAP (mg TE/g) | Chelating (mg EDTAE/g) | PBD (mmol TE/g) |
|---|---|---|---|---|---|---|---|
| *Verbascum cheiranthifolium* | Ethyl acetate | 48.35 ± 0.21$^{ab}$ | 66.38 ± 0.95$^{bc}$ | 138.83 ± 3.44$^{d}$ | 70.95 ± 5.29$^{cd}$ | 21.38 ± 1.21$^{b}$ | 2.08 ± 0.01$^{a}$ |
| | MeOH | 48.74 ± 0.02$^{a}$ | 69.57 ± 0.04$^{a}$ | 196.44 ± 3.51$^{a}$ | 94.83 ± 4.53$^{a}$ | 15.83 ± 0.87$^{d}$ | 1.93 ± 0.16$^{abc}$ |
| | MeOH/ Water | 47.42 ± 0.16$^{bc}$ | 69.54 ± 0.09$^{a}$ | 162.20 ± 2.24$^{b}$ | 89.54 ± 1.11$^{ab}$ | 24.62 ± 0.39$^{a}$ | 1.74 ± 0.09$^{cd}$ |
| | Water | 46.15 ± 0.08$^{d}$ | 67.72 ± 1.45$^{abc}$ | 109.16 ± 1.73$^{e}$ | 64.78 ± 2.09$^{de}$ | 22.10 ± 0.45$^{b}$ | 1.69 ± 0.06$^{cd}$ |
| *Verbascum myriocarpum* | Ethyl acetate | 46.57 ± 0.11$^{cd}$ | 54.00 ± 0.78$^{g}$ | 114.31 ± 3.66$^{e}$ | 59.67 ± 3.01$^{ef}$ | 14.74 ± 0.82$^{d}$ | 2.03 ± 0.18$^{ab}$ |
| | MeOH | 48.50 ± 0.05$^{a}$ | 69.42 ± 0.05$^{ab}$ | 155.13 ± 2.86$^{c}$ | 80.19 ± 6.17$^{bc}$ | 11.58 ± 0.50$^{e}$ | 1.38 ± 0.02$^{e}$ |
| | MeOH/ Water | 47.29 ± 0.10$^{c}$ | 68.99 ± 0.76$^{ab}$ | 132.94 ± 2.86$^{d}$ | 71.63 ± 1.34$^{cd}$ | 25.22 ± 0.11$^{a}$ | 1.55 ± 0.03$^{de}$ |
| | Water | 46.05 ± 0.01$^{d}$ | 60.21 ± 2.28$^{ef}$ | 93.95 ± 1.28$^{f}$ | 52.46 ± 4.71$^{fg}$ | 24.79 ± 0.64$^{a}$ | 1.06 ± 0.02$^{f}$ |
| *Verbascum pyroliforme* | Ethyl acetate | 12.77 ± 0.27$^{h}$ | 34.17 ± 0.57$^{h}$ | 59.24 ± 0.96$^{h}$ | 31.55 ± 5.98$^{h}$ | 19.33 ± 0.81$^{c}$ | 1.81 ± 0.04$^{bcd}$ |
| | MeOH | 32.43 ± 0.85$^{f}$ | 63.28 ± 0.18$^{de}$ | 89.01 ± 0.05$^{fg}$ | 67.66 ± 2.44$^{de}$ | 20.43 ± 0.46$^{bc}$ | 1.85 ± 0.14$^{abc}$ |
| | MeOH/ Water | 34.64 ± 0.19$^{e}$ | 64.77 ± 0.07$^{cd}$ | 85.56 ± 0.79$^{g}$ | 64.74 ± 0.76$^{de}$ | 25.52 ± 0.16$^{a}$ | 1.34 ± 0.01$^{e}$ |
| | Water | 23.96 ± 0.62$^{g}$ | 58.22 ± 1.82$^{f}$ | 53.10 ± 0.08$^{h}$ | 48.45 ± 1.29$^{g}$ | 25.36 ± 0.10$^{a}$ | 1.05 ± 0.04$^{f}$ |

*Values are reported as mean ± SD of three parallel measurements. TE: Trolox equivalent; EDTAE: EDTA equivalent; na: not active. Different letters in the same column indicate significant differences in the extracts ($p < 0.05$).

