ASIAN JOURNAL OF PHARMACEUTICAL AND CLINICAL RESEARCH

INNOVARE ACADEMIC SCIENCES
Knowledge to Innovation

Vol 18, Issue 7, 2025

Online - 2455-3891
Print - 0974-2441

Research Article

VOLATILE ANTIDIABETIC PROPERTIES OF *PIPER NIGRUM* L. ETHANOL EXTRACT (ORIGINAL AND PLANT STEM CELL): NETWORK PHARMACOLOGY STUDY AND ANTIOXIDANT ACTIVITY

RISHA FILLAH FITHRIA[1,2]*, MUHAMMAD BADRUL HUDA[3], FARAHIDAH MOHAMED[4], SU LINGYU[2]

[1]Department of Pharmacology and Clinical Pharmacy, Faculty of Pharmacy, Universitas Wahid Hasyim, Semarang, Indonesia. [2]Department of Pharmacology, School of Pharmaceutical Science, Central South University, Changsha, China. [3]Department of Biology Pharmacy, Faculty of Pharmacy, Universitas Wahid Hasyim, Indonesia. [4]Innovation and Commercialisation Unit, Research Management Center, International Islamic University Malaysia, Selangor, Malaysia.

***Corresponding author: Risha Fillah Fithria; Email: rishafithria@unwahas.ac.id**

Received: 16 April 2024, Revised and Accepted: 20 June 2025

ABSTRACT

Objectives: This study aims to identify and compare the active chemical components in the ethanol extracts of *Piper nigrum* L. (black pepper) plant and its callus, and to investigate their potential roles in treating diabetes mellitus (DM) through protein–protein interaction (PPI) analysis.

Methods: Ethanol extracts were prepared from both the original black pepper plant and its callus. Chemical analysis identified key active substances, including piperine and β-D-glucopyranoside, using retention times (RT). PPI investigations were conducted to determine the interactions involved in diabetes management. The antioxidant capacities of the extracts were assessed using IC_{50} values, and the biological processes and molecular functions related to diabetes treatment were evaluated.

Results: Both the original plant and callus extracts contained active substances such as piperine (37.715%, RT: 28.1967) and β-D-glucopyranoside (54.272%, RT: 16.5768). The primary biological processes identified were the P450 epoxygenase pathway and glycogen production. In addition, the organic acid metabolic process and nucleosome core were implicated in the management of DM by the extracts. The main molecular functions predicted were p53 binding and cyclin. The antioxidant capacities of the extracts were moderate for the callus extract ($IC_{50:}$ 129.92±0.83) and poor for the original plant extract ($IC_{50:}$ 156.69±1.36).

Conclusion: The study reveals that the ethanol extracts from the black pepper callus and the original plant possess distinct chemical profiles and mechanisms in treating DM. The callus extract demonstrates a more favorable antioxidant capacity compared to the original plant extract. Both extracts engage in similar biological processes but exhibit differences in their chemical composition and potential therapeutic pathways for diabetes management.

Keywords: Callus, Black pepper, Diabetes mellitus, Volatile.

 DOI: http://dx.doi.org/10.22159/ajpcr.2025v18i7.55496. Journal homepage: https://innovareacademics.in/journals/index.php/ajpcr

INTRODUCTION

Diabetes mellitus (DM) is a significant international public health concern in the twenty-first century, impacting around 347 million individuals globally [1]. If no additional measures are implemented, this issue is projected to increase double by 2030. This health problem is a chronic metabolic ailment characterized by a lack of insulin, either complete or partial, resulting in high levels of glucose in the blood. Diabetic individuals have an elevated susceptibility to several long-term health issues worldwide, such as obesity, atherosclerosis, dyslipidemia, and kidney failure [2].

At present, several artificial medications (such as sodium-glucose cotransporter-2 inhibitors, metformin, thiazolidinediones, sulfonylureas, dipeptidyl peptidase-4 [DPP-4] inhibitors, and glucagon-like peptides) are employed for the treatment of diabetes, all of which come with associated adverse effects. Thiazolidinedione and biguanide therapy can lead to an increase in body weight, and both medications have harmful effects on the kidneys. Incretin-based medications have the potential to induce gastrointestinal complications [3].

Medicinal herbs can serve as alternative therapies to synthetic medications for the management of DM. Some plants have antidiabetic properties that work by specifically targeting key pathways such as α-glucosidase, α-amylase, DPP-4, glitazone (PPAR γ), protein tyrosine phosphatase 1B, insulin-dependent glucose transporter proteins 4, and others [4]. Moreover, certain medicinal plants can regulate high blood sugar levels and improve insulin resistance by activating the adenosine monophosphate-activated protein kinase signaling pathway [4,5].

Piper nigrum L., commonly known as black pepper, has been proven to be a promising antidiabetic plant in many studies. Several studies have focused on the volatile compound content of this plant, namely piperine, an alkaloid from *P. nigrum* L., which has shown significant antidiabetic activity by synthesizing new derivatives with benzothiazole groups [6]. In addition, several compounds found in the extract of *P. nigrum* L. are known to be effective in preventing and treating diabetes, as well as demonstrating increased oxidative efficacy [7]. In addition, the extract from *P. nigrum* L. leaves has been proven to possess antioxidant activity, restoring depleted antioxidant levels in rats experiencing oxidative stress, indicating its potential in combating oxidative stress associated with diabetes [8].

Plant development can be achieved via plant stem cell techniques. The advancement of plant stem cells presents favorable prospects for improving agricultural production and the utilization of therapeutic plants. Using plant stem cells, a plant can generate secondary metabolites with different kinds and quantities, leading to pharmacological effects distinct from those of the parent plant. Plant stem cells are a collection of undifferentiated meristematic cells that possess the ability to develop into specific tissues and exhibit fast regeneration capabilities. Stem cells in plants have the role of supplying new cells for growth and the replacement of dead cells. This role enables them to undergo rapid division [9]. Plant stem cells are typically located in the growth sites of

plants, specifically in areas known as apical meristems, which include root primordia and shoot primordia [10]. The technology of plant stem cells involves isolating meristem cells and cultivating them in a medium with specific growth regulators, preventing them from regenerating into tissues or organs, but instead continuously proliferating into a mass of cells known as callus. The growth regulator used includes auxin groups such as 2,4-D, picloram, or cytokinin groups such as thidiazuron [11]. Even with the use of growth regulators, differentiated tissues such as mesophyll tissue can be reprogrammed into stem cells, which are cells that divide without differentiating. The callus is continuously propagated, and then its metabolite compounds are used as cosmetic ingredients or medicinal substances. The phytochemical content in callus has been proven to be higher and has better antioxidant activity compared to the extract from the original plant, as demonstrated by Abdulhafiz *et al*.'s research. The study showed that callus extract exhibited the highest phenolic content and had better diphenylpicryl-hydrazyl (DPPH) radical scavenging activity [12].

Verifying the mechanism of action of a compound found in a plant is necessary to determine its suitability as a pharmaceutical. Network pharmacology is a technique that integrates pharmacology and pharmacodynamics to forecast the interactions among proteins implicated in a chemical's fundamental mechanism of action [6]. The objective of this study is to determine the specific therapeutic targets and processes by which the ethanol extracts from both the plant and callus of *P. nigrum* L. act as agents to treat diabetes.

This study aims to identify the active compounds present in the ethanol extract of the original plant and callus of *P. nigrum* L., verify 10 key target proteins of the active compounds in the ethanol extract of the original plant and callus of *P. nigrum* L., conduct gene ontology (GO) functional enrichment analysis to understand the underlying mechanism of *P. nigrum* L. as an antidiabetic agent, conduct Kyoto encyclopedia of genes and genomes (KEGG) pathway enrichment analysis to understand the underlying mechanism of *P. nigrum* L. as an antidiabetic agent and determine the antioxidant activity of the ethanol extract of the original plant and callus of *P. nigrum* L.

METHODS

Culture of plant stem cells from *P. nigrum* L.

The meristem section of *P. nigrum* L. (identified by the mathematics and natural sciences faculty, Universitas Negeri Semarang, with the specimen number B/9880/UN37.1.4/PG/2021) was soaked in 30% sulfuric acid for 15 min, followed by surface sterilization with 40% Clorox for 20 min. The specimen was then rinsed with sterile distilled water to remove any residual Clorox. The disinfected seeds were aseptically inoculated into a jar containing approximately 25 mL of medium for callus induction. Medium mass spectrometry (MS) is supplemented with 3 mg/L indole-3-acetic acid (IAA). The culture was incubated at a temperature of 25±2°C under cool neon light with a light/dark cycle of 16/8 h [12].

