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Colchicine, serotobenine, and kinobeon A: novel therapeutic compounds in *Carthamus tinctorius L.* for the management of diabetes

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Abstract

Diabetes, a global health concern, poses increasing mortality risks. The pathogenesis of diabetes involves multiple mechanisms, with oxidative stress being one of the key contributors. As synthetic drugs have various side effects, which can be minimized by using herbal plants. This study focuses on the In vitro antioxidant potential, α-amylase inhibition potential, identification of bioactive compounds, and hub genes in diabetes treatment mechanism by using *C. tinctorius* Extraction of *C. tinctorious* lead and flower was performed using different solvents (Distilled water, methanol, chloroform, and Dimethyl ether). After extraction different concentrations range from 25–200 mg/mL) was made and checked against activities. The antioxidant potential was assessed using 2, 2-diphenyl-1-picryl-hydrazyl (DPPH), total phenolic contents (TPC), and total antioxidant capacity (TAC) assays, while antidiabetic activity was evaluated through α-amylase inhibition assay. Phytochemicals was identified by GC–MS analysis, followed by ADMET screening and network pharmacology analysis using Swiss Target Prediction, Gene Card, DesGeNet, DAVID, STRING, Cytoscape, and drug revitalization databases. Results revealed positive correlations with DPPH, TAC, and TPC. Methanol extract exhibited the highest inhibitory concentration. Screening of 46 compounds was performed by studying their pharmacokinetic properties which revealed 9 compounds effective against 204 diabetes targets. Moreover, their network analysis identified four hub genes, including AKT1, JUN, EGFR, and MMP9. These genes found highly associated with drugs like Colchicine and Serotobenine. Revitalization analysis also highlighted four genes (EGFR, PTGS2, AKT1, and MMP9) strongly correlated with FDA-approved drugs. The study suggests *C. tinctorius* methanol extract is a potential source for novel drugs.

Keywords *Carthamus tinctorius*, Diabetes, Phytochemicals, Network pharmacology, Drug revitalization

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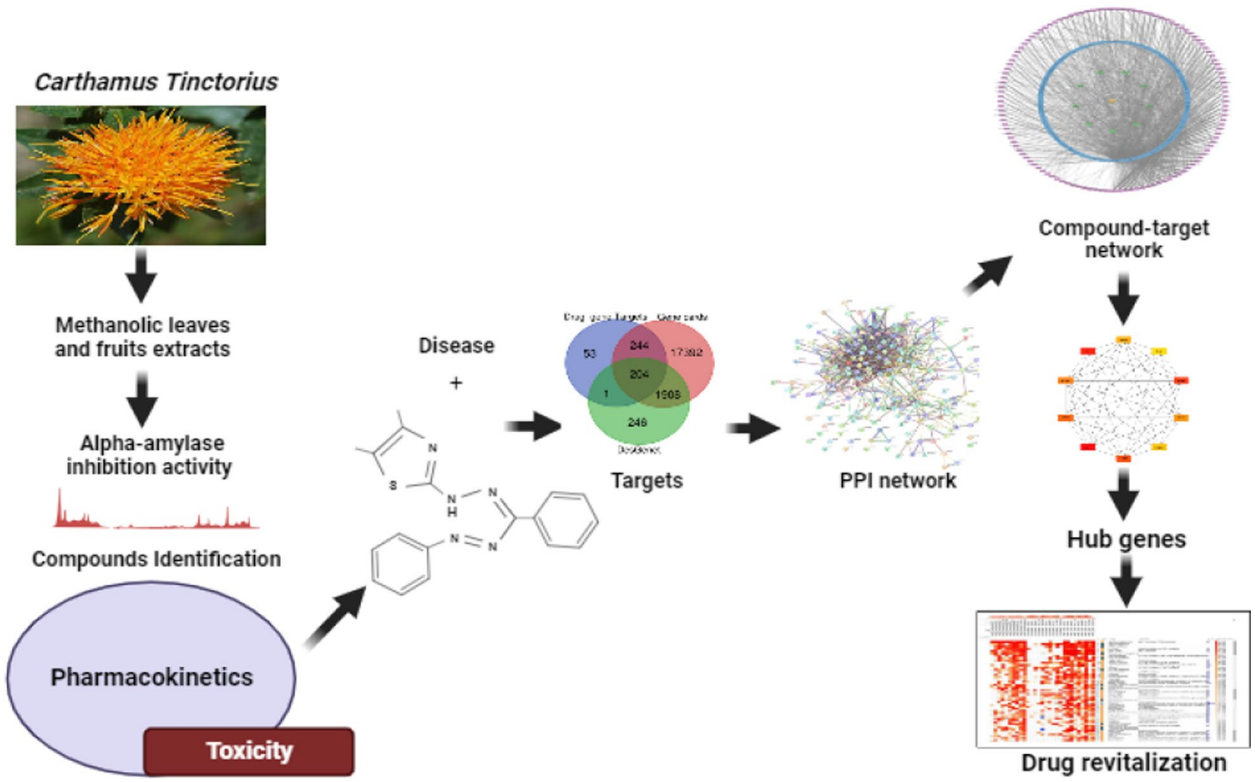
Graphical Abstract



Introduction

Diabetes represents a substantial medical concern, marked by a growing prevalence and increasingly dire outcomes [1]. According to data from the International Diabetes Federation, the global prevalence of diabetes mellitus among individuals aged 20 to 79 was estimated at 536.6 million in 2021, and this figure is anticipated to escalate to 783.2 million by 2045 [2, 3]. Diabetes encompasses a multifaceted set of metabolic disorders characterized by prolonged high blood sugar levels. Its development varies by type and involves several mechanisms, including oxidative stress, cardiovascular diseases, kidney diseases, mental health, and Alzheimer's disease [4, 5].

One of the major mechanisms involved in the pathogenesis of diabetes is oxidative stress. Oxidative stress occurs when there is an imbalance between the production of reactive oxygen species (ROS) [5, 6]. ROS are highly reactive molecules containing oxygen atoms that play a dual role in the body; they serve as essential signaling molecules in normal cellular processes but also contribute to oxidative stress when their levels become excessive[7, 8]. In diabetes, these species damage pancreatic beta cells and insulin receptors, disrupt glucose regulation, and cause inflammation. This cascade of events ultimately leads to insulin resistance and decreased insulin production [9, 10].

Numerous synthetic medications are available for managing diabetes; however, these therapies can be costly, lengthy, and comes with significant side effects. An alternative approach for managing diabetes could involve the use of medicinal plants and the natural substances they contain [3]. Consuming foods rich in natural antioxidants can be beneficial in managing diabetes. Safflower, scientifically referred to as *C. tinctorius* is a member of the Asteraceae family [11]. This plant has many healing qualities and has been utilized in various traditional medical practices. It is abundant in a diverse array of compounds, including alkaloids, phenols, flavonoids, terpenoids, tannins, and cardiac glycosides. This plant also exhibits antioxidant, anticancer, anti-inflammatory, analgesic and anti-atherogenic properties [12, 13]. Kinobeon A, a flavonoid compound from *C. tinctorius*, is noteworthy for its antioxidant and

anti-inflammatory properties. It holds potential in combating oxidative stress and inflammation, crucial factors in diabetes and its complications. By doing so, Kinobeon A can enhance insulin sensitivity and protect against retinopathy and neuropathy [14]. Serotobenine, another phenolic compound in *C. tinctorius*, exhibits anti-diabetic promise by inhibiting alpha-glucosidase, slowing glucose absorption, and reducing post-meal blood sugar spikes, making it valuable for those with impaired glucose tolerance [15]. Colchicine, derived from *C. tinctorius* and historically used for gout, has emerged as an anti-diabetic agent due to its inflammation-reducing and insulin-sensitivity-improving effects, offering hope for type 2 diabetes management, especially in the context of diabetic nephropathy [16].

Synthetic drug resistance and toxicity are the major health risk factors. Herbal drug formulation has evolved from the beginning of human civilization to the present through the development of cutting-edge research methods such as pharmacophylogeny and pharmacophylogenomics [17]. In this era of research, network pharmacology is an emerging and interdisciplinary field that has revolutionized the way we understand and approach drug discovery and development. It combines the principles of pharmacology, bioinformatics, systems biology, and network analysis to comprehensively study the complex interactions between phytochemicals, targeted genes, and biological pathways within a living organism [18, 19]. Bioactive compounds are multi-targeted. By constructing and analyzing intricate networks of compound-protein interactions, protein–protein interactions, and other molecular relationships, researchers in network pharmacology can identify novel drug targets, predict potential side effects, and even repurpose existing drugs for new therapeutic indications. This approach not only accelerates the drug discovery process but also offers a deeper understanding of the underlying mechanisms of diseases, ultimately leading to more effective and safer medications [20].

Therefore, these compounds hold potential for novel therapeutic opportunities by exceeding the currently constrained space of FDA-approved pharmaceutical and synthetic drugs. The optimization of the connectivity map investigates the functional relationship between medication, genes and diseases. This biological database has been extensively used for the revitalization of existing drugs, discovering the molecular mechanism of unidentified drugs and utilizing novel compounds for specific ailments [1, 21]. The present study aims to investigate the antioxidant and antidiabetic potential of three novel compounds (Kinobeon A, Serotobenine and Colchicine) present in *C. tinctorius* through network pharmacology and drug revitalization studies.

Materials and methods

Germplasm collection and growing condition

Seeds of *C. tinctorius* L. were collected from the local Nursery (Green Mall, 27 EME-II Canal City), Lahore. The field experiment was conducted at the Botanical Garden of Lahore College for Women University, located at 31.1418° N latitude and 72.3752° E longitude at an altitude of 563ft. The experiment was conducted using a randomized complete block design (RCBD). *C. tinctorius* cultivation requires meticulous soil preparation. The planting depth and row spacing were both set at 20–25 cm. The seeds were planted randomly, with an estimated number of 100–150 seeds. The seeds were covered with matured agricultural fertilizers, and the planting lines were covered with soil. The garden soil exhibited excellent drainage capabilities with a sandy loam texture, maintaining a pH level between 8.7 and 8.9 and an electrical conductivity of 1.59 ds/m. Germination of these seeds was achieved at an optimal temperature of 18 °C. After 6 months of growth, mature plants were harvested for further examination of their leaves and flowers, particularly in relation to their potential antioxidant and antidiabetic properties.

Extraction of leaves and flowers of *C. tintorious*

The shade-dried flowers and leaves of *C. tintorious* were used for extraction. Four different solvents were used for extraction ranged from non-polar to polar (distilled water, chloroform, methanol, and petroleum ether). Approximately 50 g of powdered leaves and flowers were extracted separately in soxhlet apparatus in 250 mL of Chloroform for 3 days their boiling temperature. Separation of compounds with remaining solvents petroleum ether methanol, and Distilled water was performed afterwards based on the solvents polarity. The solvent was evaporated by using rotary evaporater under reduced pressure and crude extract is obtained after complete evaporation of solvent. A stock solution of the crude extracts was prepared in DMSO (dimethyl sulfoxide) after obtaining desired yield. Different concentration (25, 50, 100, 200 mg/mL) were then tested against diabetes.