### 3.2. Chemical profile

The estimation of 5 phenolics compounds namely, chlorogenic acid, verbascoside, apigenin, apigenin-7-*O*-glucoside and luteolin-7-*O*-glucoside were determined via HPLC technique. Results are depicted in Table 2. Chromatograms are given in supplemental materials (Figs. S1–S24). Verbascoside was the major compound in the aerial parts of *V. cheiranthifolium* and *V. myriocarpum*. It was in the following descending order for *V. cheiranthifolium;* EtOAc > MeOH >70% MeOH > Water and for *V. myriocarpum* as; MeOH > EtOAc >70% MeOH > Water. Verbascoside was also detected in *V. pyroliforme* extracts but in relatively lower content and did not present the dominant compound. This result is in accordance with previous studies highlighting that *Verbascum* spp. are characterize by the presence of verbascoside (Blanco-Salas et al., 2021; Küçük et al., 2016; M. Amin et al., 2020). Furthermore, extracts (3/4) from *V. cheiranthifolium* contained the highest chlorogenic acid content in the following descending order; MeOH >70% MeOH > Water > EtOAc. This was followed by extracts from *V. myriocarpum* with 70% MeOH and MeOH recovered the highest content respectively. Interestingly, although all extracts from *V. pyroliforme* had the least content of these two compounds it accumulated the highest amount of apigenin-7-*O*-glucoside in the following descending order; 70% MeOH > MeOH > Water > EtOAc. The other two species had very low content in apigenin-7-*O*-glucoside but MeOH and 70% MeOH extracts from *V. cheiranthifolium* contained considerable amount of the aglycon apigenin. The highest content in luteolin-7-*O*-glucoside was recorded from the 70% MeOH of *V. pyroliforme* then followed by extracts from *V. cheiranthifolium* (EtOAc > MeOH >70% MeOH) and respectively the MeOH and water extracts of the former species. Extracts from *V. myriocarpum* had lower luteolin-7-*O*-glucoside content when compared to those from the two other species. In summary, extracts from *V. cheiranthifolium* and *V. myriocarpum* were characterized by the presence of verbascoside and chlorogenic acid, in addition, the former had considerable amount of luteolin-7-*O*-glucoside. Extracts of *V. pyroliforme* were mainly dominated by the presence of luteolin-7-*O*-glucoside and apigenin-7-*O*-glucoside. In their study on the flowers and stems extracts of *V. cheiranthifolium,* Dalar et al. (2018) showed that verbascoside was the major compound present in the flowers extract while luteolin-glucoside was mainly accumulated in the stems extract. Chlorogenic acid, and apigenin were detected as trace amount in both organs. This variation could be attributed to many factors including genetics one besides the age, part of the plants and different environmental influences among others (Yagi et al., 2020).

### 3.3. Antioxidant activity

Antioxidants have significant role in human health as it counteract the oxidative damage of cell structures and molecules by reactive oxygen species and ultimately contribute to the prevention from a wide range of diseases like diabetes, cancer, Parkinson's disease, Alzheimer's disease and cardiovascular disease (Vona et al., 2021). In the present study 6 complementary assays were performed to evaluate the antioxidant activity of extracts from aerial parts of the investigated *Verbascum* spp. Results are presented in Table 3. The four extracts in *V. cheiranthifolium* and *V. myriocarpum* were slightly different in their capacity to scavenge the free DPPH radical with values in the range of 46.05–48.74 mg TE/g. Extracts of *V. pyroliforme* displayed less anti-DPPH activity (12.77–34.64 mg

**Table 4**
Enzyme inhibition ability of *V. cheiranthifolium, V. myriocarpum* and *V. pyroliforme* extracts.

| Plant species | Extracts | AChE (mg GALAE/g) | BChE (mg GALAE/g) | Tyrosinase (mg KAE/g) | Amylase (mmol ACAE/g) | Glucosidase (mmol ACAE/g) |
|---|---|---|---|---|---|---|
| *Verbascum cheiranthifolium* | Ethyl acetate | $2.25 \pm 0.08^{cd}$ | $1.24 \pm 0.04^{a}$ | $54.47 \pm 1.06^{a}$ | $0.70 \pm 0.01^{a}$ | $0.45 \pm 0.01^{b}$ |
| | MeOH | $2.05 \pm 0.04^{de}$ | na | $43.08 \pm 1.35^{cd}$ | $0.41 \pm 0.01^{c}$ | $0.12 \pm 0.02^{def}$ |
| | MeOH/ Water | $1.89 \pm 0.05^{ef}$ | na | $49.17 \pm 0.47^{b}$ | $0.34 \pm 0.01^{d}$ | $0.09 \pm 0.01^{def}$ |
| | Water | $0.33 \pm 0.01^{h}$ | na | $14.90 \pm 1.56^{g}$ | $0.09 \pm 0.01^{e}$ | na |
| *Verbascum myriocarpum* | Ethyl acetate | $2.40 \pm 0.10^{bc}$ | $0.58 \pm 0.04^{c}$ | $46.49 \pm 0.47^{bc}$ | $0.55 \pm 0.01^{b}$ | $0.64 \pm 0.12^{a}$ |
| | MeOH | $2.57 \pm 0.12^{ab}$ | $0.54 \pm 0.09^{c}$ | $37.01 \pm 1.80^{e}$ | $0.41 \pm 0.01^{c}$ | $0.07 \pm 0.03^{ef}$ |
| | MeOH/ Water | $1.89 \pm 0.08^{ef}$ | na | $44.47 \pm 0.37^{bc}$ | $0.34 \pm 0.01^{d}$ | na |
| | Water | $0.81 \pm 0.01^{g}$ | na | $0.96 \pm 0.04^{h}$ | $0.11 \pm 0.03^{e}$ | na |
| *Verbascum pyroliforme* | Ethyl acetate | $1.90 \pm 0.11^{ef}$ | $0.85 \pm 0.12^{b}$ | $45.81 \pm 1.90^{bc}$ | $0.73 \pm 0.03^{a}$ | $0.31 \pm 0.02^{c}$ |
| | MeOH | $2.65 \pm 0.10^{a}$ | na | $29.74 \pm 4.30^{f}$ | $0.35 \pm 0.01^{d}$ | $0.20 \pm 0.06^{cd}$ |
| | MeOH/ Water | $1.73 \pm 0.04^{f}$ | na | $39.49 \pm 0.90^{de}$ | $0.37 \pm 0.03^{cd}$ | $0.15 \pm 0.02^{de}$ |
| | Water | $0.19 \pm 0.03^{h}$ | na | na | $0.08 \pm 0.01^{e}$ | na |