Preparation of extracts

The *P. nigrum* L. plant was washed and then cut into small pieces, which were then dried in an oven at a temperature of 40°C for 72 h. Then, it was pulverized using a blender. The herbal powder is macerated using 95% ethanol (1:10) for 24 h at room temperature, then filtered and extracted twice. The extracted substance was then evaporated using a rotary evaporator at a temperature of 50°C for 60 min at a speed of 50 rpm. The callus extraction process was carried out using the same method, except that the drying process was only conducted for 30 min. The extracted result was then calculated for its yield, placed in dark bottles, and stored in a desiccator to prevent compound degradation [13].

Identification of active compounds in *P. nigrum* L. using gas chromatography-MS (GC-MS) analysis

The analysis was performed utilizing the Agilent Technologies Type 1909 IS-433. The plant extract underwent filtration using a purple nylon syringe filter with a pore size of 0.45 μm. Subsequently, 2 μL of the filtered extract was injected into split mode with a ratio of 10:1. The carrier gas, helium, was employed at a flow rate of 1 mL/min. The temperature of the injector was set to 250°C. The substance of interest was subsequently isolated by use of a fused silica capillary column measuring 30 m in length, 0.25 mm in diameter, and with a thickness of 0.5 μm. The configuration parameters for the gadget were as follows: The temperature started at 40°C and was raised to 300°C over 1 min, with an increase of 10°C each min. The temperature was then kept at 280°C for 9 min, with a decrease of 5°C every min. The ionization energy employed for determining the mass spectrum was 70 electron volts, with a mass scanning range spanning from 10 to 400 mass-to-charge ratio units. The GC-MS mass spectra were identified and interpreted using the NIST mass spectrum database. Furthermore, the test extract components' retention index, name, chemical structure, and weight were verified by the information reported in the literature [12].

Network pharmacology study

The bioactive constituents derived from the plant and callus extracts of *P. nigrum* L. were acquired using GC analysis. The 2D structures of each compound were obtained from the PubChem database (www.pubchem.ncbi.nlm.nih.gov), and the target proteins for each compound were determined using (https://www.way2drug.com/passtargets/), SuperPred (https://prediction.charite.de/subpages/target_prediction.php), SEA (https://sea.bkslab.org/), and CTD (https://ctdbase.org/). The proteins associated with DM were acquired from GeneCards (https://www.genecards.org/) and DisGeNET (https://www.disgenet.org/). The shared targets between the extract and the disease were subsequently examined using a Venn diagram. The interactions among the target proteins were examined utilizing the STRING database (https://string-db.org/). The DAVID database (https://david.ncifcrf.gov/) was utilized to conduct GO analysis and KEGG pathway enrichment analysis. The network connections among the target proteins were examined using Cytoscape software (version 3.10.1). The main protein target and primary active compounds involved in addressing DM were analyzed based on the degree, Betweenness centrality, and Closeness centrality scores obtained from the Cytoscape software.

Antioxidant activity assay

The plant extract was solubilized in methanol and then diluted to create a series of concentrations: 100, 250, 500, and 750 μg/mL. Quercetin, used as a reference, was also diluted to create a series of concentrations: 1, 5, 10, and 20 μg/mL. The samples were subsequently combined with 0.5 mL of DPPH solution (1 mM dissolved in methanol) and placed in a sealed container at ambient temperature for 30 min. The measurement of absorbance was conducted at a specific wavelength of 515 nm. The extract's capacity to remove free radicals was subsequently determined using the following equation:

The DPPH scavenging effect can be calculated using the formula:

$$(A1–A0/A1) \times 100 \quad (1)$$

Let A1 represent the absorbance of the control solution containing DPPH, and A0 represent the absorbance of the extract at different concentrations in DPPH. The antioxidant activity was assessed by determining the IC_{50} value, which represents the concentration of the sample needed to block 50% of DPPH radicals. The study conducted by Abdulhafiz *et al*. utilized Quercetin as a point of comparison [12]. The antioxidant test results were obtained using linear regression analysis between % inhibition and extract concentration.

RESULTS AND DISCUSSION

Culture of plant stem cells from *P. nigrum* L.

Subasinghe *et al*. utilized leaf explants to establish callus cultures, employing different media and growth hormones. Leaves are appropriate for callus culture due to their abundance of meristematic cells, which are actively dividing and capable of undergoing dedifferentiation [14].

The media and growth hormones utilized for callus culture in this investigation were derived from the studies conducted by Subasinghe *et al*. and Hussain *et al*. [14,15]. Table 1 presents the outcomes of callus growth on MS media and different growth hormones utilized.

The concentrations of all hormones utilized in MS medium were 3 parts per million (ppm) to 1 ppm. The results indicate that the combination of NAA + Kinetin, 2,4D + Kinetin, and IAA + Kinetin hormones in the MS medium successfully promoted callus growth. Subasinghe's *et al*. study yielded callus with optimal hormone levels utilizing 2,4D, in contrast to the current findings. Hussain *et al*. achieved the most favorable callus outcomes by utilizing BA hormones [14,15]. This phenomenon might arise because plants sourced from many locations and climates possess distinct attributes, particularly in their response to nutrients or growth hormones. The callus that develops appears as an uneven and shapeless cluster, taking the appearance of spherical bumps or molds that emerge from the explant's surface. It possesses a soft and somewhat flexible feel. Fig. 1 displays the callus picture.

Extraction of the original plant and callus of *P. nigrum* L.

The original plant was harvested when it reached the age of 3 years, at which point the black pepper seeds were ready for harvest. The entire plant was utilized in this study. The entire plant was subjected to wet sorting to clean the plant and reduce the number of contaminating microorganisms. The entire plant was then cut into little pieces. The original plant was subjected to drying in an oven at a temperature of 40°C for 72 h, whereas the callus was dried for 30 min due to its simpler structure and less complex nature compared to regular plant tissues, which allows for faster evaporation of water. In addition, the callus was composed of undifferentiated cells, which allows water to exit these cells easily [16]. The purpose of the drying process is to decrease the moisture content to halt the enzymatic activity and inhibit the proliferation of fungi and bacteria [17]. Dried plants and calluses have the property of being easily compressed when pressure is applied to the fingers.

Table 1: Callus growth results at various concentrations of growth hormone combinations

No. Culture	Hormone combination*	Callus growth
1	NAA+BA	-
2	NAA+Kinetin	√
3	2,4D+BA	-
4	2,4D+Kinetin	√
5	IAA+BA	-
6	IAA+Kinetin	√

*NAA: α-naphthalene acetic acid, BA: 6-Benzylaminopurine, 2,4D: 2,4-dichlorophenoxy acetic acid, IAA: Indole-3-acetic acid

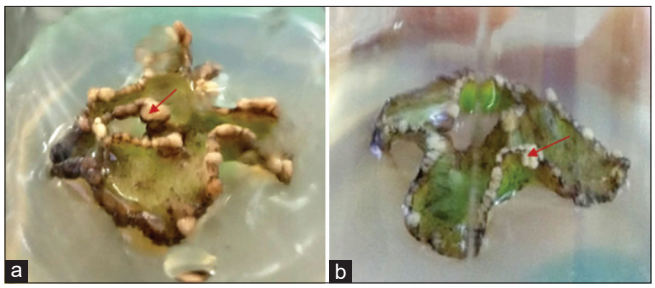

Fig. 1: Callus of *Piper nigrum* inoculated from the leaf

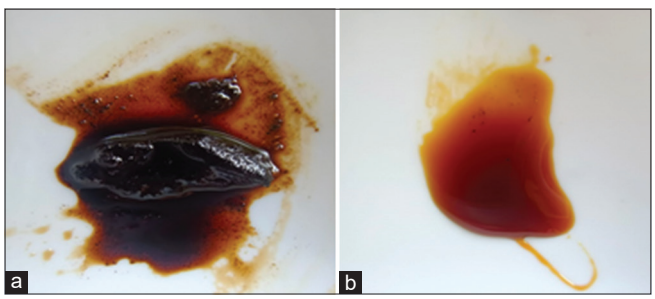

Fig. 2: (a) plant extract, (b) callus extract

The next step was the process of pollination to increase the surface area of plant pollen and callus, allowing all the compounds contained within to be fully extracted. According to the Regulation of the Food and Drug Supervisory Agency about the Quality Requirements of Traditional Medicines, the requirement for the moisture content of crude drugs is <10% [18]. The moisture content of the original plant and the obtained callus is 5% and 4%, respectively, which meets the requirements for the moisture content of a crude drug. The powdered form of black pepper obtained is 520 g, derived from the initial dry weight of the plant, which is 28,300 g. Meanwhile, the obtained callus powder amounts to 18.06 g from a dry callus weight of 25.85 g.