In vitro screening of *C. tinctorius* extracts

Antioxidant potential by free radical scavenging DPPH assay

The free radical scavenging capability of the plant extracts was assessed using the DPPH radical scavenging assay [7]. This involved evaluating the plant extracts' ability to donate hydrogen atoms, indicated by the decolorization of a methanol solution containing DPPH. Violet/purple hue was imparted to the methanol solution by DPPH. But transitions to various shades of yellow in the presence of antioxidants was also considered. To perform the assay, a methanol solution containing 0.1 mM

DPPH was prepared, and 1.6 mL of the extracts of *C. tinctorius* leaves and flowers at different concentrations (ranging from 25–200 μgmL^{-1}) was mixed with 2.4 mL of this DPPH solution. The resultant mixture was thoroughly vortexed and left to stand in the dark at room temperature for 30 min. Subsequently, the absorbance of the mixture was measured using spectrophotometry at a wavelength of 517 nm. Alpha tocopherol was used as a reference. The percentage of DPPH radical scavenging activity was calculated using the following formula:

$$\%RSA = \frac{Ac - As}{Ae} \times 100$$

In this context, Ac represents the absorbance of the control, and As stands for the absorbance of the extractives/standard. This experimental procedure was replicated three times for each concentration.

Estimation of total phenolic content (TPC)

The total phenolic content in the leaf and flower extracts of *C. tinctorius* was quantified using a modified Folin-Ciocalteu method [22]. The total reaction mixture of 10 ml was prepared. 2 mL from each concentration (25–200 mgmL^{-1}) was combined with 2 mL of Folin-Ciocalteu reagent, diluted with water at a 1:10 v/v ratio, and 2 mL of a 75 g/L sodium carbonate solution. The tubes were then vigorously vortexed for 15 s and allow to stand for 20 min at 25 °C for color development. The absorbance was measured at 760 nm using a UV-spectrophotometer (Shimadzu, USA). The extract samples were assessed at final concentrations of 0.1 and 0.15 mg/mL. The total phenolic content was expressed in terms of gallic acid equivalent (GAE) with the standard curve equation:

$$y = 0.0276 \times +1.986,$$

with an R-squared value of 0.996, represented as milligrams of gallic acid per gram of dry extract (mg of GA/g). This experiment was repeated three times at each concentration.

Total antioxidant capacity by phosphomolybdate assay

The phosphomolybdate method was used to determine the overall antioxidant capacity of leaf and flowers extracts of *C. tinctorius*, with alpha tocopherol serving as the standard [23]. A 0.1 mL of the sample solution was combined with 1 mL of a reagent solution containing 0.6 M sulphuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate. The sample tubes were securely capped and incubated in a water bath at 95 °C for 90 min. After cooling to room temperature, the absorbance of the resulting mixture was measured at 695 nm using a UV–visible spectrophotometer, with control as reference. The control solution consisted of 1 mL of the reagent solution and the appropriate solvent volume, subjected to the same incubation conditions. The antioxidant capacity of the plant extract was calculated using the following formula:

$$TAC(\%) = \frac{Controal\ absorbance - sample\ absorbance}{Control\ absorbance} \times 100$$

Antidiabetic activity by α-amylase inhibition assay

Alpha-amylase (0.5 mg mL^{-1}) was mixed in 500 mL of 0.02 M of sodium phosphate buffer (pH adjusted to 6.9 with 0.006 M of sodium chloride) and incubated for 10 min at 25 °C. After incubation period, various concentrations (25, 50, 100, 200 mg/mL) of leaf and flower extract along with 500 mL of a 1% starch solution (prepared in 0.02 M PBS) were added to each test tube. This was followed by incubation at 25 °C for 10 minutes. To stop the reaction, 1.0 mL of dinitrosalicylic acid (DNSA) was added to the reaction mixture and a final incubation was carried out at 25 °C for 5 min. The test tubes were then cooled to room temperature, and 5 mL distilled water was added to dilute the reaction mixture. The absorbance was measured using a UV–visible spectrophotometer at the wavelength of 540 nm. The absorbance measurements were compared with standard control Acarbose [24]. The percentage of alpha-amylase inhibition, indicating antidiabetic activity, was determined using following formula:

$$\%\ \text{Inhibition} = [(A_c - A_s)/A_c] \times 100.$$

Gas chromatography-mass spectrometry (GC–MS) analysis

GC–MS analysis of methanolic leaf and flower extract of *C. tintorious* was performed by following the methodology of Sindhu et al. [25]. The analysis was conducted using a Shimadzu Nexis GC-2030 coupled to the MS detector (Shimadzu GC-MS-QP2020 NX).The conditions for analysis were as follow: a stabilized wax column (60 m, 0.25 mm ID, film thickness of 0.25 μm), an injector temperature of 80 °C, and temperature gradient for the column, was raised by 4 °C per minute until it reaches 150 °C after being held at 40 °C for 5 min. The temperature was then increased by 30 °C per minute until it reached its final setting of 250 °C, which was held for 5 mines. In split injection mode, helium was used as the mobile phase at flow rate of 1 mL min^{-1}. The MS conditions were run in EI ionization mode; with the detector and interface temperatures set at 230 °C and 250 °C, respectively. It ran for 40 min. Compounds were identified by comparing the mass spectra with NIST library

(NIST14 standard version) and the LRI values with external standards. The Linear Retention Indices (LRI) of each component were calculated using an identical series of an n-alkane solution (C10-40, Polyscience, Niles, IL, USA; 5 mg L^{-1}).

Networking pharmacology analysis of *C. tinctorius*

Compounds selection by pharmacokinetic properties and ADME analysis

The compounds identified by GC–MS analysis were used to extract the physiochemical and pharmacokinetic properties. The three-dimensional (3D) structures and Canonical SMILES of compounds were downloaded from SpiderChem (SpiderChemhttp://www.chemspider.com/ accessed on 17 August 2023) and PubChem (http://www.pubchem.ncbi.nlm.nih.gov/ accessed on 17 August 2023) databases by using compound CID/SID number and name. In-depth pharmacokinetic and ADME properties of compounds were studied by using Swiss ADME (http://www.swissadme.ch/ accessed on 18 August 2023), Molinspiration (https://www.molinspiration.com/ accessed on 18 August 2023), and Tox prediction (https://tox-new.charite.de/protox_II/ accessed on 19 August 2023) for toxicity assessment by inputting compounds Canonical SMILES and structures. According Lipinski's rule of five for drug discovery, compounds that exhibit oral bioavailability (OB ≥ 30), molecular weight (MW < 500 Da), Druglikeness (DL ≥ 0.18), hydrogen bond donors (H donor < 5), octanl water coefficient (P < 5) and hydrogen bond acceptors (H acceptor < 10) are ideal for study. Targets was predicted by using these ideal compounds [26].

Compounds and disease target prediction

The target of the effective compounds were identified by using STITCH (http://stitch.embl.de/ accessed on 20 August 2023) and Swiss Target Prediction (http://www.swisstargetprediction.ch/ accessed on 20 August 2023). Two data bases, GeneCards and DisGeNET were explored for putative antidiabetic targets information by selecting species as "*Homo sapiens*". The common names of all the genes was searched from UniProtKB. After removing repetition in genes, a Venn diagram created to find the common targets by using Bioinformatics tool (https://bioinformatics.psb.ugent.be/webtools/Venn/ accessed on 20 August 2023) [27].

Gene ontology and enrichment analysis

The functional annotation bioinformatics tool DAVID (https://david.ncifcrf.gov/ accessed on 23 August 2023) was used to study genes ontology and enriched pathways. A total of 204 common targets were entered into DAVID to collect data by selecting species "*Homo sapiens*" and employing cutoff method for probability score ($P < 0.05$). Data on gene molecular function (MF), biological process (BP) and cellular components were used to characterize the Gene ontology and KEEG pathway for enrichment analysis. Top 20 pathways were selected to draw dot plots for visual representation of pathways involved by Shiny GO V0.77.

Development of compound-target network

The compound-target network was developed by Cytoscape V3.10.1 (https://cytoscape.org/ accessed on 25 August, 2023) to illustrate the interaction between *C. tinctorius* components and the antidiabetic target. The network analyzer assessed the network's properties using "degree," a node property that indicate the number of nodes associated with a specific node.

Protein–protein interaction (PPI) network and hub genes screening

The functional Protein association network was constructed by STRING database V12.0 (https://string-db.org/ accessed on 27 August 2023), with the organism set to *Homo sapiens*. The minimum interaction level was set at a threshold of 0.4 or greater. The network was visualized in Cytoscape V3.10.1 (https://cytoscape.org/ accessed on August 30, 2023). The CytoHubba plugin was used to identify hub genes and high-degree nodes, indicating stronger associations among the targeted genes for further analysis [28].

Construction of target-compound-pathway network

Using DAVID, data from all functional genes KEGG pathways were employed to construct the target-compound-pathway network in Cytoscape [29].

Network based drug revitalization

For the identification of target drugs linked with top hub genes. The drug Repurposing Hub and DGIdb 3.0 databases were assessed and a model was constructed [30, 31].

Statistical analysis

The data was presented as means ± standard deviations. Two-way analysis of variance (ANOVA) was used for statistical analysis, followed by the Turkey-Kramer multiple comparisons test. Differences were considered significant at $P < 0.05$. The Pearson correlation coefficients of DPPH radical scavenging (%); Total Phenolic Content (TPC), Total antioxidant assay (TAA), and alpha-amylase

inhibitory assay were calculated using SPSS Version 24.0 (SPSS, Chicago, IL, USA) [32].

Results

Plant extraction yield

The yields of leaf extraction varied across different solvents: chloroform (0.38%), methanol (1.22%), aqueous extract (2.5%), and petroleum ether (0.82%). Similarly, for flower extraction, the yields differed in various solvents: chloroform (0.43%), methanol (1.08%), aqueous extract (0.44%), and petroleum ether (3.1%). Different concentrations (25, 50, 100 and 200 mg/mL) was checked against antioxidant and antidiabetic activities.

Antioxidant profile of *C. tinctorius*

In the present study various extract of *C. tinctorius* were explored for their antioxidant capacity (DPPH free radical scavenging activity), total phenolic content and total antioxidant assay and results are presented in Table 1. Significant antioxidant activity was observed by various extracts of *C. tinctorius*. 2, 2-diphenyl-1-picrylhydrazl (DPPH) is a stable free radical. The DPPH antioxidant assay is based on DPPH's capacity to decolorize in the presence of antioxidant compounds [33]. Results depicted that both parts of the *C. tinctorius* observed to have slightly higher scavenging (%) as compared to standard α-tocopherol. Among different leaves extracts maximum scavenging (%) was observed by methanol (92.2 ± 0.57d); followed by chloroform (91.2 ± 1.3d); petroleum ether (90.2 ± 0.57d) and aqueous extract (90.3 ± 0.5d) at 200 $mgmL^{-1}$ whereas, aqueous extract observed to have minimum scavenging (%) i.e. 10.6 ± 0.57^{b} at 25 $mgmL^{-1}$ (Table 1). Methanol extract of flowers observed to have maximum scavenging (87.6 ± 0.5^{d}) at 200 $mgmL^{-1}$. Highest phenolic content was observed in methanol extract of