*Values are reported as mean ± SD of three parallel measurements. GALAE: Galantamine equivalent; KAE: Kojic acid equivalent; ACAE: Acarbose equivalent; na: not active. Different letters in the same column indicate significant differences in the extracts ($p < 0.05$).

TE/g). On the other hand, all extracts of the three species displayed higher activity to scavenge the ABTS radical (34.17–69.57 mg TE/g) than the DPPH one, even, most extracts of *V. pyroliforme* were nearly twice active. Variation in the capacity of extracts to scavenge the DPPH and ABTS radicals could be attributed to many factors like stereoselectivity of the radicals or the solubility of the extracts in different testing systems might affect the capacity of extracts to react and quench different radicals (Jimoh et al., 2010; Zheng and Wang, 2001). All extracts of the three species exerted better capacity to reduce the $Cu^{+2}$ ion than $Fe^{+3}$ one with highest activity observed in the extracts from *V. cheiranthifolium* (109.16–196.44 mg TE/g) followed by those from *V. myriocarpum* (93.95–155.13 mg TE/g) while *V. pyroliforme* extracts (53.10–89.01 mg TE/g) revealed the least capacity. Moreover, the MeOH and 70% MeOH of the three species exerted significantly ($p < 0.05$) the highest metal reducing capacity. Extracts also showed considerable iron chelating power with the 70% MeOH extracts of the three species (24.62–25.52 mg EDTAE/g) in addition to the water extracts from *V. myriocarpum* (24.79 mg EDTAE/g) and *V. pyroliforme* (25.36 mg EDTAE/g) exhibited significantly ($p < 0.05$) the highest activity. Moreover, they had the capacity to reduce Mo (VI) with significant ($p < 0.05$) highest total antioxidant activity recorded from the EtOAc extracts from *V. cheiranthifolium* (2.08 mmol TE/g) and *V. myriocarpum* (2.03 mmol TE/g) in addition to the MeOH extracts of the former (1.93 mmol TE/g) and *V. pyroliforme* (1.85 mmol TE/g). Results of *V. cheiranthifolium* aerial part extracts supported previous study by Dalar et al. (2018) who reported that the flower and stem revealed considerable FRAP and peroxyl radicals ORAC activity. Furthermore, the anti-DPPH and metal reducing property of *V. cheiranthifolium* and *V. myriocarpum* could be in part to their high concentration in verbascoside (Aldini et al., 2006; Chen et al., 2012; Quirantes-Piné et al., 2013) and chlorogenic acid (Ali et al., 2022). Additionally, studies showed that chlorogenic acid activates endogenous antioxidant systems to defence and scavenge free radicals and hence improve oxidative damage (Bender and Atalay, 2021).