The extraction of plant-origin powder and callus was performed using the maceration extraction method with 95% ethanol solvent. Maceration is a method of extraction used to extract heat-resistant and non-heat-resistant beneficial substances. The principle of maceration extraction is the achievement of equilibrium by immersing powdered crude drugs in a solvent solution, with or without agitation. Immersion of a substance can enhance the permeability of cell walls by allowing solvents to enter the cell walls, resulting in cell swelling. Swelling of cells can result in the release of compounds found in the cell walls of plants, which then enter the solvent. This swelling causes the diffusion of compounds extracted by the solvent to exit the cell walls of plant cells. The maceration method involves two rounds of extraction, namely the maceration process and the re-maceration process. In general, the maceration method is not significantly different from the maceration method. The difference in the method of maceration lies in the use of a partial solvent for maceration, the collection of residual components during the maceration stage, followed by re-immersion with the remaining solvent, and subsequent filtration. The processes of filtration, maceration, and re-maceration are combined in the final stage [19].

Ethanol 95% was chosen as the solvent because it is more effective at extracting non-polar compounds than ethanol 70%. One of the active compounds found in black pepper plants is *piperine*, which is non-polar in nature and, therefore, expected to be extracted more optimally [20].

The liquid extract was concentrated using a rotary evaporator, resulting in a concentrated extract of 57 g from an initial weight of 520 g of black pepper plant powder. Meanwhile, 18.06 g of callus powder yielded a concentrated extract of 1.75 g. The yield of the extract from the original plant and the callus was 10.96% and 9.68%, respectively. The purpose of producing a concentrated extract is to remove the solvent through evaporation, resulting in an extract that contains only the active compounds present in the extract. Macroscopically, the ethanol extract of the black pepper plant was dark brown, whereas the callus was also brown (Fig. 2). The concentrated extract obtained was stored in a brown container and covered with aluminum foil to protect it from sunlight, which might damage the active components of the extract.

Identification of active compounds in the extract using GC-MS analysis

The black pepper plant contains most of the volatile compounds that can be detected by GC-MS. GC-MS analysis of extracts from the original plant and callus of black pepper revealed the presence of various groups of bioactive compounds, as presented in Tables 2 and 3. The GC-MS chromatogram is shown in Figs. 3 and 4.

Based on the results of GC-MS analysis, it can be observed that the type and quantity of active compounds present in the ethanol extract of the original plant and the ethanol extract of black pepper callus are different.

Table 2: Active compounds of ethanol extract from black pepper plants

No.	Active compound	Retention time (RT)	Area Pct	Pct max	Pct total
1	(+)-3-Carene	7.7168	0.1265	0.34	0.127
2	(+)-3-Carene	7.8176	0.0974	0.26	0.097
3	D-Limonene	8.1579	0.1265	0.34	0.127
4	Linalool	9.2796	0.1862	0.49	0.186
5	Cyclohexene, 4-ethenyl-4-methyl-3-(1-methylethenyl)-1-(1-methylethyl)-, (3R-trans)-	12.7076	1.444	3.83	1.444
6	.alpha.-Cubebene	12.8714	0.0851	0.23	0.085
7	Copaene	13.2999	0.9046	2.4	0.905
8	Cyclohexane, 1-ethenyl-1-methyl-2,4-bis (1-methylethenyl)-, [1S-(1.alpha.,2.beta.,4. beta.)]-	13.4512	0.7749	2.05	0.775
9	1H-Cyclopropa[a] naphthalene, 1a, 2,3,3a, 4,5,6,7b-octahydro-1,1,3a, 7-tetramethyl-, [1aR-(1a.alpha.,3a.alpha.,7b.alpha.)]-	13.7284	0.1651	0.44	0.165
10	Caryophyllene	13.9427	7.4556	19.77	7.456
11	.alpha.-Guaiene	14.0561	0.3644	0.97	0.364
12	1,4,7,-Cycloundecatriene, 1,5,9,9-tetramethyl-, Z, Z, Z-	14.3838	0.9548	2.53	0.955
13	Naphthalene, 1,2,3,4,4a, 5,6,8a-octahydro-7-methyl-4-methylene-1-(1-methylethyl)-, (1.alpha.,4a.beta.,8a.alpha.)-	14.5729	0.192	0.51	0.192
14	Cyclohexene, 6-ethenyl-6-methyl-1-(1-methylethyl)-3-(1-methylethylidene)-, (S)-	14.6359	0.0674	0.18	0.067
15	Germacrene D	14.6989	0.7914	2.1	0.791
16	Naphthalene, decahydro-4a-methyl-1-methylene-7-(1-methylethenyl)-, [4aR-(4a. alpha.,7.alpha.,8a.beta.)]-	14.8249	1.3605	3.61	1.361
17	Naphthalene, 1,2,3,4,4a, 5,6,8a-octahydro-4a, 8-dimethyl-2-(1-methylethenyl)-, [2R-(2. alpha.,4a.alpha.,8a.beta.)]-	14.8879	1.467	3.89	1.467
18	Naphthalene, 1,2,3,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-, (1S-cis)-	15.1148	1.3801	3.66	1.38
19	Naphthalene, 1,2,3,4,4a, 7-hexahydro-1,6-dimethyl-4-(1-methylethyl)-	15.3038	0.1685	0.45	0.169
20	(2E,4S,7E)-4-Isopropyl-1,7-dimethylcyclodeca-2,7-dienol	15.8836	0.1662	0.44	0.166
21	Caryophyllene oxide	15.9844	0.5016	1.33	0.502
22	Aromadendrene	16.123	0.0959	0.25	0.096
23	1,1,7,7a-Tetramethyl-1a, 2,6,7,7a, 7b-hexahydro-1H-cyclopropa[a] naphthalene	16.2995	0.2724	0.72	0.272
24	.gamma.-Muurolene	16.4003	0.2511	0.67	0.251
25	Isospathulenol	16.4759	0.728	1.93	0.728
26	1-Naphthalenol, 1,2,3,4,4a, 7,8,8a-octahydro-1,6-dimethyl-4-(1-methylethyl)-, [1S-(1. alpha.,4.alpha.,4a.beta.,8a.beta.)]-	16.6776	1.3486	3.58	1.349
27	Naphthalene, 1,2,3,5,6,7,8,8a-octahydro-1,8a-dimethyl-7-(1-methylethenyl)-, [1S-(1. alpha.,7.alpha.,8a.alpha.)]-	16.8414	0.283	0.75	0.283
28	1H-Indole-3-carboxylic acid, 4-hydroxy-	16.9926	0.3344	0.89	0.334
29	Eudesma-4 (15),7-dien-1.beta. -ol	17.1439	0.5625	1.49	0.562
30	(3aS,4R,7R)-1,4,9,9-Tetramethyl-5,6,7,8-tetrahydro-4H-3a, 7-methanoazulene	17.5094	0.0938	0.25	0.094
31	cis-Z-.alpha.-Bisabolene epoxide	17.6102	0.0708	0.19	0.071
32	Cyclohexane, 1,2-dimethyl-3,5-bis (1-methylethenyl)-, (1.alpha.,2.beta.,3.beta.,5.alpha.)-	17.7362	0.594	1.57	0.594
33	5-Isopropenyl-2-hydroxy-2,4,6-cycloheptatrien-1-one	18.1395	0.4016	1.06	0.402
34	2,2,6-Trimethyl-1-(2-methyl-cyclobut-2-enyl)-hepta-4,6-dien-3-one	18.3664	0.1996	0.53	0.2
35	4,6,6-Trimethyl-2-(3-methylbuta-1,3-dienyl)-3-oxatricyclo[5.1.0.0 (2,4)]octane	19.1477	0.061	0.16	0.061
36	Hexadecanoic acid, methyl ester	19.5258	0.172	0.46	0.172
37	2,4-Decadienamide, N-isobutyl-, (E, E)-	19.7401	1.998	5.3	1.998
38	n-Hexadecanoic acid	19.9291	0.6629	1.76	0.663
39	(2E,4E)-N-Isobutylundeca-2,4-dienamide	20.8365	0.1098	0.29	0.11
40	E-15-Heptadecenal	21.076	0.0619	0.16	0.062
41	9-Octadecenoic acid, methyl ester, (E)-	21.2146	0.3877	1.03	0.388
42	Phytol	21.3029	0.1685	0.45	0.168
43	Methyl stearate	21.4415	0.0329	0.09	0.033
44	9,12-Octadecadienoic acid (Z, Z)-	21.6431	1.6224	4.3	1.622
45	(2E,4E)-N-Isobutyldodeca-2,4-dienamide	21.7692	1.6096	4.27	1.61
46	1-(Piperidin-1-yl) dodecan-1-one	22.1977	0.1887	0.5	0.189
47	(E)-1-(Piperidin-1-yl) dodec-2-en-1-one	22.7774	0.0638	0.17	0.064
48	(2E,4E)-1-(Pyrrolidin-1-yl) dodeca-2,4-dien-1-one	23.4832	0.2411	0.64	0.241
49	(2E,4E)-N-Isobutyltetradeca-2,4-dienamide	23.5714	0.1786	0.47	0.179
50	(2E,4E)-1-(Piperidin-1-yl) dodeca-2,4-dien-1-one	23.6848	0.3858	1.02	0.386
51	1-(Piperidin-1-yl) tetradecan-1-one	24.0125	0.101	0.27	0.101
52	.gamma.-Sitosterol	24.1763	0.1005	0.27	0.1
53	.gamma.-Sitosterol	24.252	0.203	0.54	0.203
54	2-Benzo[1,3]dioxol-5-yl-indolizine	24.4536	0.1207	0.32	0.121
55	Bis (2-ethylhexyl) phthalate	24.9073	0.078	0.21	0.078
56	(2E,4E)-N-Isobutylhexadeca-2,4-dienamide	25.2224	0.6163	1.63	0.616
57	9-Octadecenamide, n-butyl-	25.4114	0.3606	0.96	0.361
58	Octadecanamide, N-butyl-	25.4619	0.0987	0.26	0.099
59	(2E,4E,6E)-7-(Benzo[d][1,3]dioxol-5-yl)-1-(piperidin-1-yl) hepta-2,4,6-trien-1-one	25.6005	0.3371	0.89	0.337
60	Furane-2-carbohydrazide, 5-phenylethynyl-	25.7013	0.697	1.85	0.697