Table 1 Antioxidant profile of *C. tinctorius*

Sample	Concentrations ($mgmL^{-1}$)	200	100	50	25
DPPH radical scavenging (%)					
Leaves	Chloroform	91.2 ± 1.3^{d}	62.3 ± 0.5^{c}	47.6 ± 0.57^{b}	27.6 ± 0.5^{a}
	Methanol	92.2 ± 0.57^{d}	88.6 ± 0.5^{d}	81.33 ± 0.5^{d}	46.6 ± 0.57^{b}
	Petroleum ether	90.6 ± 0.57^{d}	76 ± 1^{d}	37.4 ± 0.75^{b}	18.6 ± 0.57^{d}
	Aqueous extract	90.3 ± 0.5^{d}	65.2 ± 0.69^{c}	16.6 ± 0.5^{b}	10.6 ± 0.57^{b}
Flowers	Chloroform	86.1 ± 1.15^{d}	62.6 ± 0.5^{c}	28.3 ± 0.5^{b}	13.3 ± 0.5^{a}
	Methanol	87.6 ± 0.5^{d}	75.1 ± 1.15^{c}	43 ± 1^{b}	26.2 ± 0.17^{a}
	Petroleum ether	85.6 ± 0.5^{d}	65 ± 1^{c}	41.1 ± 1.15^{b}	22.3 ± 0.5^{a}
	Aqueous extract	86.6 ± 0.5^{d}	73 ± 1^{c}	44.6 ± 0.5^{b}	27.6 ± 0.5^{a}
Alpha-tocopherol	Alpha-tocopherol	84 ± 1^{d}	28.6 ± 0.5^{a}	26.66 ± 0.57^{a}	20.3 ± 0.57^{a}
Total phenolic content (mg GAE g^{-1} dry extract wt.)					
Leaves	Chloroform	731 ± 1^{d}	648 ± 1^{d}	528 ± 1^{d}	311 ± 1^{a}
	Methanol	754 ± 1^{d}	618 ± 1^{d}	525 ± 1^{d}	307 ± 1^{b}
	Petroleum ether	493.6 ± 0.5^{c}	352.3 ± 0.5^{b}	246.3 ± 2.1^{a}	98 ± 1^{a}
	Aqueous extract	607 ± 1^{d}	495.3 ± 0.5^{c}	333 ± 1^{b}	103.3 ± 0.5^{a}
Flowers	Chloroform	548 ± 1^{d}	401 ± 1^{d}	227 ± 1^{d}	86.6 ± 1^{b}
	Methanol	597 ± 1^{d}	648 ± 1^{d}	176 ± 1^{d}	99.6 ± 1^{b}
	Petroleum ether	266.6 ± 1^{d}	385 ± 0.5^{b}	102.3 ± 2.1^{a}	87 ± 1^{a}
	Aqueous extract	507 ± 1^{d}	152.3 ± 0.5^{c}	200 ± 1^{b}	72.6 ± 0.5^{a}
Total antioxidant assay ascorbic acid equivalent (AAE)/g dry weight					
Leaves	Chloroform	0.42 ± 0.002^{b}	0.28 ± 0.02^{a}	0.24 ± 0.04^{a}	0.15 ± 0.022^{a}
	Methanol	1 ± 0.002^{d}	0.74 ± 0.03^{d}	0.42 ± 0.0005^{b}	0.22 ± 0.005^{a}
	Petroleum ether	0.99 ± 0.10^{d}	0.84 ± 0.001^{d}	0.52 ± 0.01^{b}	0.25 ± 0.015^{a}
	Aqueous extract	0.99 ± 0.02^{d}	0.46 ± 0.001^{b}	0.2 ± 0.002^{a}	0.15 ± 0.004^{a}
Flowers	Chloroform	0.21 ± 0.05^{a}	0.18 ± 0.004^{a}	0.16 ± 0.002^{a}	0.14 ± 0.001^{a}
	Methanol	1.43 ± 0.36^{d}	0.82 ± 0.05^{d}	0.26 ± 0.03^{a}	0.16 ± 0.02^{a}
	Petroleum ether	0.53 ± 0.02^{c}	0.64 ± 0.015^{d}	0.2 ± 0.01^{a}	0.14 ± 0.0015^{a}
	Aqueous extract	0.57 ± 0.006^{c}	0.42 ± 0.01^{b}	0.16 ± 0.001^{a}	0.14 ± 0.001^{a}
Standard	Ascorbic acid	0.51 ± 0.15^{c}	0.77 ± 0.01^{c}	0.91 ± 0.01^{d}	1.01 ± 0.017^{d}

Same letter indicating the same significance level among mean values. Whereas, different letter indicating the difference in significance level among mean values

leaves of *C. tinctorius* (754 ± 1^{d} mg GAE g^{-1} dry extract wt.) followed by leaf chloroform extract (731 ± 1^{d} mg GAE g^{-1} dry extract wt.); leaf aqueous extract (607 ± 1^{d} mg GAE g^{-1} dry extract wt.); flower methanol extract (597 ± 1^{d} mg GAE g^{-1} dry extract wt.) at 200 $mgmL^{-1}$ concentrations. Methanol extract of leaves (1 ± 0.002^{d} ascorbic acid equivalent (AAE)/g dry weight) and flowers (1 ± 0.002^{d} ascorbic acid equivalent (AAE)/g dry weight) also showed highest total antioxidant content (1.43 ± 0.36^{d} ascorbic acid equivalent (AAE)/g dry weight). The results indicated that most of the extracts showed significant results in all activities, with the methanolic extract of leaves showing highly significant results. As statistically, there is no significant difference between extracts. But methanolic extract of leaves can be considered the most effective among all extracts.

Antidaibetic potential of various extracts of *C. tinctorius*

Inhibition of alpha amylase by various extracts of *C. tinctorius* was also explored among all the extracts of both parts of plants, methanol extract observed to have maximum α-amylase inhibition (%) as compared to standard and other extracts. Maximum α-amylase inhibition (%) was found in methanol extracts of flowers (92 ± 1^{d}); followed by methanol extract of leaves; petroleum ether extract of leaves and flowers (92 ± 1^{d}; 91 ± 1^{d}; 76 ± 1^{d} and 71.3 ± 0.5^{d}) at 200 $mgmL^{-1}$ (Table 2). The findings of this activity indicate that the methanolic extract exhibited statistically significant effects. Correlation coefficient analysis revealed strong positive and negative relation between different variables, antioxidant assays and alpha amylase inhibitory activity. The alpha amylase inhibitory assay exhibited positive correlation with DPPH ($r = 0.76$), TPC ($r = 0.53$), and TAC ($r = 0.68$). Whereas, concentrations offered a weak correlation against antioxidant and alpha amylase inhibition assays (Fig. 1).

Active compounds screening and ligand/compound selection

Total 46 compounds were identified from leaves and flowers methanol extracts of *C. tinctorius* through GC–MS analysis. These compounds were further subjected to network pharmacology analysis. After studying pharmacokinetic properties and ADME analysis, 9 compounds found effective i.e. licarbazepine, pterin-6-carboxylic acid, N-Coumaroyl serotonin, Nb-p-Coumaroyltryptamine, moschamine, colchicine, cartorimine, serotobenine and kinobeon A (Table 1). Lipinski's rules of five was applied to conform the drug discovery criteria. According to this rule, all the 9 compounds have zero Lipinski's rule violation and meet the standard criteria i.e. molecular weight (MW < 500 Da), Drug Likeness (DL ≥ 0.18), hydrogen bond donors (H donor < 5), octanl water coefficient (P < 5) and hydrogen bond acceptors (H acceptor < 10) (Table 3).

Compounds ADME properties study suggest that compounds pterin-6-carboxylic acid, N-Coumaroyl serotonin, moschamine, kinobeon A, colchicine and cartorimine were found unable to cross the blood brain barriers, while other compounds licarbazepine, Nb-p-Coumaroyltryptamine and serotobenine can easily cross the blood brain barriers. Blood brain barriers are actually the protective barriers developed by endothelial cells in brains blood vessels that prevents many toxins to enter brain tissues. Four compounds licarbazepine, Nb-p-Coumaroyltryptamine, moschamine and colchicine showed positive results for permeability glycoprotein substrates (P-gp substrates) while the remaining compounds showed negative efficacy. It is indicated by

Table 2 The inhibition of α -amylase activity of various extracts and concentrations of *C. tinctorius*

Alpha amylase inhibition assay (%)					
Sample	**Concentrations ($mgmL^{-1}$)**	**200**	**100**	**50**	**25**
Leaves	Chloroform	67 ± 1^{d}	52.3 ± 1.5^{c}	24.6 ± 0.5^{b}	17.3 ± 0.5^{a}
	Methanol	91 ± 1^{d}	66.3 ± 0.5^{d}	48.3 ± 0.5^{c}	28.6 ± 0.5^{b}
	Petroleum ether	76 ± 1^{d}	65.3 ± 0.5^{d}	47.6 ± 0.5^{c}	28.6 ± 0.5^{b}
	Aqueous extract	66 ± 1^{d}	52 ± 1^{c}	24.6 ± 0.5^{b}	17.3 ± 0.5^{a}
Flowers	Chloroform	57.3 ± 0.5^{d}	42 ± 1^{c}	17.3 ± 0.5^{a}	12 ± 1^{a}
	Methanol	92 ± 1^{d}	77 ± 1^{d}	53.6 ± 0.5	33.3 ± 0.5^{b}
	Petroleum ether	71.3 ± 0.5^{d}	55 ± 1^{c}	43 ± 1^{c}	25 ± 1^{b}
	Aqueous extract	66.3 ± 0.5^{d}	42 ± 1^{c}	18 ± 1^{a}	13 ± 1^{a}
Standard	Acarbose	68.2 ± 0.6^{d}	54.5 ± 0.5^{c}	34.3 ± 0.5^{b}	16.6 ± 0.5^{a}

Same letter indicating the same significance level among mean values. Whereas, different letter indicating the difference in significance level among mean values

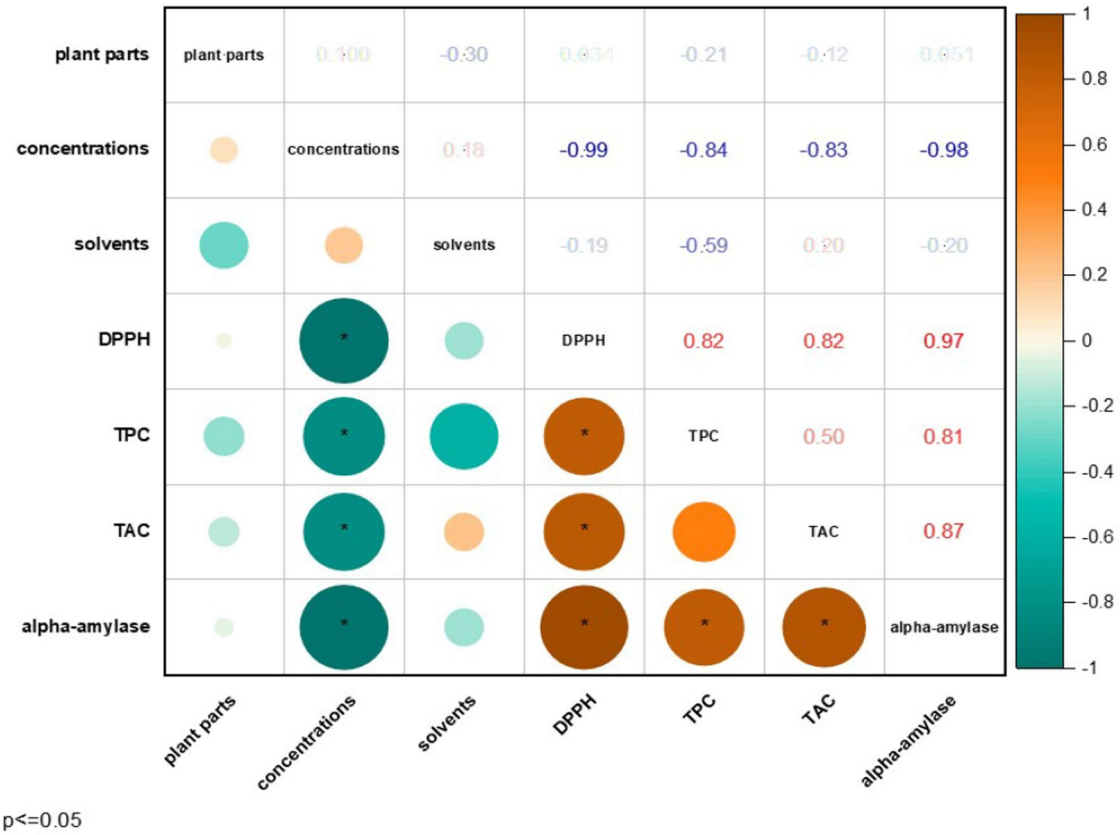


Fig. 1 Pearson correlation revealed strong positive and negative relation between different variables, antioxidant assays and alpha amylase inhibitory activity. Figure (insert number of figure) showed a significant negative correlation of concentrations against several antioxidant and alpha-amylase inhibitory assays. The alpha amylase inhibitory assay was found to have a significant relationship with DPPH (r = 0.76), TPC (r = 0.534), and TAC (r = 0.68)