### *3.4. Enzyme inhibition activity*

Enzymes, beside their catalytic activity, are readily susceptible to inhibition by small metabolites and thus represent potential therapeutic alternative for some human diseases like Alzheimer's disease, diabetes and some skin disorders (Orhan, 2019). In the present study extracts of the investigated *Verbascum* spp. aerial parts were examined for their enzyme inhibition property against AChE, BChE, Tyr, α-amylase, and α-glucosidase enzymes. Results are presented in Table 4. All extracts of the three species had the capacity to inhibit the AChE with their MeOH (2.05–2.65 mg GALAE/g) and EtOAc (1.90–2.40 mg GALAE/g) extracts displayed significantly ($p < 0.05$) the highest activity. However, only 4 extracts; EtOAc extracts (0.58–1.24 mg GALAE/g) of the three species and MeOH extract of *V. myriocarpum* (0.54 mg GALAE/g) showed anti-BChE property with highest significant ($p < 0.05$) value recorded from the EtOAc extract of *V. cheiranthifolium.* All the three organic extracts from the studied species revealed considerable Try inhibition activity and the EtOAc extract of *V. cheiranthifolium* exerted significantly ($p < 0.05$) the highest activity (54.47 mg KAE/g). Water extracts were either not active or displayed weak activity (≤14.90 mg KAE/g) when compared with the other extracts. The enzyme inhibition property of the studied species against the enzymes associated with diabetes showed that they exerted moderate activity against α-amylase and α-glucosidase enzymes with highest significant ($p < 0.05$) activity obtained from the EtOAc extracts of *V. cheiranthifolium* (0.70 mmol ACAE/g) and *V. pyroliforme* (0.73 mmol ACAE/g) against the former enzyme and that of *V. myriocarpum* (0.64 mmol ACAE/g) against the latter. Generally, there are few studies reporting the enzyme inhibition properties of *Verbascum* spp. It was noted that the three investigated species displayed higher anti- AChE activity but lower anti-BChE and -Tyr activity than that of

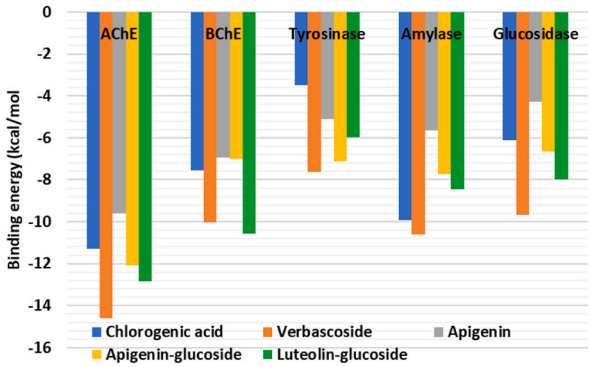


**Fig. 1.** Docking score of bioactive compounds from different extracts of three Verbascum species from Turkey.

*V. euphraticum* and *V. oocarpum* (Zengin et al., 2021). Only one study performed by Dalar et al. (2018) on the flower and stem extracts of *V. cheiranthifolium* showed that they displayed moderate α-glucosidase inhibition property. Also, Leaf of *V. thapsus* possessed considerable α-amylase inhibition capacity (Sharma et al., 2023). Moreover, the AChE inhibitory activity of the studied *Verbascum* spp. extracts could be partly due the presence of verbascoside which was previously demonstrated to possess moderate anti-AChE activity (Kahraman et al., 2010).

### 3.5. Molecular modeling

The molecular docking score estimated in terms of binding energy values revealed that chlorogenic acid, verbascoside, apigenin, apigenin-glucoside, and luteolin-glucoside were potentially bound to all the target enzymes (Fig. 1). Verbascoside displayed a high binding energy score (<− 7.5 kcal/mol) against all the enzymes, with the greatest binding propensity against AChE (<− 14.2 kcal/