(Contd...)

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

No.	Active compound	Retention time (RT)	Area Pct	Pct max	Pct total
61	(E)-5-(Benzo[d][1,3]dioxol-5-yl)-1-(piperidin-1-yl) pent-2-en-1-one	25.8652	3.6436	9.66	3.644
62	3'-Amino-5,6'-dimethyl-2,8'-dioxo-1H-spiro[indole-3,1'-pyrano[4,3-b] pyran]-2'-carbonitrile	25.9786	1.8587	4.93	1.859
63	(E)-1-(Piperidin-1-yl) hexadec-2-en-1-one	26.155	0.8727	2.31	0.873
64	Bicyclo[4.1.0]heptan-3-one, 4,7,7-trimethyl-, [1R-(1.alpha.,4.alpha.,6.alpha.)]-	26.6214	0.5238	1.39	0.524
65	Bicyclo[4.1.0]heptan-3-one, 4,7,7-trimethyl-, [1R-(1.alpha.,4.beta.,6.alpha.)]-	26.7474	6.2004	16.44	6.2
66	Piperine	26.9994	0.2802	0.74	0.28
67	Piperine	27.0498	0.4341	1.15	0.434
68	1-(Piperidin-1-yl) octadecan-1-one	27.2263	0.6148	1.63	0.615
69	Uridine, 2'-deoxy-, 3',5'-diacetate	27.3397	0.8722	2.31	0.872
70	Piperine	27.4658	0.6197	1.64	0.62
71	1,3-Benzodioxole-4-methanamine, N-[2-[tetrahydro-2,2-dimethyl-4-(1-methylethyl)-2H-pyran-4-yl] ethyl]-	27.5918	0.788	2.09	0.788
72	(E)-1-(Piperidin-1-yl) octadec-2-en-1-one	27.7178	1.0516	2.79	1.052
73	Pyrrolidine, 1-[5-(1,3-benzodioxol-5-yl)-1-oxo-2,4-pentadienyl]-, (E, E)-	27.8312	1.1056	2.93	1.106
74	Piperine	28.1967	37.7153	100	37.715
75	Piperine	28.348	0.6151	1.63	0.615
76	(2E,6E)-7-(Benzo[d][1,3]dioxol-5-yl)-1-(piperidin-1-yl) hepta-2,6-dien-1-one	28.4362	1.7426	4.62	1.743
77	Piperidine, 1-[5-(1,3-benzodioxol-5-yl)-1-oxo-2,4-pentadienyl]-, (Z, Z)-	28.6883	0.6566	1.74	0.657
78	Retrofractamide-A	29.2428	0.3737	0.99	0.374
79	2 (3H)-Furanone, 3,4-bis (1,3-benzodioxol-5-ylmethyl) dihydro-, (3R-trans)-	29.3688	0.4877	1.29	0.488
80	(E)-9-(Benzo[d][1,3]dioxol-5-yl)-1-(pyrrolidin-1-yl) non-8-en-1-one	29.5201	0.6098	1.62	0.61
81	(3R,4R)-3-(Benzo[d][1,3]dioxol-5-ylmethyl)-4-(3,4-dimethoxybenzyl) dihydrofuran-2 (3H)-one	29.6083	0.732	1.94	0.732
82	(E)-9-(Benzo[d][1,3]dioxol-5-yl)-1-(piperidin-1-yl) non-8-en-1-one	30.0998	3.2124	8.52	3.212
83	Piperine	30.4653	0.2455	0.65	0.245
84	Piperine	30.7803	0.0705	0.19	0.07

Table 3: Active compounds of ethanol extract from callus of black pepper

No.	Active compound	Retention time (RT)	Area Pct	Pct max	Pct total
1	.beta.-D-Glucopyranoside, methyl	16.5768	54.2722	100	54.272
2	.beta.-D-Glucopyranoside, methyl	16.9297	8.332	15.35	8.332
3	Hexadecanoic acid, methyl ester	19.5637	9.0332	16.64	9.033
4	9,12-Octadecadienoic acid (Z, Z)-, methyl ester	21.2021	9.2647	17.07	9.265
5	cis-13-Octadecenoic acid, methyl ester	21.2651	8.4448	15.56	8.445
6	Phytol	21.3534	2.2428	4.13	2.243
7	Methyl stearate	21.492	1.2659	2.33	1.266
8	.gamma.-Sitosterol	24.5167	7.1444	13.16	7.144

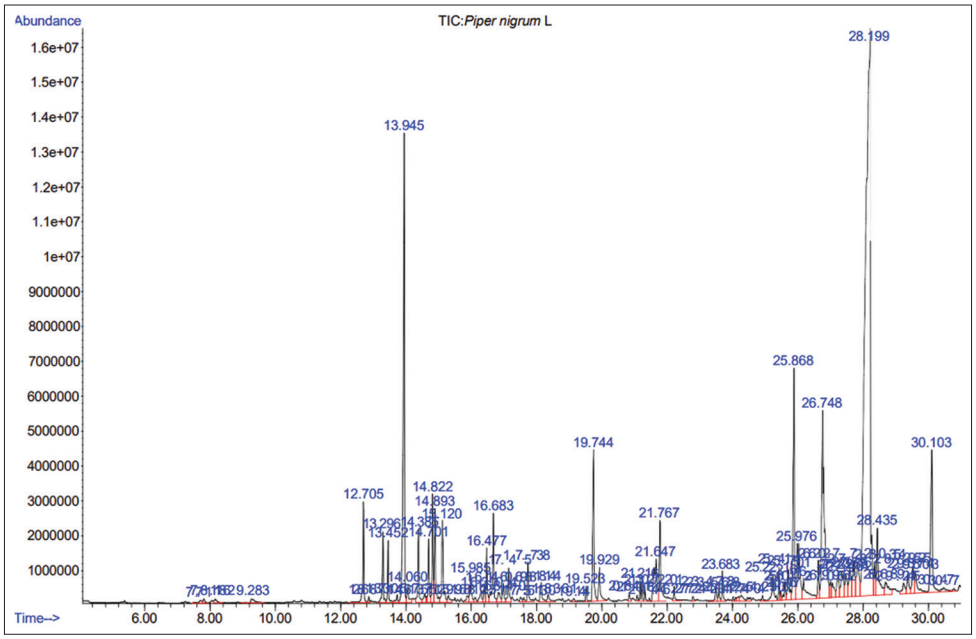

Fig. 3: Gas chromatography-mass spectrometry chromatogram of ethanol extract from black pepper plants