the results that non-Pgp substrates can longer persists in the cells with greater efficacy. To achieve continuous plasma levels and improved bioavailability, it was anticipated that the tested compounds would exert inhibitory effects on all five cytochrome classes P450, i.e. CYP2C9, CYP2C19, CYP3A4, CYP1A2 and CYP2D6. Results revealed that only one compound moschamine showed inhibitory effect against all cytochrome classes CYP2C9, CYP2C19, CYP3A4, CYP1A2 and CYP2D6. Moreover, N-Coumaroyl serotonin can inhibit CYP2D6, Nb-p-Coumaroyltryptamine can inhibit CYP2C19 and CYP2D6; serotobenine can inhibit CYP2C19 and CYP2C9; and colchicine can inhibit CYP3A4 and CYP2D6. The remaining compounds did not show any inhibition potential against these cytochrome classes. All compounds, except for Pterin-6-carboxylic acid, exhibited significant gastrointestinal absorption, indicating a high level of absorption in the human intestinal tract (Table 4).

Toxicity potential

Four different types of toxicity factors (Tumorigenic, mutagenic, irritant and reproductive toxicity) were predicted for identified compounds. All the compounds showed non-toxic effects against all factors except two compounds. Cartorimine showed mild mutagenic and licarbazepine showed highly toxic reproductive effects (Table 5). Compounds LD_{50} and toxicity class prediction results showed that only one compound Kinobeon A have drug toxicity class V (LD_{50} = 2842 mg/kg), if swallowed may be harmful or not; four compounds licarbazepine, N-Coumaroyltryptamine, Nb-p-Coumaroyltryptamine and moschamine have drug toxicity class IV (LD_{50} = 856, 500, 500 and 500 mg/kg, respectively) if swallowed harmful; two compounds pterin-6-carboxylic acid and colchicine have drug toxicity class III (LD_{50} = 1500 and 290 mg/kg, respectively), if swallowed can be toxic; and two compounds serotobenine and colchicine have drug toxicity class II (LD_{50} = 38 and 6 mg/kg, respectively), if swallowed fatal (Table 6).

Table 3 Three-dimensional structure, physiochemical and pharmacokinetic properties of active compounds of *C. tinctorius*

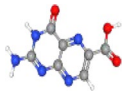
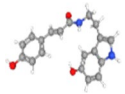
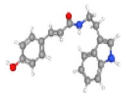
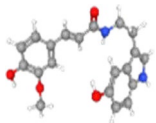
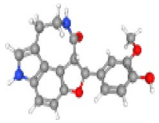
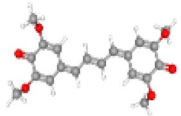
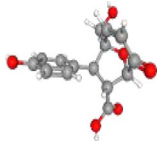
Sr. No.	Phytochemicals	Molecular weight (MW < 500 Da)	3D structures	H-bond donor (< 5)	No. of rotatable bonds (< 10)	H-bond acceptors (< 10)	clog P (p < 5)	Polar surface area (Å^2)	Oral bioavailability (OB ≥ 30)	Lipinski's rule of five violations
1	Licarbazepine	254.288		2	0	4	2.4449	66.56	0.55	0
2	Pterin-6-Carboxylic acid	207.149		3	1	8	− 1.7866	134.85	0.56	0
3	N-Coumaroyl serotonin	322.363		4	5	5	2.6238	85.35	0.55	0
4	Nb-p-Coumaroyltryptamine	306.364		3	5	4	2.9695	65.12	0.55	0
5	Moschamine	352.389		4	6	6	2.5538	94.58	0.55	0
6	Serotobenine	350.373		3	2	6	2.5703	83.58	0.55	0
7	Kinobeon A	356.373		0	6	6	2.239	71.06	0.55	0
8	Cartorimine	290.27		3	3	6	0.0234	104.06	0.56	0

Table 3 (continued)

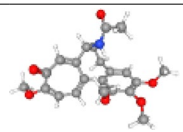
Sr. No.	Phytochemicals	Molecular weight (MW < 500 Da)	3D structures	H-bond donor (< 5)	No. of rotatable bonds (< 10)	H-bond acceptors (< 10)	clog P (p < 5)	Polar surface area (Å^2)	Oral bioavailability (OB ≥ 30)	Lipinski's rule of five violations
9	Colchicine	399.442		1	5	7	1.863	83.09	0.55	0

Table 4 ADME properties of active compounds of *C. tinctorius*

Compounds	CYP1A2 inhibitor	CYP3A4 inhibitor	CYP2C19 inhibitor	CYP2D6 inhibitor	CYP2C9 inhibitor	BBB permeant	P-gp substrate	Log K_p (skin permeation) (cm/s)	GI absorption
Licarbazepine	No	No	No	No	No	Yes	Yes	− 6.28	High
Pterin-6-Carboxylic acid	No	No	No	No	No	No	No	− 8.38	Low
N-Coumaroyl serotonin	Yes	No	No	Yes	No	No	No	− 6.14	High
Nb-p-Coumaroyltryptamine	Yes	No	Yes	Yes	No	Yes	Yes	− 6.26	High
Moschamine	Yes	Yes	Yes	Yes	Yes	No	Yes	− 6.34	High
Serotobenine	No	No	Yes	No	Yes	Yes	No	− 6.86	High
Kinobeon A	No	No	No	No	No	No	No	− 6.19	High
Cartorimine	No	No	No	No	No	No	No	− 8.12	High
Colchicine	No	Yes	No	Yes	No	No	Yes	− 8.01	High

Network pharmacology profiling

Compounds and diabetes targets prediction A total of 893 targets were obtained by Swiss Target Prediction against *C. tintorious* 9 compounds. Potential diabetic targets were collected from Gene Card (19749) and DisGeNet (2360) databases. Targets duplication was removed, merged and after Venn diagram illustration, 204 common targets was obtained (Fig. 2).

Compound-target network A compound-target network was constructed between 9 active compounds and 204 common genes. Degree circle green color showing active compounds, blue color showing their interaction with targets and purple color showing their function in diverse biological pathways (Fig. 3).

Protein–protein-interaction Protein–protein interaction network of 204 common genes was built and visualized in cytoscape. The topological parameters was studied by network analyzer, 204 nodes and 2505 edges were found (Fig. 5a).

Hub genes The top 10 genes identified were AKT1 (30), JUN (29), EGFR (28), CASP3 (25), GAPDH (22), PTGS2 (21), STAT3 (20), MMP9 (17), CTNNB1 (17) and TLR4 (10) (Table 7, Fig. 4B). Further, these gene were subjected to fist-stage node targets evaluation against most effective compounds. 6 compounds pterin-6-Carboxylic acid, moschamine, Kinobeon A, Serotobenine, Cartorimine, N-Coumaroyl serotonin and Colchicine found to be highly interactive against EGFR, MMP9, PTGS2, AKT1 (Fig. 5a).

Gene ontology and enrichment pathways Top 20 pathways were selected for GO analysis and KEGG pathway study. This selection was made based on a significance threshold of $P < 0.05$, as determined by DAVID. GO analysis found 170 molecular functions (MF) such as protein serine/threonine/tyrosine kinase activity, enzyme binding, RNA polymerase II sequence-specific DNA binding transcription factor binding, heme binding, iron ion binding, G-protein coupled adenosine receptor activity and nitric-oxide synthase regulator activity (Fig. 6a); 659 biological processes (BP), protein auto phosphorylation, trans membrane receptor protein tyrosine kinase signaling pathway, regulation of cell growth, insulin-like growth factor receptor signaling pathway, regulation of angiogenesis, cellular response to reactive oxygen species, regulation of blood pressure, positive regulation of vasoconstriction and protein functions (Fig. 6b); and 97 cellular components (CC), such as cytoplasm, receptor complexes, plasma membrane function, integral component of plasma membrane, membrane raft and cell surface function (Fig. 6c). KEGG analysis predicted 172 pathways regarding the anti-diabetics targets including Lipid and atherosclerosis, EGFR tyrosine kinase inhibitor resistance, AGE- RAGE signaling pathway in diabetic complications, ErbB signaling pathway, Endocrine resistance and PI3K-Akt signaling pathway (Fig. 6d).

Compound–target–pathway network A compound–target–pathway network was also built for visual representation of KEEG pathway. This pathway represents that the common targets have ability to regulate many functions in diverse biological systems (Fig. 5b).

Table 5 Toxicity prediction of active compounds of *C. tinctorius*

Phytochemicals	Tumorigenic	Mutagenic	Irritant	Reproductive effect
Licarbazepine				
Pterin-6-Carboxylic acid				
N-Coumaroyl serotonin				
Nb-p-Coumaroyltryptamine				
Moschamine				
Serotobenine				
Kinobeon A				
Cartorimine				
Colchicine				
Licarbazepine				

Non-Toxic
Highly toxic
Less Toxic

Table 6 Anticipation of LD_{50} predictions and characterization of compounds toxicity grades

Sr. No.	Compounds	LD_{50} prediction	Toxicity class prediction
1	Licarbazepine	856 mg/kg	Class IV
2	Pterin-6-Carboxylic acid	1500 mg/kg	Class III
3	N-Coumaroyl serotonin	500 mg/kg	Class IV
4	Nb-p-Coumaroyltryptamine	500 mg/kg	Class IV
5	Moschamine	500 mg/kg	Class IV
6	Serotobenine	38 mg/kg	Class II
7	Kinobeon A	2842 mg/kg	Class V
8	Cartorimine	290 mg/kg	Class III
9	Colchicine	6 mg/kg	Class II

* Class I: fatal ($LD_{50} \leq 5$); Class II:fatal ($5 < LD_{50} \leq 50$); Class III: toxic ($50 < LD_{50} \leq 300$); Class IV: harmful ($300 < LD_{50} \leq 2000$); Class V: may be harmful ($2000 < LD_{50} \leq 5000$); Class VI: non-toxic ($LD_{50} > 5000$)

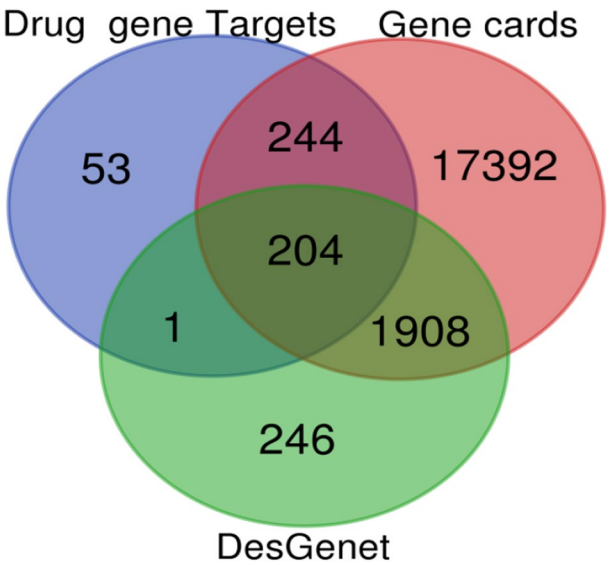


Fig. 2 Illustration of common genes by Venn diagram

Drug revitalization Increasing trend that several pharmacological molecules show their effects by interacting with a multiple target is boosting the advancement in research domains that challenge the data reductionism approach. The drug gene interaction network results revealed that EGFR, PTGS2, AKT1 and MMP9 were highly correlated with the diseased genes. Figure 7 depicts the various regulatory pathways associated with the top hub genes and drugs used in regulation of hub genes or to treat mutations in hub genes with median score ≥ 90. The FDA approved candidate drugs associated with the hub genes are presented in Tables 8, 9.