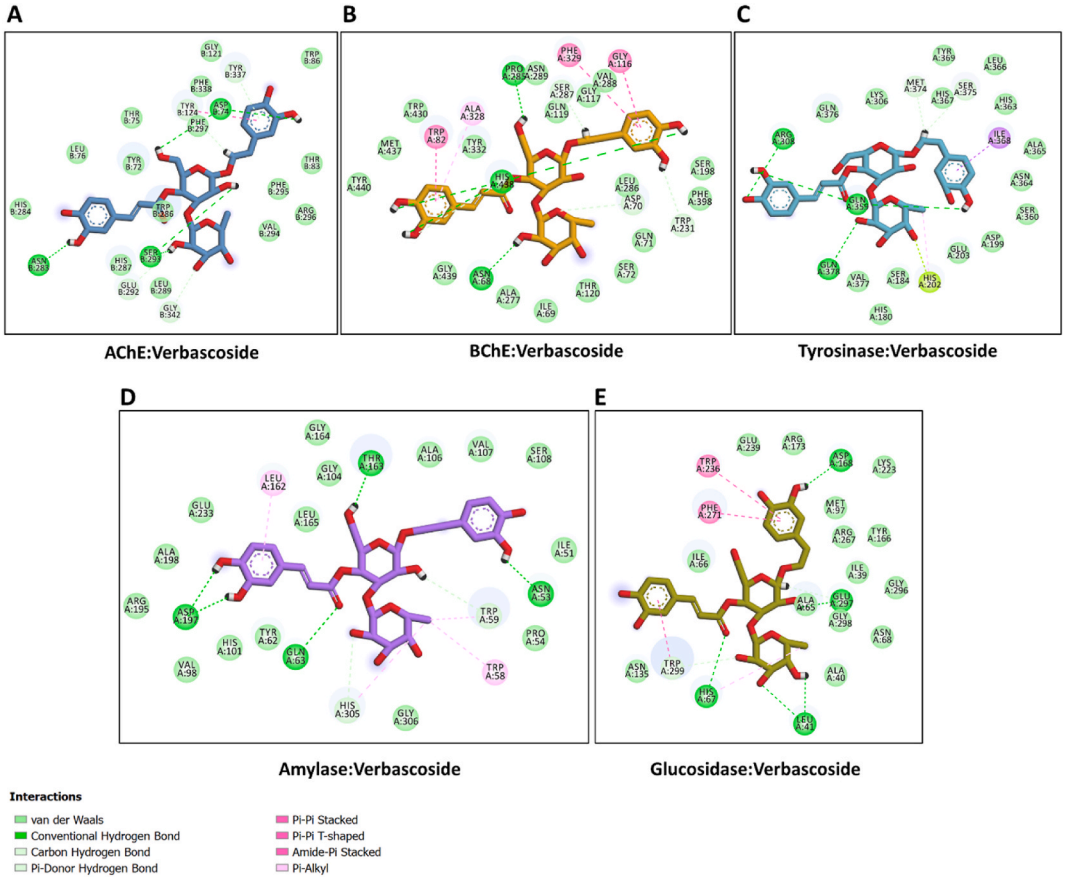


**Fig. 2.** Interaction of verbascoside with (A) AChE, (B) BChE, (C) tyrosinase, (D) amylase, and (E) glucosidase.

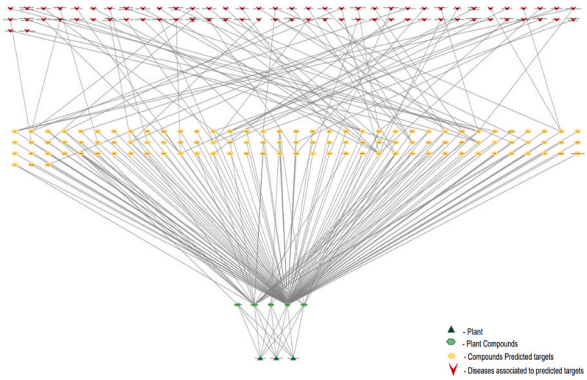


**Fig. 3.** *Verbascum cheiranthifolium, Verbascum myriocarpum* and *Verbascum pyroliforme* target network.

mol). Therefore, the interaction of verbascoside with all the proteins was examined. Verbascoside bound to the active site of AChE via H-bonding with Asp74, Asn298, and Ser298; a hydrophobic contact with Tyr124; and several van der Waals interactions all over the active site (Fig. 2A). Similarly, verbascoside demonstrated very strong interaction with BChE via H-bonding with Asn68, Pro285, and His438; π-π stacked interactions with Trp82 and Phe329; an amide-π stacked interaction with the backbone of Gly116; and multiple van der Waals interactions with different amino acid residues lining the active site (Fig. 2B). Interestingly, verbascoside also engaged the active site amino acid residues of tyrosinase by forming H-bonding with Arg308, Gln359, and Gln378, as well as many van der Waals interactions all over the channel (Fig. 2C). The same compounds bound to the active site of amylase via H-bonding with Asn53, Gln63, Thr163, and Asp197; hydrophobic contacts with Trp58, Trp59, Leu162, and His305; and van der Waals interactions (Fig. 2D). In case of glucosidase, verbascoside formed interactions that comprises H-bonds with His67, Asp168, Glu297, as well as the backbone of Leu41. Other interactions include π-π stacked interactions with Trp236, Phe271, and Trp299; and multiple van der Waals interactions (Fig. 2E). with a similar binding mode—one dihydroxyphenyl moiety interacted with the surface recognition residues and the other was buried deep inside the pocket, forming several interactions from which H-bonds and multiple van der Waals interactions appeared to be common in all the complexes. Collectively, these interactions likely added up to block the activity of the enzymes.