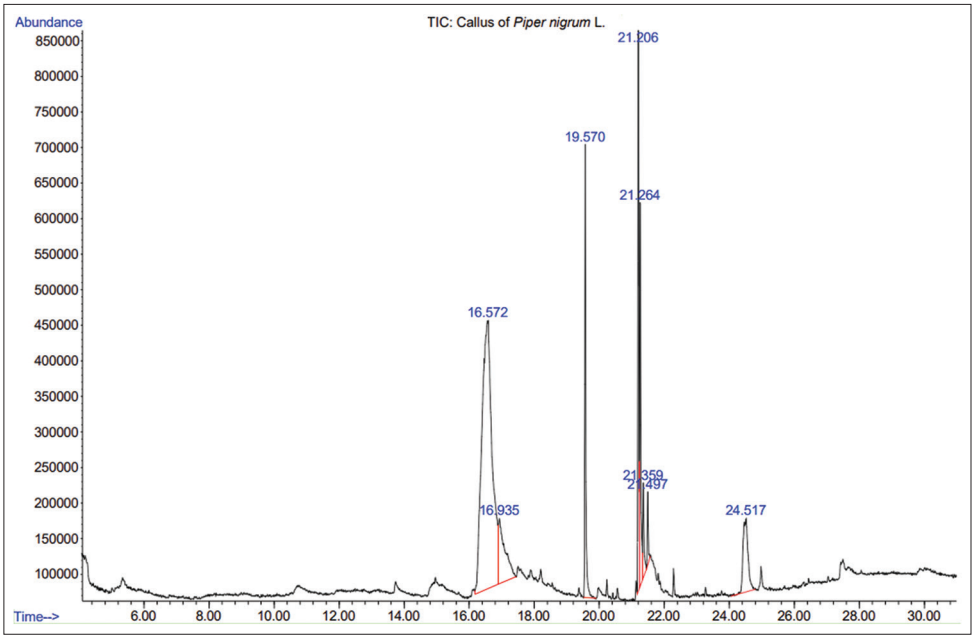

Fig. 4: Gas chromatography-mass spectrometry chromatogram of ethanol extract from callus of black pepper

This difference is consistent with the findings of previous researchers, including Sireen and Anbumalarmathi, who have demonstrated that the callus extract of *Abutilon indicum* (L.) contains active compounds with different types and levels, as well as exhibiting superior antioxidant and antibacterial activities compared to the extract from the parent plant [21]. The active compound found in the extract of the black pepper plant is piperine, with a concentration of 37.715% and a retention time (RT) of 28.1967. Meanwhile, in the extract of black pepper callus, it is beta-D-Glucopyranoside, methyl, with a concentration of 54.272% with an RT of 16.5768.

Network pharmacology study

All the active compounds in the extract of the original plant, as well as the black pepper callus, were detected using GC-MS analysis, and their two-dimensional structures were then searched for using the PubChem database (www./pubchem.ncbi.nlm.nih.gov). Based on the protein target search results of each active compound through (https://www.way2drug.com/passtargets/), SuperPred (https://prediction.charite.de/subpages/target_prediction.php), SEA (https://sea.bkslab.org/), and CTD (https://ctdbase.org/), a total of 7,437 protein targets were obtained for the extract of black pepper plants and 1,723 protein targets were obtained for the extract of black pepper callus. A total of 14,655 protein targets for DM were obtained from GeneCards (https://www.genecards.org/) and DisGeNET (https://www.disgenet.org/). The same target between the extract and illness was analyzed using a Venn diagram, resulting in 5,396 targets in the extract from the original plant and 1,254 targets in the callus extract. The results are presented in Fig. 5.

The interaction between the target proteins is analysed using the STRING database (https://string-db.org/) and then Cytoscape software (version 3.10.1) to study protein interactions. The results of the interaction are presented in Figs. 6 and 7.

Based on the results in the image, an increasing degree of each protein is indicated by the progressively more intense color of the nodes (moving toward the right in the legend). The interaction between the target proteins and the callus extract results in a variable number of degrees, ranging from 1 to 96. The Betweenness Centrality values range from 0 to 0.3104, while the Closeness centrality values range from 0.13468 to 0.33435. Meanwhile, the extract from the original plant produces a range of degrees from 1 to 127, Betweenness centrality from 0 to 0.1847, and Closeness centrality from 0.120582 to 0.375921. The protein–protein targets are then filtered using MCODE to obtain 10 main protein targets in each extract. The result is presented in Fig. 8.

The main protein targets of both extracts were subjected to GO analysis and KEGG pathway enrichment analysis using the DAVID database (https://david.ncifcrf.gov/). The obtained results are presented in Figs. 9-12.

The ethanol extract of black pepper callus and the ethanol extract of black pepper plants are predicted to have distinct mechanisms of action in overcoming DM, as indicated by the results of the GO analysis. The primary biological process in black pepper callus extract is the epoxygenase P450 pathway, which is responsible for the production and metabolism of epoxy fatty acids, including epoxyeicosatrienoic acids. This pathway plays a significant role in the pathophysiology of metabolic diseases, including diabetes. This pathway can be modulated to provide therapeutic benefits by increasing insulin sensitivity, reducing inflammation, and enhancing lipid metabolism [22]. In the interim, the primary biological process of black pepper plant extract, which involves the stimulation of insulin and the inhibition of GSK-3β to increase glycogen biosynthesis, is a promising approach to the management and treatment of diabetes. These strategies are designed to alleviate diabetes symptoms and lower blood glucose levels by enhancing insulin sensitivity and glycogen storage [23,24].

The primary cellular component that is regulated by ethanol callus synthesis in the treatment of diabetes is the organic acid metabolic process. Organic acids, such as those found in various types of food, can enhance insulin sensitivity by reducing interstitial pH. It is hypothesized that this mechanism can enhance glucose control and reduce insulin resistance in patients with type 2 diabetes [25]. However, in the case of the primary original plant extract, the primary cellular component that is involved in the treatment of DM is the nucleosome core. The nucleosome's presence increases the accessibility of the transcription factor to the promoter gene. The alteration of gene expression in diabetes can result from the nucleosomal position. For instance, the presence of nucleosomes in the promoter region of SGLT2 is more prevalent in the human body than in other regions, which may explain why SGLT2 is more frequently expressed in the body. Customization can increase nucleosomal abundance, thereby facilitating the access of transcription factors such as HNF1α to promoter regions. As a result, the increased access enhances SGLT2 expression and improves glucose absorption [26].

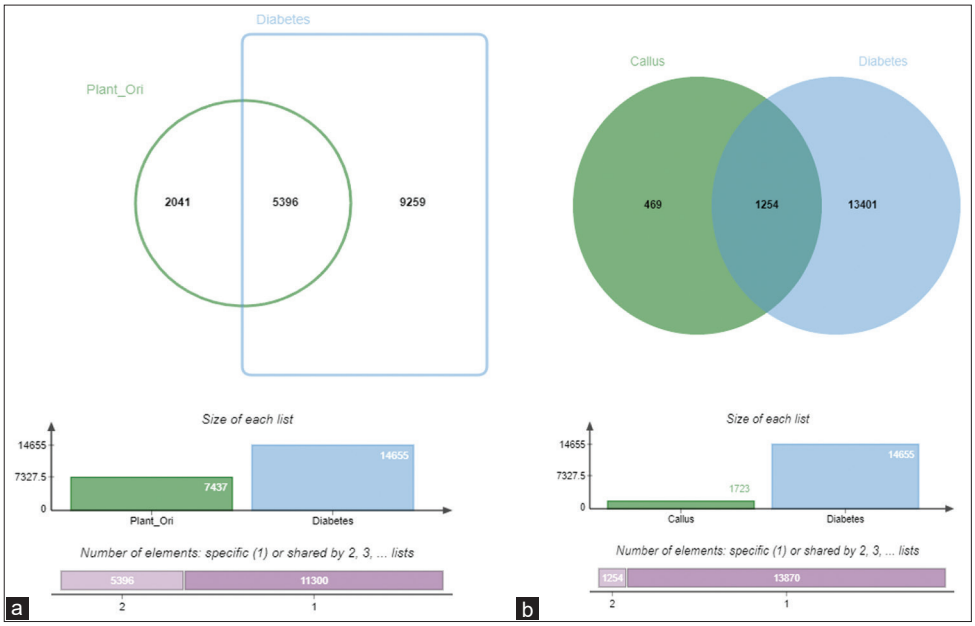

Fig. 5: The Venn diagram illustrates the extraction of ethanol from the original plant and the callus of black pepper