Discussion

Diabetes is a metabolic disorder induced by decreased insulin production or the onset of insulin resistance. Worldwide, it is now regarded as one of the leading causes of death. If diabetes is not treated or managed can lead to long-term health issues such as cardiopathy, blindness, and hepatic and renal abnormalities [34, 35]. Many synthetic therapies require the prescription of two distinct medications when a single drug would be more desirable, leading to improved adherence and potentially enhanced safety by simplifying treatment into a single medication [36]. Hence, the demand for natural products is increasing daily. Many diabetic patients are currently interested in complementary treatments that use herbal products. Various bioactive compounds have a direct impact on the selection criteria of herbal products for disease treatment. These factors include the stage of diabetes progression, the type

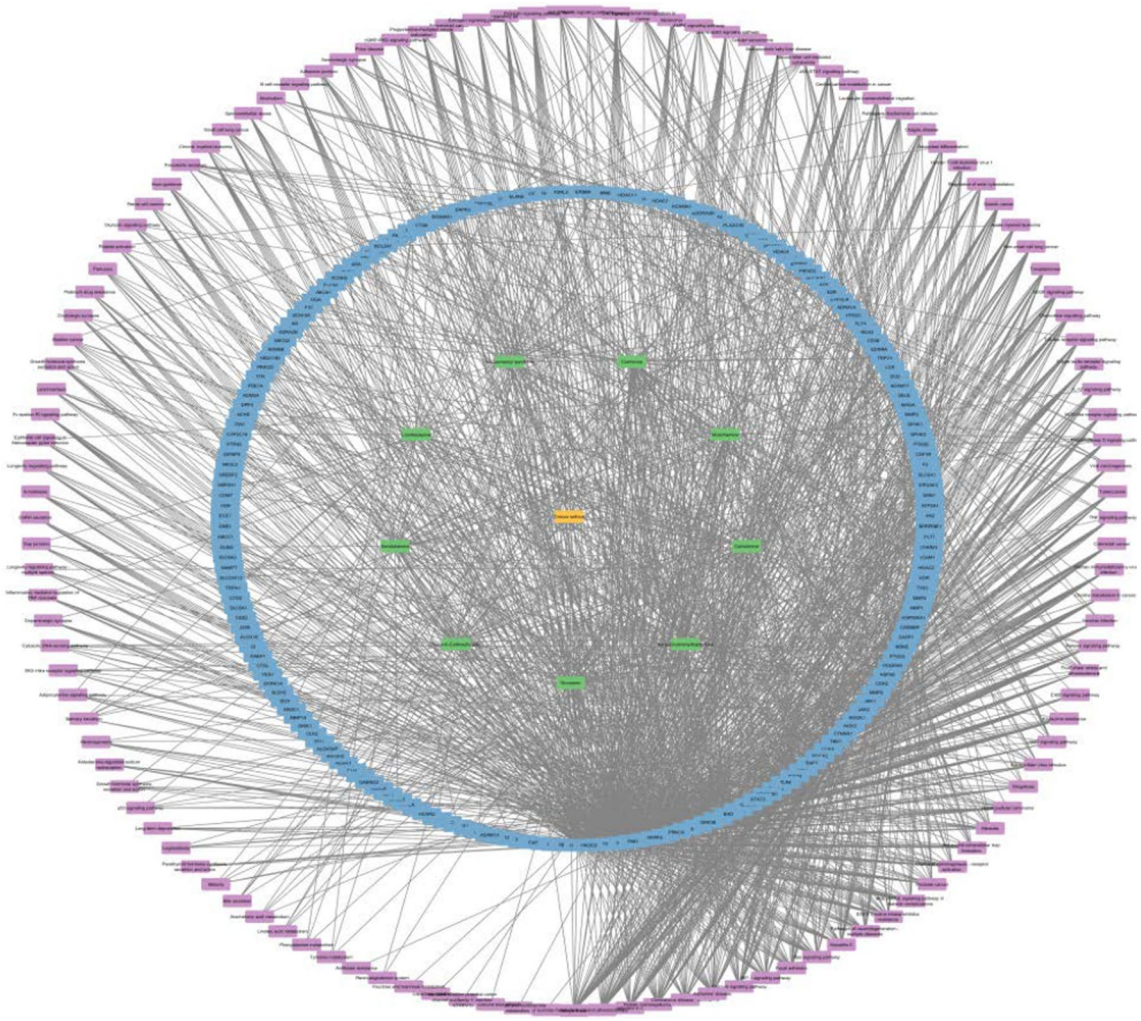


Fig. 3 Compound-target network

Table 7 Identifications of highly interactive cytohub compounds and genes

Rank	Genes	Degree
1	AKT1	30
2	JUN	29
3	EGFR	28
4	CASP3	25
5	GAPDH	22
6	PTGS2	21
7	STAT3	20
8	MMP9	17
9	CTNNB1	17
10	TLR4	10

of comorbidities the patient has, availability, price, and the safety profile of the herbs and herbal formulation [37]. The generation of free radicals as a result of oxidative stress is a detrimental process that hastens the development of diseases such as diabetes [38]. The efficacy of medicinal plants to reduce free radicals, donate hydrogen ions, and quench singlet oxygen can all be utilized to assess their anti-oxidant properties. Potential anti-oxidants found in medicinal and food plants may be able to mitigate the adverse effects of oxidative stress in illnesses such as diabetes [39, 40]. Based on these findings and the use of herbal items for hypoglycemic activity *C. tinctorius* was chosen for the study.

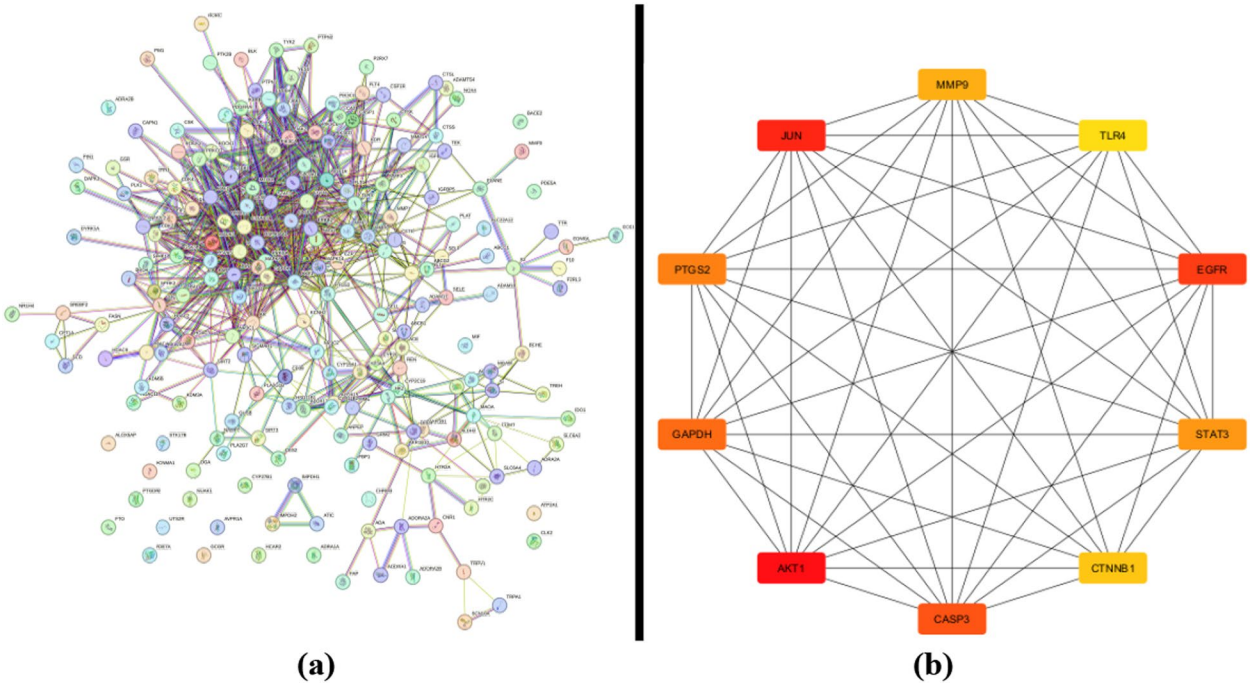


Fig. 4 **a** Protein–protein interaction (PPI) network **b** hub genes

The present study compared the antioxidant and alpha amylase inhibitory potential of various extracts (chloroform, methanol, petroleum ether, and distilled water) of *C. tinctorius* via in vitro models (Tables 1 and 2). The DPPH free radical scavenging (%) of methanol extract was higher i.e. 92.2 ± 0.57^{d} % as compared to alpha tocopherol (standard). Additionally, the methanol extract found to have a significant amount of total phenolic content and total antioxidant content (Table 1). A Previous study documented that methanol extract of *Asystasia gangetica* exhibited noticeable total phenolic content and free radical scavenging percentage, which may play a vital role in controlling antioxidants [35]. The presence of a high amount of bioactive compounds, may be responsible for normalizing insulin secretion, decreasing gluconeogenesis, enhanced pancreatic-cell proliferation, increasing the expression of glucose transporters, and protecting these cells from oxidative stress and inflammation [41, 42].

Amylase is an intestine enzyme that helps in carbohydrate degradation and glucose absorption in the human body. The hyperglycemic situation in diabetic patients produces oxidative stress and damage pancreatic cells. Inhibition of alpha amylase enzyme serves as an anti-nutritive, reducing glucose absorption and digestion [43]. In the present study *C. tinctorius* methanol extract exhibited the highest In vitro anti-diabetic efficacy due to the presence of Colchicine, Serotobenine, and Kinobeon A which have anti-diabetic activity (Table 2) (Fig. 1). In Persian traditional medicine, *C. tinctorius* has been used to treat diabetes, phlegmatic fever, melancholia, and dropsy. According to studies, all parts of the plant have long been used to stimulate libido in Pakistan and India [44–46].