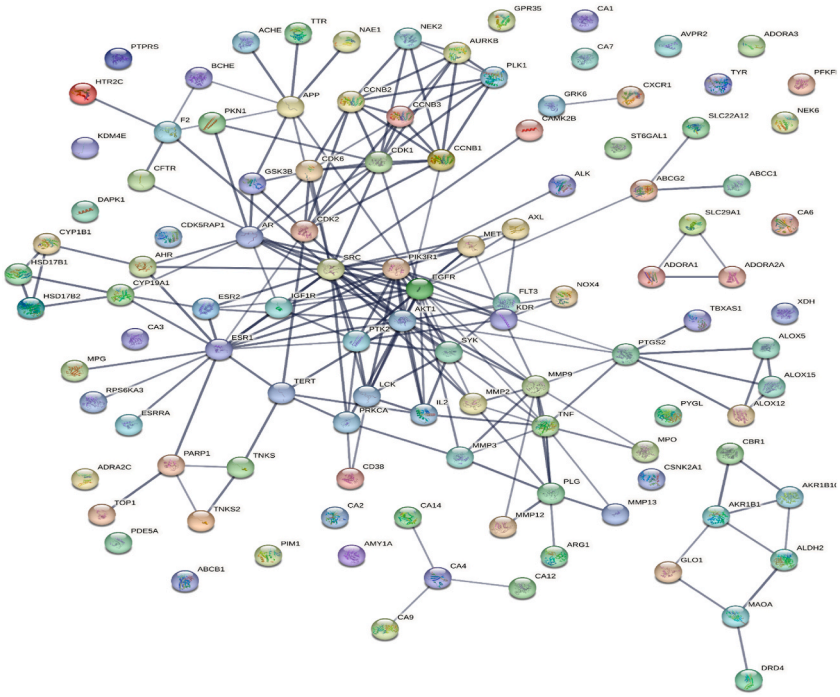


**Fig. 4.** The protein-protein interaction network with high confidence >0.7 for the predicted targets. The thickness of the line indicates the strength of the data.

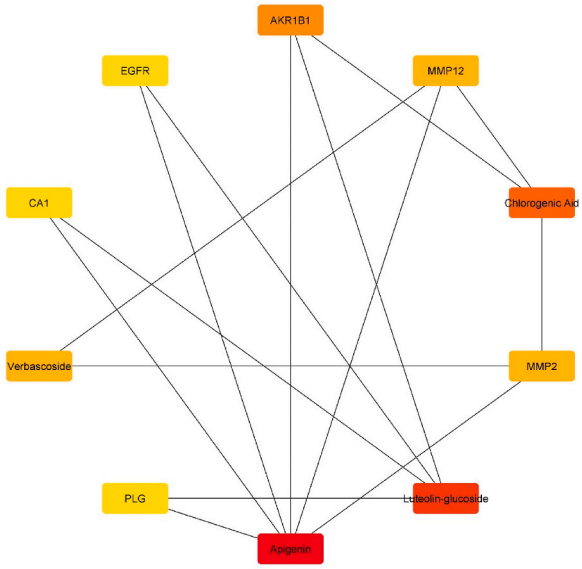


**Fig. 5.** The top 10 closely related node obtained from the Cytohubba plug-in.

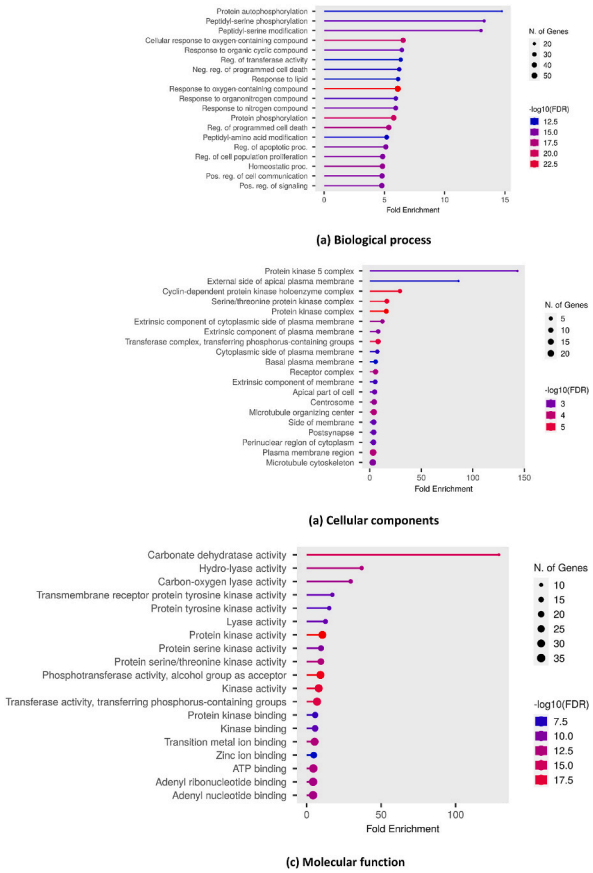


**Fig. 6.** The Lollipop plot representation for the gene ontology of the predicted targets. Lollipop plot of the biological process(A), cellular components(B), and molecular function (C) category terms from GO enrichment analysis.