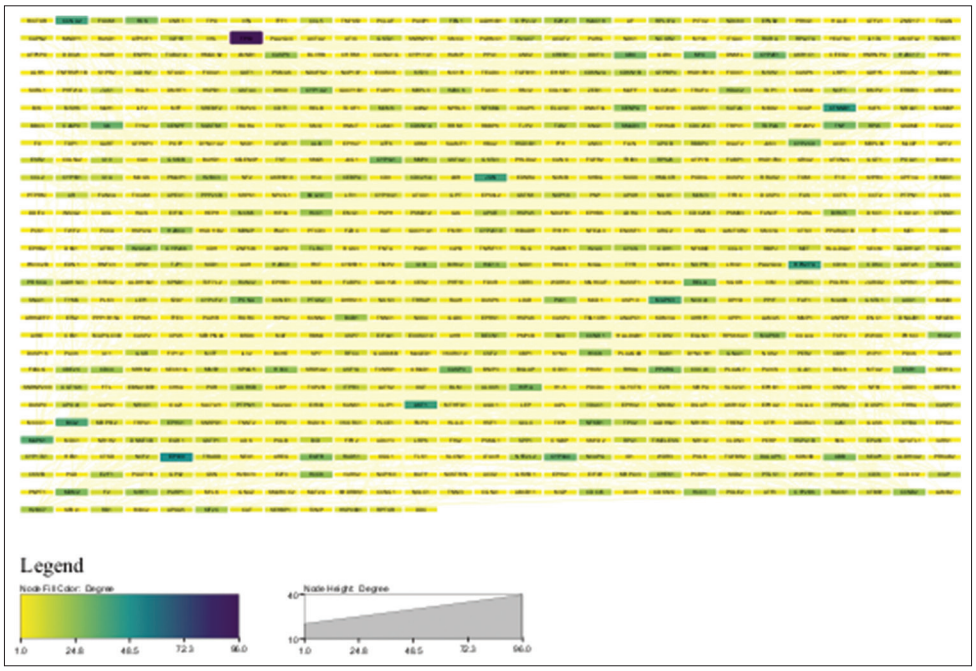

Fig. 6: Interactions among target proteins of the ethanol extract derived from the callus of black pepper

The primary molecular function that is described in the ethanol callus extract for the treatment of diabetes is p53 binding, which is involved in the multifaceted process of diabetes treatment by regulating apoptosis, growth, metabolism, adipose function, and adipose tissue function. Identifying p53 or its deletion offers a potential therapeutic strategy for improving glucose control and reducing diabetes complications [27,28]. Conversely, the primary molecular function that is implicated in the treatment of DM is cyclin. Cyclins, particularly cyclin D1, induce diabetes by altering glucose metabolism, enhancing the function of β-cells, modulating insulin secretion, inhibiting glucose production in the liver, and addressing the lipid reorganization associated with diabetes complications [29,30].

Antioxidant activity assay

Antioxidants can aid in managing diabetes by reducing oxidative stress, preserving beta cell function, enhancing insulin sensitivity, preventing complications, modulating signaling pathways, and dietary intake or supplementation. However, it is important to note that antioxidants are best used in conjunction with standard diabetes management strategies [31]. The ability of an extract to treat diabetes is diverse due to the presence of multiple active compounds with different mechanisms in the extract to address a particular disease.

Based on the IC_{50} value standard, a sample is considered to have a very strong antioxidant activity if its IC_{50} value is <50 ppm, strong if the value is between 50 and 100 ppm, moderate if the value is between 101 and 150 ppm, and weak if the value is between 151 and 200 ppm [32]. Based on the antioxidant test results in Table 4, it can be observed that when the concentration of the sample and the comparator increases, the obtained absorbance value decreases, resulting in a higher percentage of inhibition. Based on the IC_{50} values, it is evident that

Fig. 7: Interactions among target proteins of the ethanol extract derived from the black pepper plant

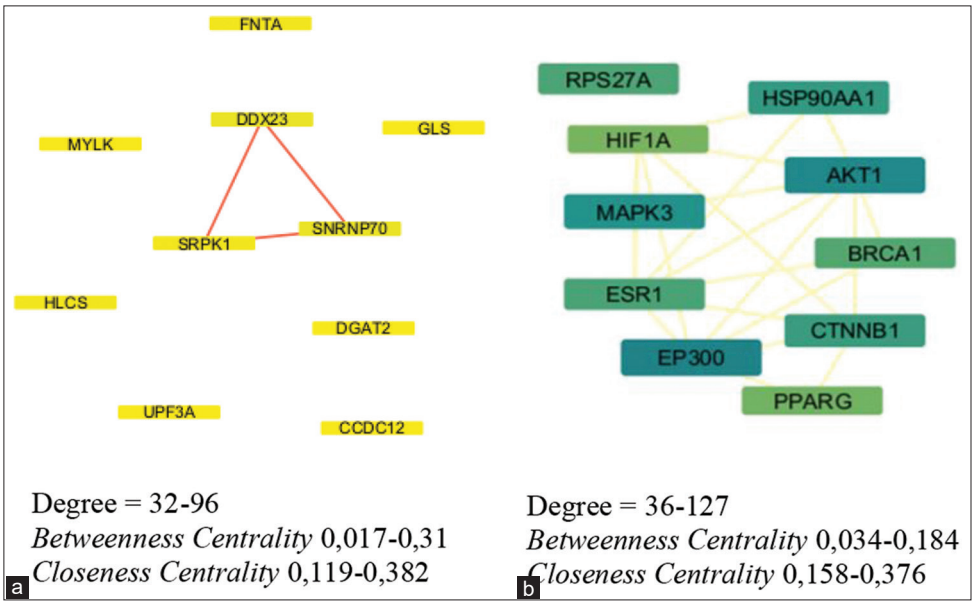

Fig. 8: (a) 10 main protein targets of ethanol extract from callus. (a) The 10 main protein targets of the ethanol extract from the black pepper plant

Table 4: Antioxidant test results

No.	Sample	Concentration (ppm)	Absorbance	Inhibition (%)	IC_{50} (μg/mL) (mean±SD)	Category
1	Quercetin (comparator)	10	1.61	15.65	4.08±0.01	Very strong
		50	1.11	41.81		
		100	0.24	87.24		
		200	0.20	89.71		
2	Original plant extract	100	1.19	46.86	156.69±1.36	Weak
		250	0.99	55.90		
		500	0.56	75.08		
		750	0.11	95.22		
3	Callus extract	100	0.96	49.38	129.92±0.83	Moderate
		250	0.91	52.38		
		500	0.81	57.61		
		750	0.72	62.42		