The findings of the present study suggested that one of the mechanisms by which the *C. tinctorius* various extracts (chloroform, methanol, petroleum ether and aqueous) may exert their hypoglycemic impact is through the inhibition of alpha amylase activity, leading to a delay in starch hydrolysis. Inhibiting alpha-amylase remains a powerful strategy for developing new anti-diabetic drugs [47, 48]. Modulation of alpha amylase activity by bioactive in these extracts would eventually result in a reduction in postprandial blood glucose levels (Fig. 8). The presence of an alpha amylase inhibitor disrupts the normal pathway of dietary starch conversion to maltose, maltotriose, and oligosaccharides, and finally to glucose in the gut, which is absorbed in the blood [49]. Thus, alpha-amylase inhibitors can prevent glucose production in hyperglycemic situations. This alpha-amylase suppression could occur in a dose-dependent or dose-independent manner. The identified phytoactive compounds (Colchicine, Serotobenine, and Kinobeon A) responsible for alpha-amylase inhibition must be isolated. Said et al. [50] documented that the efficacy of alpha-amylase inhibitors inhibition can be boosted by combining bioactive components of

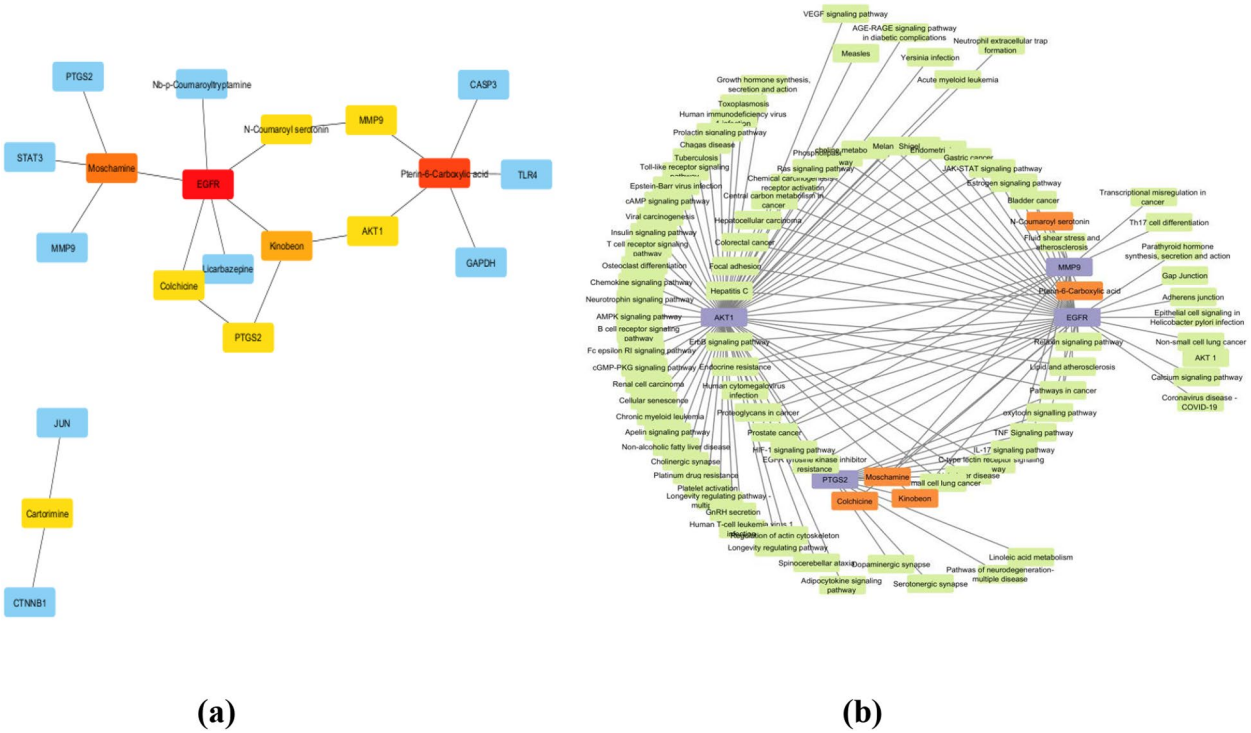


Fig. 5 **a** Fist-stage node genes **b** compound-target network

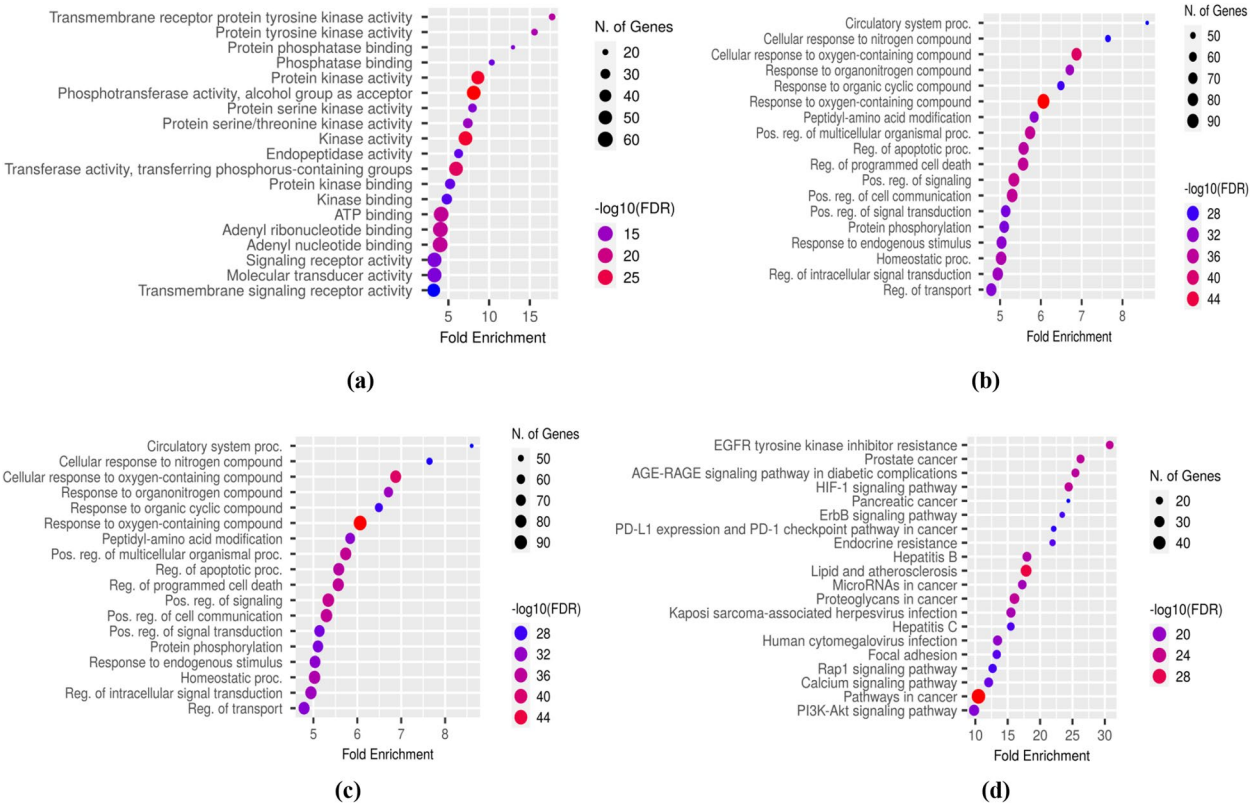


Fig. 6 Gene ontology and enrichment analysis (**a**) GO molecular function (MF) ` (**b**) GO biological process (BP) (**c**) GO cellular components (**d**) KEEG pathway

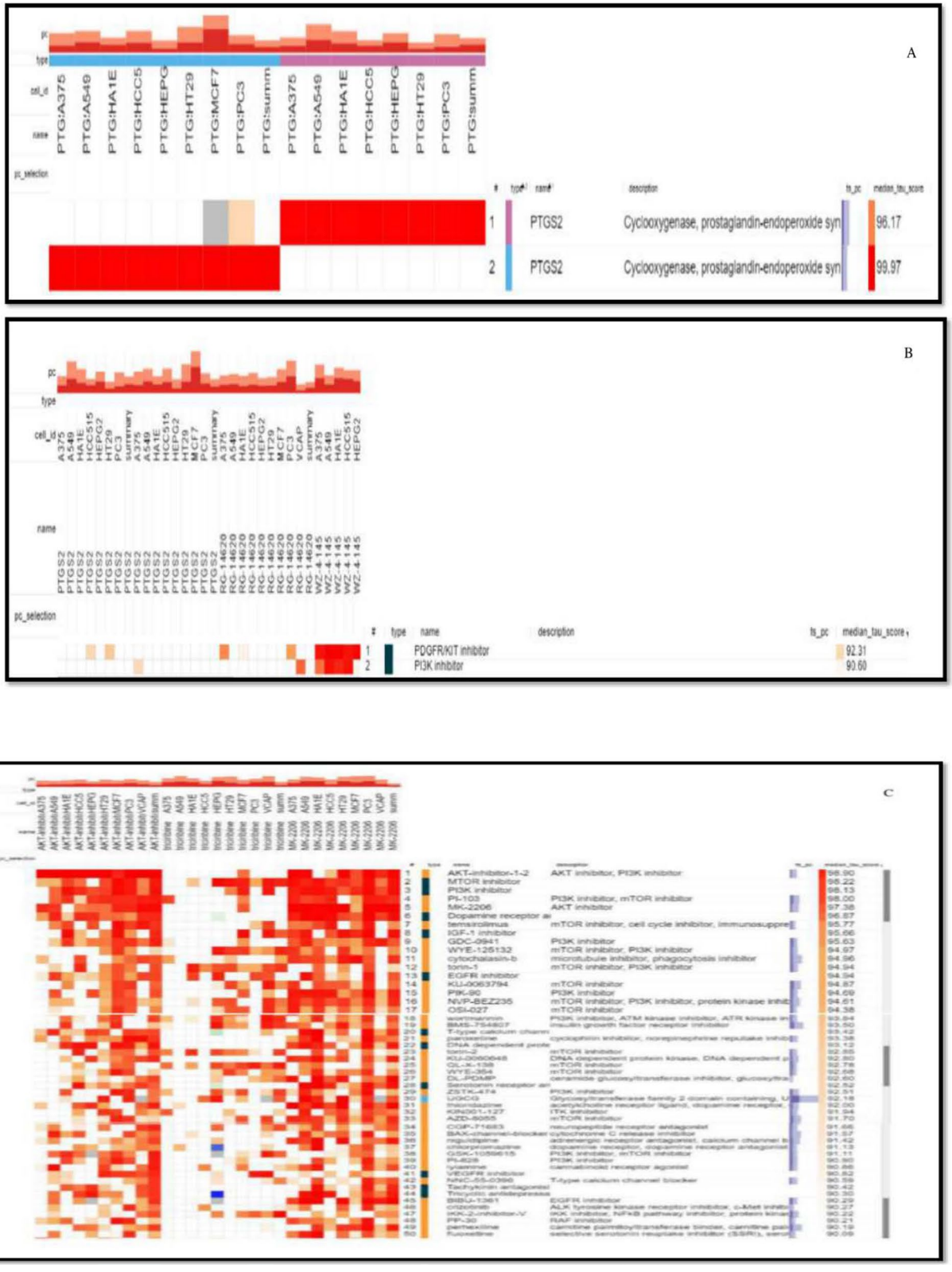


Fig. 7 Different FDA Approved drugs associated with top hub genes with median score greater than 90

Table 8 Candidate drugs with score greater than 90 associated with hub genes from drug repurposing hub:

Hub genes	Rank	Score	Type	Drug-ID	Name	Description	MOA
PTGS2	1	100	kd	CGS001-5743	PTGS2	Cyclooxygenase	
	175	96.6	cp	BRD-K28849549	Mesalazine	Cyclooxygenase inhibitor	Cyclooxygenase inhibitor, Lipoxygenase inhibitor, Prostanoid receptor antagonist
	408	92	cp	BRD-K92870997	Pterostilbene	Cyclooxygenase inhibitor	Cyclooxygenase inhibitor, PPAR receptor agonist
	452	90.9	cp	BRD-K10670311	Sulfasalazine	Antirheumatic	Antirheumatic, NFkB pathway inhibitor
EGFR	33	99.7	cp	BRD-K96778649	Tyrphostin-47	EGFR inhibitor	EGFR
	72	99.4	cp	BRD-A44551378	LFM-A12	EGFR inhibitor	EGFR
	305	96.9	cp	BRD-K97399794	Quercetin	Polar auxin transport inhibitor	PIK3CG, AKR1B1, ATP5A1, ATP5B, ATP5C1, CYP2C8, EGFR, GAA, HCK, HIBCH, MAOA, PIM1, PTPN1, SCN5A, SIRT1, STK17B, UGT3A1, XDH
	578	92.6	cp	BRD-K35573744	Erbstatin-analog	EGFR inhibitor	EGFR
	697	90.8	cp	BRD-K32292990	CGP-53353	EGFR inhibitor	EGFR, PRKCB
AKT1	1	99.9	cp	BRD-K80431395	Triciribine	AKT inhibitor	AKT1, AKT2, AKT3
	49	98.3	cp	BRD-K13049116	BMS-754807	IGF-1 inhibitor	IGF1R, AKT1
	96	96.9	cp	BRD-K28296557	AKT-inhibitor-IV	AKT inhibitor	AKT1
	143	95.7	cp	BRD-K61401890	Deguelin	NADH-ubiquinone oxidoreductase (Complex I) inhibitor	AKT1, PTGS2
	534	90.3	cp	BRD-K50168500	Canertinib	EGFR inhibitor	EGFR, ERBB2, ERBB4, AKT1

four herbal plants. Alpha-Amylase inhibitors are important for managing in postprandial blood glucose level in diabetic patient. [51]. Some ethnic and indigenous communities in undeveloped nations use various naturally available therapeutic plants in the form of crude extracts or distinct formulations. Meanwhile, the various adverse effects of well-known allopathic pharmaceuticals are driving the public, especially in industrialized countries, to herbal therapy [52].