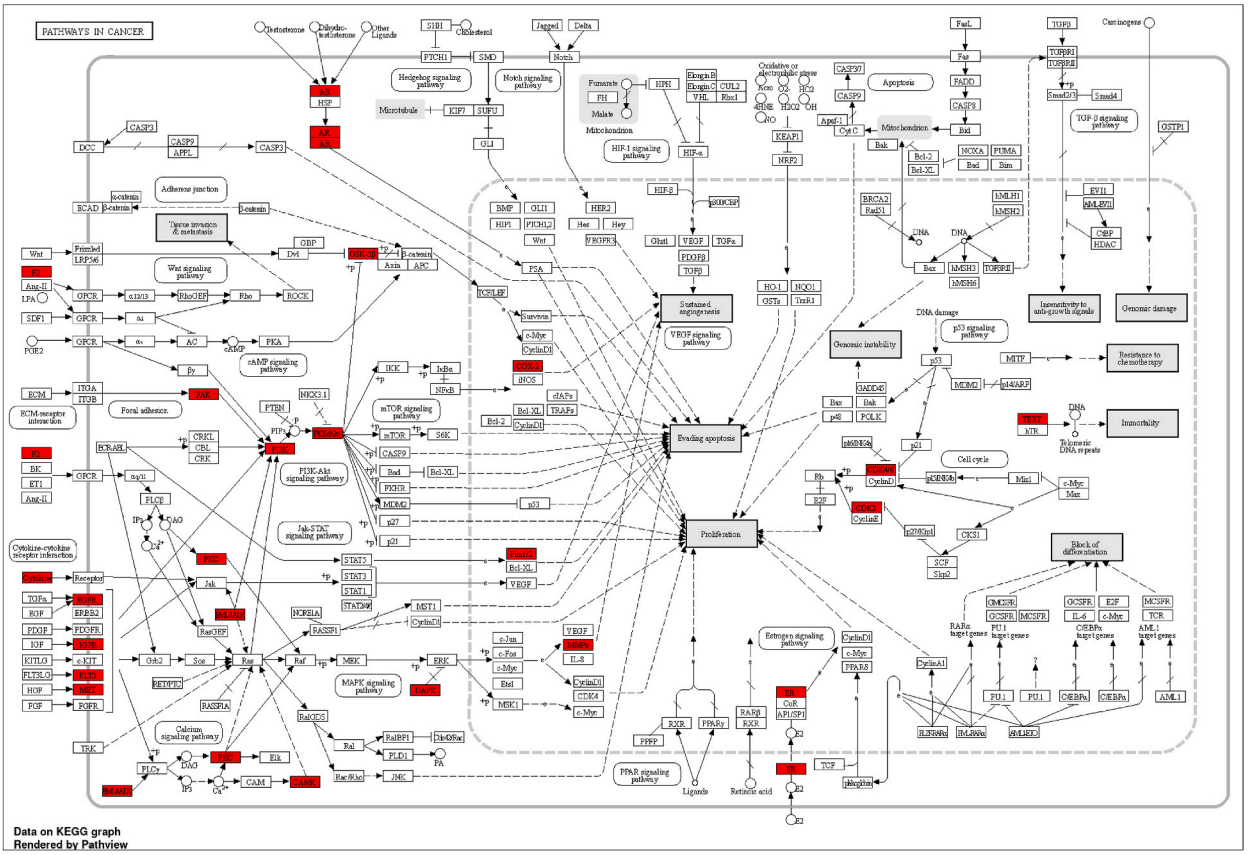


**Fig. 7.** The compound's predicted targets were highlighted in pathways in Cancer.

### 3.6. Network pharmacology

From Swiss TargetPrediction the targets for the compounds were predicted and filtered based upon the probability score, totally 132 targets were predicted for the five phenolic compounds (Chlorogenic acid, Verbascoside, Apigenin, Apigenin-glucoside and Luteolin-glucoside). For the 132 predicted targets the associated diseases were obtained from DisGeNET, totally 77 diseases with the annotated score greater than and equal to 0.7 were annotated to the predicted target. The network was constructed and visualized in hierarchal layout (Fig. 3) from plant compounds to diseases through predicted targets using Cytoscape software. Further, Cytohubba plugin was used to choose the top-ranking nodes with respect to degree and closeness were obtained. The top hub genes associated to the 5 phenolic compounds with respect to their degree were AKR1B1, MMP12, MMP2, PLG, CA1, EGFR (Fig. 5). The top closely related genes to the plant were EGFR, AKT1, AKR1R1, PLG, CA2 and CA4.

The protein-protein interaction network was constructed with the high confidence value $> 0.7$ using STRING database (Fig. 4). The protein-protein interaction network represents the functional and physical interaction between the proteins. The resulted network of the predicted targets had more significant interactions than the expected interactions. This means that the targeted proteins were more functional, as well as the physical interaction could biologically connect them as a group. The target proteins were clustered based on how functionally proteins were associated. It was clustered into 3 cluster using K means clustering option.