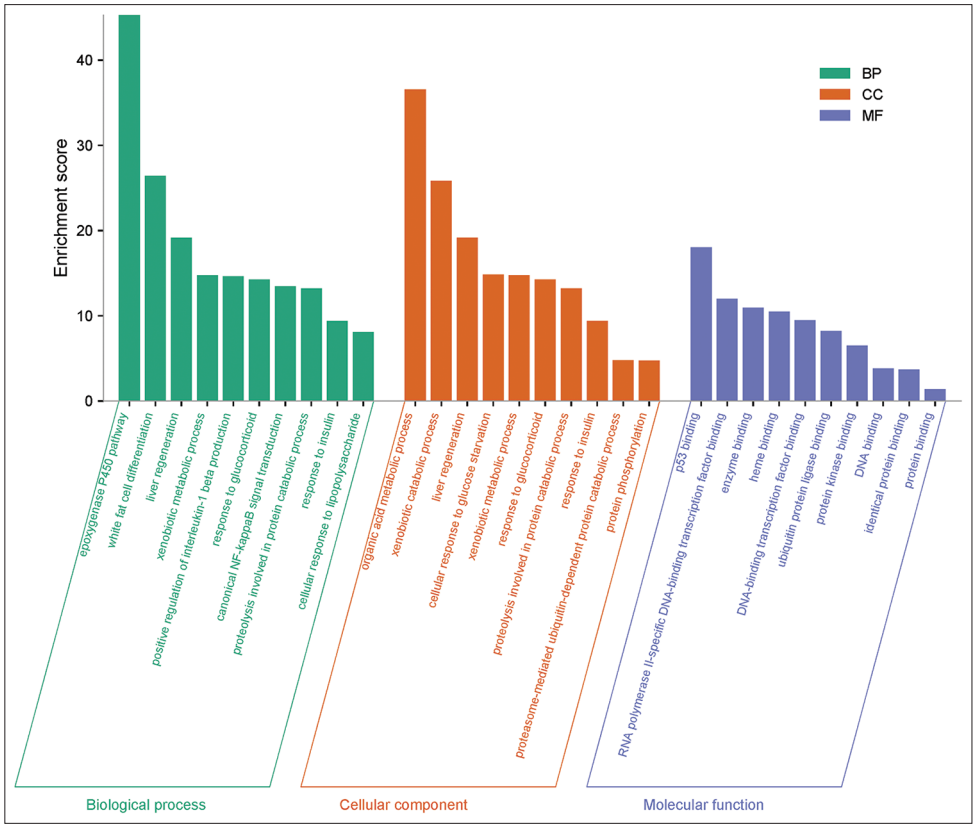

Fig. 9: Gene ontology functional enrichment analysis ethanol extract of the callus of black pepper

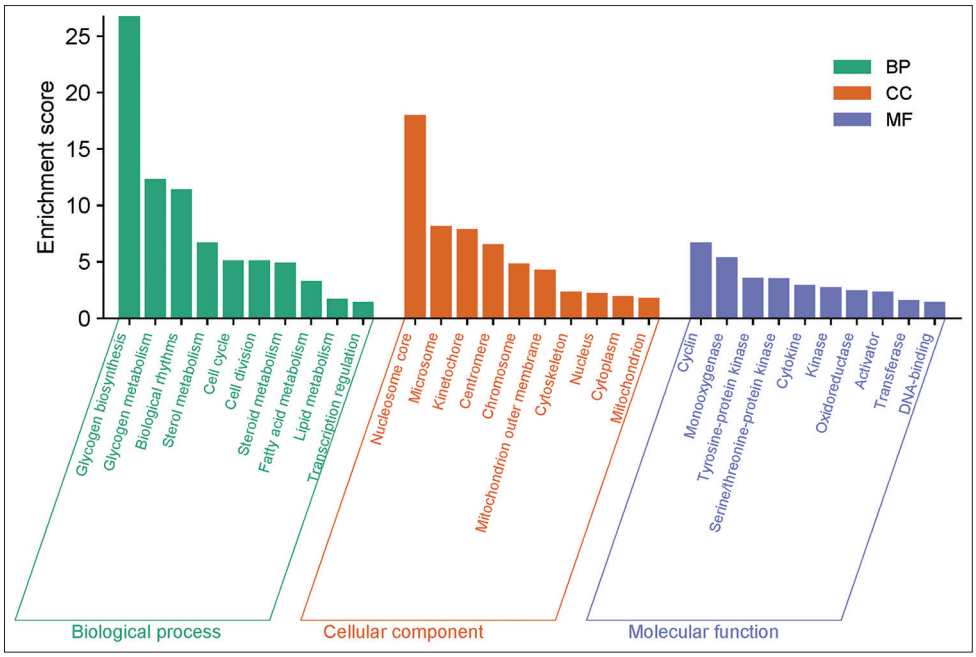

Fig. 10: Gene ontology functional enrichment analysis of ethanol extract of black pepper plant

the ethanol extract of black pepper callus exhibits better antioxidant activity compared to the ethanol extract of the original black pepper plant, although it is not superior to quercetin. These findings align with several studies that have compared the antioxidant activity of callus extracts to that of the parent plant extracts. For instance, a study conducted by Sireen and Anbumalarmanthi demonstrated that the antioxidant activity of callus extract from *A. indicum* L. was superior to that of the original plant extract. Similarly, Ranade *et al.* demonstrated that the callus extract of *Barleria prionitis* L. has antioxidant activity, as evidenced by the FRAP assay, which is 7 times more effective than the extract of the parent compound. In addition, the DPPH assay showed that it has 3 times better activity compared to the extract of the original plant [21,33].

This research demonstrates that the ethanol extract of callus and the original plant of black pepper have the potential to act as antidiabetic

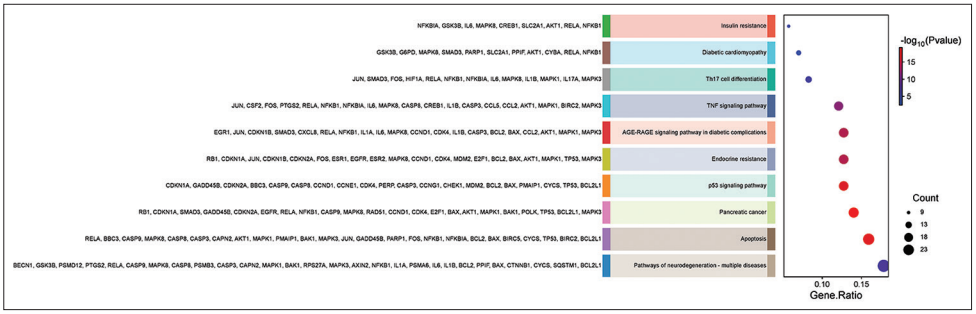

Fig. 11: Kyoto encyclopedia of genes and genomes pathway enrichment analysis, black pepper callus ethanol extract

Fig. 12: Kyoto encyclopedia of genes and genomes pathway enrichment analysis, black pepper plant ethanol extract

agents with different mechanisms. Furthermore, to provide further evidence, it is necessary to conduct antidiabetic activity tests on both extracts through *in vitro* and *in vivo* studies.

CONCLUSION

This research concludes that the ethanol extract of the original plant and callus of *P. nigrum* L. includes many different active compounds and is predicted to be able to address DM. The ethanol extract of the plant contains ten primary protein targets, namely RPS27A, HIF1A, MAPK3, ESR1, EP300, PPARG, CTNNB1, BRCA1, AKT1, and HSP90AA1. The 10 primary targets of ethanol callus extract are FNTA, MYLK, DDX23, GLS, SRPK1, SNRNP70, HLCS, DGAT2, UPF3A, and CCDC12.

This study demonstrates that the ethanol extract of callus and the original plant of black pepper have the potential to act as antidiabetic agents with different mechanisms. The P450 epoxygenase pathway is the main biological process in the extraction of black pepper callus, while in the extract of the original black pepper plant, it enhances glycogen biosynthesis. The main predicted cellular component involved in the ethanol extract of black pepper callus in treating diabetes is the organic acid metabolic process, while in the ethanol extract of the original black pepper plant, it is the nucleosome core. The main predicted molecular activity involved in the ethanol extract of black pepper callus in treating diabetes is p53 binding. Meanwhile, in the ethanol extract of black pepper, the active compound is cyclin. To further demonstrate the potential of both extracts in addressing DM, it is necessary to conduct antidiabetic activity tests through *in vitro* and *in vivo* studies.

ACKNOWLEDGMENT

The authors express their gratitude to Wahid Hasyim University for providing financial support for this research.

AUTHORS' CONTRIBUTIONS

Risha Fillah Fithria and Su Lingyu contributed to the collection of data, manuscript preparation, idea generation, conceptualization, supervision, and critical evaluations.

Muhammad Badrul Huda is responsible for gathering data and creating the initial version of the article.

Prof. Dr. Farahidah Mohamed specializes in idea generation and conceptualization, critical evaluation, and supervisory roles.

CONFLICTS OF INTERESTS

The authors do not have any conflicts of interest to disclose.