For the past few decades, despite of rapid advancements in technologies, the pharmaceutical business has been plagued by low drug productivity. The main reason behind this low productivity is the pharmacologic paradigm of "single drug, single target, and single disease" necessitates the development of multi target drugs. Network pharmacology is a major component of drug discovery and development as drug-target crosstalk may result in novel drug-target interactions [53]. Phytochemicals play a crucial role in network pharmacology by actively participate in the intricate network of molecular interactions and synergistic mechanisms that underlie the therapeutic potential of naturally occurring plant-derived compounds [54, 55].

Total 46 compounds were identified from GC–MS analysis. Initial screening of compounds was performed through pharmacokinetic and ADMET properties study. Nine compounds Licarbazepine, Pterin-6-carboxylic acid, N-Coumaroyl serotonin, Nb-p-Coumaroyltryptamine, Moschamine, Colchicine, Cartorimine, Serotobenine and Kinobeon A found effective with zero Lipinski's rule violation (Table 3). Analyzing the pharmacokinetic characteristics of substances is essential for comprehending the processes through which drugs enter the body, get distributed, undergo metabolism, and are ultimately get eliminated. This understanding plays a crucial role in refining drug dosage regimens and improving their effectiveness in therapy [56]. A perfect pharmaceutical compound is characterized by its complete compliance with Lipinski's Rule, with no violations [57].

The study on ADMET properties of various compounds revealed that pterin-6-carboxylic acid, N-Coumaroyl serotonin, moschamine, kinobeon A, colchicine and cartorimine were unable to cross the blood brain barriers (Table 4). The blood–brain barrier acts as a protective shield, regulating the passage of substances into the brain, including phytochemicals with potential neuroprotective effects [58, 59].

Among the compounds tested, Licarbazepine, Nb-p-Coumaroyltryptamine, Moschamine, and Colchicine exhibited favorable outcomes as permeability glycoprotein (P-gp) substrates, while the other compounds did not show efficacy in this regard (Table 4). P-glycoprotein, also referred to as ABCB1, is a protein belongs to the ATP-binding cassette (ABC) transporter family. It is found in the cell membranes of various tissues, including the intestines, liver, kidney, and the blood–brain barrier. It functions as an efflux pump, actively transporting a wide variety of substances out of cells [60]. Assessing

Table 9 Candidate drugs targeting top three hub genes from DGIdb database

Gene	Drug	Interaction types	Sources	PMIDS
PTGS2	Hydroxychloroquine	–	NCI	14963695
	Thalidomide	Antagonist	TdgClinicalTrial	12710892; 15446566; 15982930; 21507989; 15598423; 15892618
	Oxaliplatin	–	PharmGKB	19219602
	Capecitabine	–	PharmGKB	19219602
EGFR	Ibrutinib	Inhibitor	DTC	24915291
	Everolimus	–	JAX-CKB	23629727
	Pemetrexed	–	CIViC	31605797; 24636847
	Carboplatin	–	CIViC|PharmGKB	22370314; 20573926; 19692680; 22581822; 21783417; 22285168; 21900837; 22139083; 20022809
	Cisplatin	–	JAX-CKB|CIViC	23764753; 27040853
	Temozolomide	–	JAX-CKB	25910950; 26846818
	Irinotecan	–	JAX-CKB	23209031; 25185971
	Bevacizumab	–	JAX-CKB	28408243
	Temsirolimus	–	JAX-CKB	24470557
	Sirolimus	–	JAX-CKB|DoCM|CIViC	24934779; 24813888; 25157968; 18089823
	Bosutinib	–	JAX-CKB	28416483
	Paclitaxel	–	JAX-CKB|CIViC|PharmGKB	24886365; 22370314; 20573926; 19692680; 22581822; 21783417; 22285168; 21900837; 22139083; 20022809
	Fluorouracil	–	PharmGKB	23816762
	Gemcitabine	–	PharmGKB	21783417; 22285168
	Decitabine	–	JAX-CKB	24874286
	Ponatinib	–	JAX-CKB	22238366
	Etoposide	–	JAX-CKB	27216155
	Imatinib	–	JAX-CKB|PharmGKB	28762371; 22323597
	Sunitinib	–	JAX-CKB	27149458
	Sorafenib	–	DTC|JAX-CKB|TTD	23629727; 26743856; 26318998; 24166906
	Trastuzumab	–	TEND	11752352
	Dasatinib	–	JAX-CKB	28416483
	Docetaxel	–	PharmGKB	21783417; 22285168
AKT1	Everolimus	inhibitor	JAX-CKB; MyCancerGenomeClinicalTrial	
	Carboplatin	–	PharmGKB	22901187
	Cisplatin	–	JAX-CKB; PharmGKB	22901187; 25519148
	Irinotecan	–	JAX-CKB	25714871
	Temsirolimus	–	JAX-CKB	27016228
	Sirolimus	–	JAX-CKB	18708578
	Paclitaxel	–	JAX-CKB	
	Gemcitabine	–	JAX-CKB	
	Sorafenib	–	JAX-CKB	
	Doxorubicin	–	JAX-CKB	18708578
	Topotecan	–	DTC	21440338

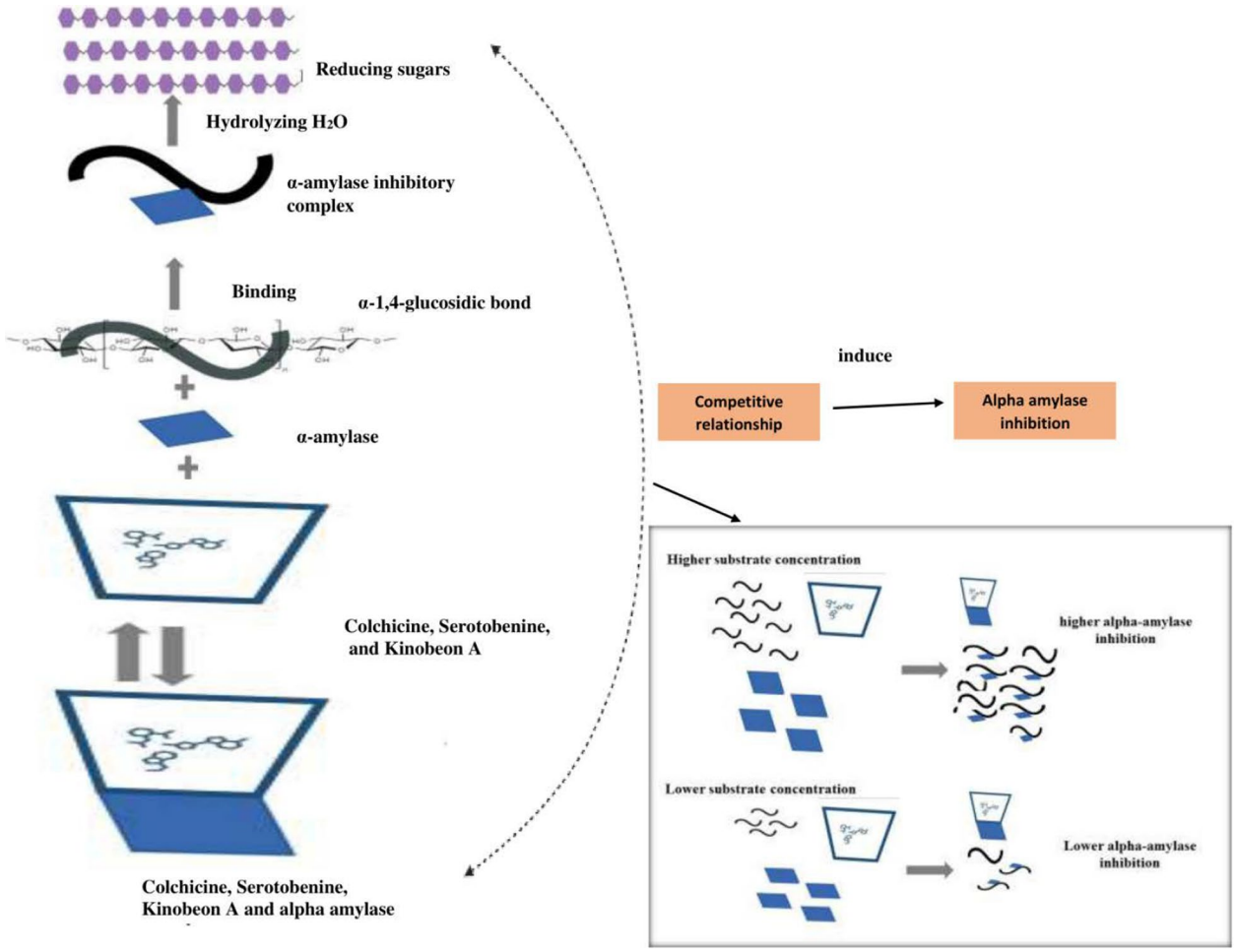


Fig. 8 The diagram describing the behaviors of Colchicine, Serotobenine, and Kinobeon A; α-amylase and substrate in the ternary digestion system

P-glycoprotein is crucial in drug discovery to predict potential interactions and overcome drug resistance, thereby enhancing therapeutic efficacy [61, 62]. Inhibitory effects of five cytochrome classes P450, i.e. CYP2C9, CYP2C19, CYP3A4, CYP1A2 and CYP2D6 were studied. The study found that only moschamine, effectively inhibited all cytochrome classes (CYP2C9, CYP2C19, CYP3A4, CYP1A2, and CYP2D6). Additionally, several other compounds displayed inhibitory effects on specific cytochrome classes: N-Coumaroyl serotonin on CYP2D6, Nb-p-Coumaroyltryptamine on CYP2C19 and CYP2D6, serotobenine on CYP2C19 and CYP2C9, and colchicine on CYP3A4 and CYP2D6 (Table 4). The Cytochrome P450 (CYP450 or CYP) enzyme family is instrumental in the metabolic processing of drugs, toxins, and a range of naturally occurring substances within the human body [63]. With the exception of Pterin-6-carboxylic acid, all compounds demonstrated substantial absorption in the gastrointestinal system, indicating notable uptake within the human intestinal tract (Table 4). Insufficient absorption of a drug within the gastrointestinal tract can lead to inadequate bloodstream concentration, thus diminishing its therapeutic efficacy. Inadequate or inconsistent absorption can result in the drug's ineffectiveness [63].