The gene names of the predicted targets were imported to the online web tool ShinyGo 0.77 for enrichment analysis. The Gene ontology of the targeted proteins for biological processes, cellular components, and molecular function were represented in the form of lollipop plot with X-axis and Y-axis show the fold enrichment and full names of the processes, respectively, and the colour and size of each lollipop represent the gene count and –log10FDR, respectively (Fig. 6). The top 20 biological pathway and process with FDR cut-off value 0.05 was obtained. From the gene and pathway enrichment analysis the bioactive 5 phenolic compounds were mainly involved in the cancers, non-small cell lung cancer, VEGF signalling pathways and metabolic pathways. The biological pathways were analyzed using KEGG pathway map. The compound's predicted target genes in the cancer pathway were highlighted in red colour and shown in Fig. 7.

The Swiss ADME tool predicted the physicochemical and pharmacokinetics properties of the five phenolic compounds and mentioned in Table 5. The compound 'Apigenin' showed the null Lipinski rule violation with good GI absorption and had good bioavailability score compared to other phenolic compounds. But Apigenin inhibit CYP1A2, CYP2D6 and CYP3A4 of CPYP450 sub-types enzymes. It had null violations for lead-likeness.

The various biological and medicinal properties were reported for the *Verbascum* species (Blanco-Salas et al., 2021; Donn et al.,

**Table 5**
The physicochemical and Pharmacokinetic properties of the phenolic compounds.

| Physicochemical and Pharmacokinetic properties | ADME properties for 5 phenolic compounds | | | | |
|---|---|---|---|---|---|
| Compounds | **Chlorogenic Aid** | **Verbascoside** | **Apigenin** | **Apigenin-glucoside** | **Luteolin-glucoside** |
| Pubchem_ID | 1794427 | 5281800 | 5280443 | 131750832 | 5280637 |
| Formula | C16H18O9 | C29H36O15 | C15H10O5 | C26H28O14 | C21H20O11 |
| MW | 354.31 | 624.59 | 270.24 | 564.49 | 448.38 |
| #Rotatable bonds | 5 | 11 | 1 | 5 | 4 |
| XLOGP3 | −0.42 | −0.5 | 3.02 | −1.64 | 1.46 |
| GI absorption | Low | Low | High | Low | Low |
| BBB permeant | No | No | No | No | No |
| CYP1A2 inhibitor | No | No | Yes | No | No |
| CYP2C19 inhibitor | No | No | No | No | No |
| CYP2C9 inhibitor | No | No | No | No | No |
| CYP2D6 inhibitor | No | No | Yes | No | No |
| CYP3A4 inhibitor | No | No | Yes | No | No |
| Lipinski #violations | 1 | 3 | 0 | 3 | 2 |
| Bioavailability Score | 0.11 | 0.17 | 0.55 | 0.17 | 0.17 |
| Leadlikeness #violations | 1 | 2 | 0 | 1 | 1 |

2023). Nwafor et al. reviewed the Chlorogenic acid in cancer and fibrosis (Nwafor et al., 2022), Wu et al. reported the effect of verbascoside in prostate cancer (Wu et al., 2021), and Ahmed et al. reviewed the therapeutic effect of Apigenin against the cancer (Ahmed et al., 2021). Through network pharmacology as well as previous studies evidenced that *Verbascum cheiranthifolium, Verbascum myriocarpum* and *Verbascum pyroliforme* has anticancer effect and their bioactive compounds plays a vital role in cancer pathways.

## 4. Conclusion

Extracts from the aerial parts of the three *Verbascum* spp. varied in their TPC,TFC, content of estimated compounds, antioxidant and enzyme inhibition activity. *V. cheiranthifolium* and *V. myriocarpum* were closer in each other than *V. pyroliforme* in term of chemical constituent and tested biological activity. Verbascoside followed by chlorogenic acid were the major compounds in *V. cheiranthifolium* and *V. myriocarpum* while *V. pyroliforme* was dominated by luteolin-glucoside and apigenin-glucoside respectively. It was also observed that the best antioxidant activity was generally exerted by the methanol and hydromethanolic extracts of the three species while the EtOAc extracts exhibited relatively the best enzyme inhibition property. The network pharmacology studies establish the multi-target therapeutic effect of *Verbascum* compounds. The bioactive phenolic compounds were primarily involved in various cancer pathways. This paved the way for more enzyme inhibition studies for new targets of multiple diseases. Thus, the three investigated *Verbascum* spp. represent a potential source of bioactive metabolites with interesting antioxidant and enzyme inhibition properties worth further exploration and pharmaceutical applications.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Data availability

Data will be made available on request.

## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bcab.2023.102834.

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