REFERENCES

1. Moodley K, Joseph K, Naidoo Y, Islam S, Mackraj I. Antioxidant, antidiabetic and hypolipidemic effects of *Tulbaghia violacea* harv. (Wild garlic) rhizome methanolic extract in a diabetic rat model. BMC Complement Altern Med. 2015;15:408. doi: 10.1186/s12906-015-0932-9, PMID 26577219
2. Chen SC, Tseng CH. Dyslipidemia, kidney disease, and cardiovascular disease in diabetic patients. Rev Diabet Stud. 2013;10(2-3):88-100. doi: 10.1900/RDS.2013.10.88, PMID 24380085
3. He JH, Chen LX, Li H. Progress in the discovery of naturally occurring anti-diabetic drugs and in the identification of their molecular targets. Fitoterapia. 2019;134:270-89. doi: 10.1016/j.fitote.2019.02.033, PMID 30840917
4. Balbaa M, El-Zeftawy M, Abdulmalek SA. Therapeutic screening of herbal remedies for the management of diabetes. Molecules. 2021;26(22):6836. doi: 10.3390/molecules26226836, PMID 34833928
5. Tereshchuk L, Starovoytova K, Babich O, Dyshlyuk L, Sergeeva I, Pavsky V, *et al*. Sea buckthorn and rosehip oils with chokeberry extract to prevent hypercholesterolemia in mice caused by a high-fat diet *in vivo*. Nutrients. 2020;12(10):2941. doi: 10.3390/nu12102941, PMID 32992796
6. Gou GH, Liu L, Abdubakiev S, Xin XL, Akber Aisa H, Li J. Anti-diabetic effects and molecular mechanisms of amide alkaloids from *Piper longum* based on network pharmacology integrated with cellular assays. Chem Biodivers. 2023;20(1):e202200904. doi: 10.1002/cbdv.202200904, PMID 36469428
7. Kharbanda C, Alam MS, Hamid H, Javed K, Bano S, Ali Y, *et al*. Novel piperine derivatives with antidiabetic effect as PPAR-γ agonists. Chem Biol Drug Des. 2016;88(3):354-62. doi: 10.1111/cbdd.12760, PMID 27037532
8. Khaliq T, Sarfraz M, Ashraf MA. Recent progress for the utilization of *Curcuma longa*, *Piper nigrum* and *Phoenix dactylifera* seeds against type 2 diabetes. West Indian Med J. 2015;64(5):527-32. doi: 10.7727/wimj.2016.176, PMID 27399905
9. Kazmierski Ł, Roszkowski S. Plant stem cells culture - a new tool for skin protection and regeneration. Med Res J. 2019;4(1):52-7.

doi: 10.5603/MRJ.a2018.0030
10. Naseem M, Dandekar T. Plant stem cells methods and protocols; 2020. In: Methods in Molecular Biology. United States: Humana Press; 2094. doi: 10.1007/978-1-0716-0183-9
11. Sena G. Stem cells and regeneration in plants. Nephron Exp Nephrol. 2014;126(2):35. doi: 10.1159/000360658, PMID 24854637
12. Abdulhafiz F, Mohammed A, Kayat F, Bhaskar M, Hamzah Z, Podapati SK, *et al.* Xanthine oxidase inhibitory activity, chemical composition, antioxidant properties and GC-MS analysis of keladi candik (*Alocasia longiloba* Miq). Molecules. 2020;25(11):2658. doi: 10.3390/molecules25112658, PMID 32521624
13. Ho YL, Huang SS, Deng JS, Lin YH, Chang YS, Huang GJ. *In vitro* antioxidant properties and total phenolic contents of wetland medicinal plants in Taiwan. Bot Stud. 2012;53:55-66.
14. Subasinghe S, Swamathilaka DB, Fernando KM. *In vitro* propagation of black pepper (Piper nigrum). In: Proceedings of the Forestry and Environmental Symposium; 27–28 February 2004; Sri Lanka. Issue: Eco-friendly Approaches Towards Sustainable Development; Section: Forest and Natural Resource Management. Sri Lanka: University of Sri Jayewardenepura; 2004. doi: 10.31357/fesympo.v0i0.1530
15. Hussain A, Naz S, Nazir H, Shinwari ZK. Tissue culture of black pepper (*Piper nigrum* L.) in Pakistan. Pak J Bot. 2011;43:1069-78.
16. Ikeuchi M, Sugimoto K, Iwase A. Plant callus: Mechanisms of induction and repression. Plant Cell. 2013;25(9):3159-73. doi: 10.1105/tpc.113.116053, PMID 24076977
17. Thamkaew G, Sjöholm I, Galindo FG. A review of drying methods for improving the quality of dried herbs. Crit Rev Food Sci Nutr. 2021;61(11):1763-86. doi: 10.1080/10408398.2020.1765309, PMID 32423234
18. BPOM. Badan pengawas obat dan makanan republik Indonesia nomor 32 tahun 2019 tentang persyaratan keamanan dan mutu obat tradisional. Bpom RI. 2019;11:1-16.
19. Hidayat R, Wulandari P. Methods of extraction: Maceration, percolation and decoction. Eu Herb Indones. 2021;2(1):68-74. doi: 10.37275/ehi.v2i1.15
20. Azam S, Park JY, Kim IS, Choi DK. Piperine and its metabolite's pharmacology in neurodegenerative and neurological diseases. Biomedicines. 2022;10(1):154. doi: 10.3390/biomedicines10010154, PMID 35052833
21. Sireen AA, Anbumalarmathi J. A comparative study between plant and callus extracts of *Abutilon indicum* (L.) Sweet: Antioxidant, antibacterial, antidiabetic and anti-proliferative activity. Int J Biochem Res Rev. 2020;29:13-24. doi: 10.9734/ijbcrr/2020/v29i930220
22. Xu X, Li R, Chen G, Hoopes SL, Zeldin DC, Wang DW. The role of cytochrome P450 epoxygenases, soluble epoxide hydrolase, and epoxyeicosatrienoic acids in metabolic diseases. Adv Nutr. 2016;7(6):1122-8. doi: 10.3945/an.116.012245, PMID 28140329
23. Wang L, Li J, Di LJ. Glycogen synthesis and beyond, a comprehensive review of GSK3 as a key regulator of metabolic pathways and a therapeutic target for treating metabolic diseases. Med Res Rev. 2022;42(2):946-82. doi: 10.1002/med.21867, PMID 34729791
24. Mangaki A, Malviya N. Synthesis, characterization and biological evaluation of glycogen synthase kinase-3β inhibitors as antidiabetic agents. Int J Pharm Qual Assur. 2023;14(2):330-3. doi: 10.25258/ijpqa.14.2.15
25. Marunaka Y. The proposal of molecular mechanisms of weak organic acids intake-induced improvement of insulin resistance in diabetes mellitus via elevation of interstitial fluid pH. Int J Mol Sci. 2018;19(10):3244. doi: 10.3390/ijms19103244, PMID 30347717
26. Takesue H, Hirota T, Tachimura M, Tokashiki A, Ieiri I. Nucleosome positioning and gene regulation of the SGLT2 gene in the renal proximal tubular epithelial cells. Mol Pharmacol. 2018;94(3):953-62. doi: 10.1124/mol.118.111807, PMID 29959222
27. Kung CP, Murphy ME. The role of the p53 tumor suppressor in metabolism and diabetes. J Endocrinol. 2016;231(2):R61-75. doi: 10.1530/JOE-16-0324, PMID 27613337
28. Gu J, Wang S, Guo H, Tan Y, Liang Y, Feng A, *et al*. Inhibition of p53 prevents diabetic cardiomyopathy by preventing early-stage apoptosis and cell senescence, reduced glycolysis, and impaired angiogenesis. Cell Death Dis. 2018;9(2):82. doi: 10.1038/s41419-017-0093-5, PMID 29362483
29. Bhalla K, Liu WJ, Thompson K, Anders L, Devarakonda S, Dewi R, *et al*. Cyclin D1 represses gluconeogenesis via inhibition of the transcriptional coactivator PGC1α. Diabetes. 2014;63(10):3266-78. doi: 10.2337/db13-1283, PMID 24947365
30. Saavedra-Ávila NA, Sengupta U, Sánchez B, Sala E, Haba L, Stratmann T, *et al*. Cyclin D3 promotes pancreatic β-cell fitness and viability in a cell cycle-independent manner and is targeted in autoimmune diabetes. Proc Natl Acad Sci U S A. 2014;111(33):E3405-14. doi: 10.1073/pnas.1323236111, PMID 25092329
31. Akpoveso OP, Ubah EE, Obasanmi G. Antioxidant phytochemicals as potential therapy for diabetic complications. Antioxidants (Basel). 2023;12(1):123. doi: 10.3390/antiox12010123, PMID 36670985
32. Molyneux P. The use of the stable free radical diphenylpicryl-hydrazyl (DPPH) for estimating antioxidant activity. Songklanakarin J Sci Technol. 2004;26:211-9.
33. Ranade R, Joshi N, Kudale S. Comparative secondary metabolite expression in callus cultures and mother plant in *Barleria prionitis* L. Plant Cell Tiss Organ Cult. 2023;155(3):653-63. doi: 10.1007/s11240-023-02585-5