Toxicity assessment of phytochemicals involves evaluating their potential harmful effects on living organisms. Rapid in silico processes are best way to predict toxicity of active compounds derived from plants. These assessments aim to determine safe consumption levels and identify potential health risks [64]. In present study, each compounds exhibited low toxicity across all factors, except for two. Specifically, Cartorimine displayed a mild mutagenic effect, while licarbazepine exhibited highly toxic reproductive effects. Among the tested compounds, Kinobeon A falls into drug toxicity class V (LD_{50} = 2842 mg/kg), possibly causing harm if ingested. Four compounds, including licarbazepine, N-Coumaroyltryptamine, Nb-p-Coumaroyltryptamine, and moschamine, are categorized as drug toxicity class

IV (LD_{50} = 856, 500, 500, and 500 mg/kg, respectively), indicating potential harm if swallowed. Two compounds, pterin-6-carboxylic acid and colchicine, belong to drug toxicity class III (LD_{50} = 1500 and 290 mg/kg, respectively), suggesting toxicity when ingested. Lastly, two compounds, serotobenine and colchicine, are classified as drug toxicity class II (LD_{50} = 38 and 6 mg/kg, respectively), posing a fatal risk if swallowed (Table 5).

Utilizing advanced computational techniques can help identify possible interactions between chemical compounds and specific genes, facilitating the discovery of new drug candidates and therapeutic pathways [65]. Protein–protein interaction network was built between 204 common genes of compounds and diabetes (Figs. 2, 4a), representing the intricate web of physical connections between proteins within a cell. Active ingredients that interact with multiple target proteins could potentially offer improved clinical effectiveness while minimizing adverse side effects, compare to ingredients that target a single protein [66]. By mapping these interactions, researchers gain a deeper understanding of how proteins collaborate to regulate biological processes [67].

Furthermore, compound-target network was constructed between 9 effective compounds and 204 targets to study their functions in diverse biological systems (Fig. 3). Identifying hub genes identify crucial genes within a biological network that hold central positions in governing a wide array of cellular processes, providing valuable insights into disease mechanisms and potential targets for therapeutic interventions [68]. This is typically achieved through network analysis techniques, prioritizing genes with high connectivity and influence in the network [69]. The top 10 genes identified were AKT1, JUN, EGFR, CASP3, GAPDH, PTGS2, STAT3, MMP9, CTNNB1 and TLR4 (Fig. 4b) (Table 7).

Gene ontology is a structured record that categorizes genes into biological processes, molecular functions, and cellular components, facilitating the functional annotation of genes [60] GO analysis identified several molecular functions (MF) including protein serine/threonine/tyrosine kinase activity; which is involve in the regulation of glucose metabolism and insulin signaling enzyme binding [70]; RNA polymerase II sequence-specific activity, which does not have directly cause or prevent diabetes but contribute to the regulation of genes associated with diabetes [71]; heme binding, where hemoglobin A1c (HbA1c) serves as a type of hemoglobin used for assessing prolonged glycemic regulation in people with diabetes [71]; G-protein coupled adenosine receptor activity, which is involved in regulation of glucose metabolism, insulin secretion, and inflammation [72] and nitric-oxide synthase regulator activity, which is particularly relevant in type II diabetes involving endothelial dysfunction [73] (Fig. 6a).

Biological processes (BP), identified including protein auto phosphorylation, which is involve in insulin signaling pathways and glucose metabolism [74]; trans membrane receptor protein tyrosine kinase signaling pathway, regulation of cell growth, Insulin-like growth factor receptor signaling pathway, regulation of angiogenesis, cellular response to reactive oxygen species, regulation of blood pressure, positive regulation of vasoconstriction and protein functions[75] (Fig. 6b); and cellular components (CC) identified inclosing cytoplasm, receptor complexes, plasma membrane function, Integral component of plasma membrane, membrane raft and cell surface function [76] (Fig. 6c).

Enrichment analysis is a statistical method used to identify overrepresented gene ontology terms in a set of genes, helping researchers uncover the biological significance of their experimental results [76]. Same observations were made by [42, 55]. KEGG analysis includes pathways such as Lipid and atherosclerosis, lipids imbalance contributes insulin resistance and atherosclerosis builds plaques in arteries cause resistance in blood circulation, people with diabetes can be at higher risk of atherosclerosis [77]; EGFR tyrosine kinase inhibitor resistance, elevation in blood glucose levels activate the EGFR, leading to kidney damage [78]; AGE-RAGE signaling pathway, which involve in chronic inflammation, oxidative stress, and tissue damage in diabetes [79]; ErbB signaling pathway, influence insulin resistance and pancreatic beta-cell function [80]; Endocrine resistance, cell become less responsive to insulin function, a hormone which is released by pancreases to control blood sugar [81]; and PI3K-Akt signaling pathway, regulate glucose homeostatic[82] (Fig. 6d).

6 compounds pterin-6-Carboxylic acid, moschamine, Kinobeon A, Serotobenine, Cartorimine, N-Coumaroyl serotonin and Colchicine found to be highly interactive against EGFR, MMP9, PTGS2 and AKT1 (Fig. 5a). PTGS (prostaglandin-endoperoxide synthase) commonly known as cyclooxygenase is the main enzyme in prostaglandin synthesis. It acts as a peroxidase and dioxygenase. PTGS1 (a constitutive) and PTGS2 (an inducible) are the two isozymes of prostaglandins. Both isozymes have different regulatory and tissue distribution mechanisms. PTGS2 is a primary enzyme responsible for converting arachidonic acid into prostaglandin. It is exposed in response to acute inflammation. Thus prostaglandin in return promotes angiogenesis and cell division [83]. Inhibition of PTGS/COX proved effective in reverting diabetes [84]. Activation of PTGS2/COX2 isoform in beta cells induces pathogenic processes in diabetes. Studies documented that the administration of hyperglycemic

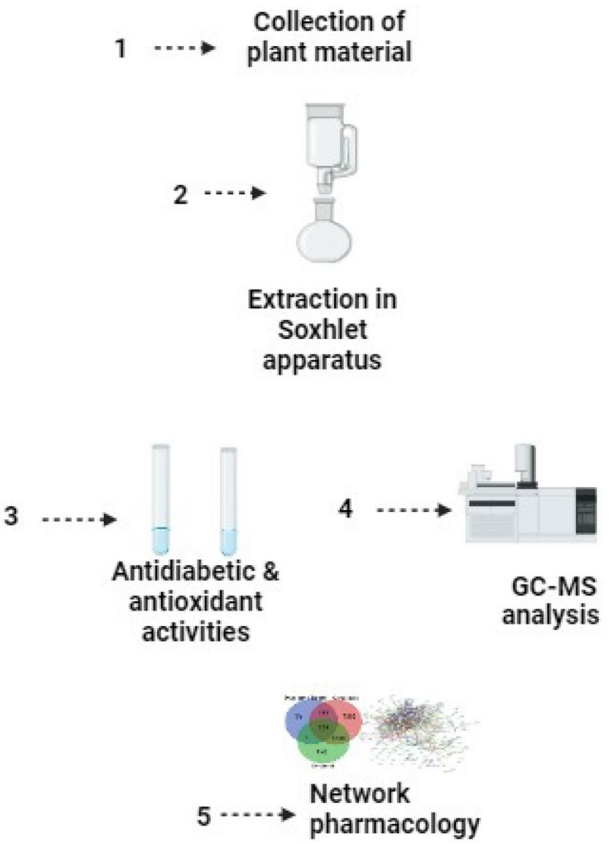


Fig. 9 Flowchart of experimental design

conditions in vivo (in mice) and in vitro (human islets in culture cells) conditions cause activation of PTGS2/COX2 [85, 86]. Table 4 shows that mesalazine (mesalamine) and pterostilbene are the two FDA-approved drugs that inhibit cyclooxygenase. Bertin et al. [85] documented that the drug mesalamine (5-ASA) lowers blood sugar level. The results of recent study showed that bioactive compounds viz; Moschamine, kinobeon, colchicine observed to have affinity with PTGS2 and thus might be involved in inhibition of cycloxygenase.

EGFR (epidermal growth factor receptor) gene encodes trans membrane glycoprotein that belongs to the protein kinase superfamily. It is a growth factor receptor that, when activated upon binding with its ligands, induces proliferation and cell differentiation. Mutation in EGFR induce formation of cancer [87]. High glucose concentration in blood trans-activates the EGFR which causes kidney damage. EGFR inhibition can reduce the size of the kidney in Streptozotocin (STZ) treated diabetic mice [88]. Tables 8, 9 shows that tyrphostin-47 inhibits the expression of EGFR. Davoodi-Semiromi et al. [89] documented that tyrphostin (AG490) reverses or cures type 1 diabetes in NOD mice. The recent study showed that Moschamine and colchicine might inhibit the expression of EGFR.

AKT1 kinase performs certain cellular functions viz; regulate cell growth, proliferation, and differentiation. AKT1 is among the three closely related serine/threonine protein kinases, located on chromosome 14q32. It regulates a number of cellular processes (metabolism, proliferation, cell survival, growth, and angiogenesis). It also controls the absorption of glucose by facilitating the SLC2A4/GLUT4 glucose transporters in response to insulin [90]. Studies showed that Kuwanon C (phytoactive compounds in mulberry leaves) is observed to have greater affinity with AKT1 gene and can be involve in glucose metabolism and insulin signaling [91]. Whereas the network pharmacological profile showed that bioactive compounds Pterin-6-carboxylic acid and Kinobeon showed a higher affinity with AKT1. Our findings assessed the potential of the bioactive compounds of *C. tinctorius*, for herbal compound prediction and found viable candidates. The results also shed light on the antidiabetic effects of *C. tinctorius* with promising potential as novel drug.

Medicinal plants rich in antioxidant compounds may help manage diabetes by reducing oxidative stress and improving α-amylase inhibition. In present study, *C. tinctorius* leaf and flower extract antioxidant and In vitro tests checked antidiabetic potential. Flow chart of experimental design is presented in Fig. 9. Leaves and flowers methanolic extract at a concentration 200 $mgmL^{-1}$ showed promising effects. The active fractions of *C. tinctorius* contain a rich phytochemical profile with numerous therapeutic compounds. Network pharmacology study revealed that three compounds Colchicine, Serotobenine and Kinobeon A are potential compounds that have antioxidant as well as antidiabetic potential. The study also highlighted new candidate genes AKT1, PTGS2, EGFR, and MMP9, and their function in molecular, biological and cellular pathways. The results obtained open up new avenues for the identification of novel molecular markers and therapeutic targets for diabetes and its associated complications.

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Author contributions
Conceptualization, Z.Y., R.U and Z.I.; methodology, Z.Y., S.H., Z.S. and S.J.; software, Z.S., Z.M. and S.H.; validation, Z.Y., A.A. and Z.S.; formal analysis, I.A., A.A., S.H. and S.., investigation, Z.Y. and A.A.; resources, Z.Y., R.U; data curation, Z.S., Z.M. and S.H.; writing—original draft preparation, S.H. and Z.S.; writing—review and editing, S.H., M.A.R. and Z.S.; visualization, A.A.; supervision, Z.Y.; project administration, Z.Y. and Z.I.; funding acquisition, Z.S. All authors have read and agreed to the published version of the manuscript.

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Availability of data and materials
The data will be available upon reasonable request from the corresponding author.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
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Competing interests
The authors have no conflict of interest.

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