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Unveiling the Phytopharmacological Potential of *Cucurbita maxima* Seeds through Docking, Network Pharmacology, and Antioxidant Assays

Partha Sen[1], Partha Pratim Mahata[2], Saswati Tarafdar Sasmal [2], Milan Kumar Maiti[*]

[1,2,*]*Bcda college of pharmacy & Technology, Hridaypur, Barasat, West Bengal, Kol: 700127*
[*]*Corresponding Author's E-mail: milan_juphar@yahoo.co.in*

Abstract:
The Cucurbita maxima (CMS)are a widely cultivated plant valued for its nutritional and medicinal benefits. Traditionally valued for its medicinal properties, CMS seeds contain diverse bioactive compounds with therapeutic potential. This study employed a systematic in-silico literature review, phytochemical analysis, and in vitro assays to assess their antioxidant activity. Extracts were evaluated for Total Phenolic Content (TPC), Total Flavonoid Content (TFC), DPPH scavenging, and metal-chelating capacity. Network pharmacology and docking suggested that major constituents—chlorogenic acid, isosorbide, sedoheptulosan, and cianidanol—may modulate inflammation, cancer, and type II diabetes–associated targets.The results revealed that methanol and aqueous extracts exhibited the highest TPC (21,248 ± 310 mgGAE/gm) and TFC (1148 ± 28 mgQE/gm) values in water, indicating significant concentrations of phenolic and flavonoid compounds. DPPH (IC_{50} value :227.37 ± 0.0028 µg/ml of methanol extract) and Metal Chelation (IC_{50} = 766.16 ± 4.53 µg/ml in water extract) assays confirmed strong free radical scavenging and metal ion binding capacities, especially for polar extracts, supporting the link between solvent polarity and antioxidant yield.The seeds of CMS possess substantial phytochemical and antioxidant potentials, validating their traditional use and supporting further exploration as a source for neutraceuticals, functional foods and pharmaceutical applications.

Keywords: *Cucurbita maxima, TPC, TFC, DPPH assay, Metal Chelation, antioxidant activity, network pharmacology, molecular docking.*

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I.Introduction

Cucurbita maxima (*CMS),* is a nutritionally and economically important *Cucurbitaceae* species native to South America and widely cultivated for its edible and medicinally valued fruits and seeds. The plant exhibits substantial morphological diversity and provides carbohydrates, proteins, minerals, and bioactive phytochemicals. Traditionally used for managing intestinal parasites, urinary disorders, and inflammation, it remains integral to folk medicine. Contemporary studies further highlight its functional food potential, with pumpkin seeds showing antihypertensive, hypoglycaemic, and hepatoprotective activities. Additionally, the plant's polysaccharides and carotenoids contribute to its immunomodulatory and anti-cancer properties (Jahan, Farhana, et al., 2023).CMS is increasingly valued for its broad pharmacological, neutraceuticals, and industrial applications, supported by its adaptability and agricultural significance

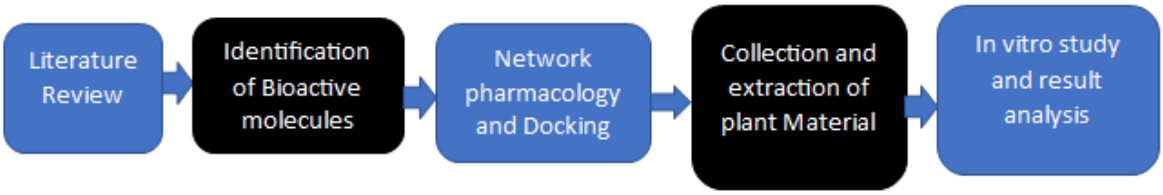


Fig.1. Graphical abstract of research work

Its by-products offer opportunities for value-added food and cosmetic products, yet scientific evidence on its phytochemical diversity and mechanisms of action remains limited. Advancing research on the isolation, characterization, and standardization of its bioactive constituents is essential for therapeutic development. Sustainable extraction approaches and comprehensive in vivo studies may further enhance its commercial and clinical relevance. This review summarizes the chemical profile and pharmacological potential of CMS, with

emphasis on its seeds as a promising bio resource. Through systematic *in vitro* and *in vivo* research, this study hopes to confirm traditional claims and provide scientific evidence supporting the development of safe and effective phytopharmaceuticals from this versatile plant (Faturoti, A. O., & Ogidi, C. O. et al.,2025).

II.Materials and Methods

2.1. Materials

2.1.1. Network Pharmacology

In network pharmacology, PubChem provides compound ADME data and SMILES files, GeneCards offers disease-related target genes, STRING supplies protein–protein interaction networks, KEGG maps targets to biological pathways, and SuperPred aids in screening and predicting active components.

2.1.2. Molecular docking

In network pharmacology and drug discovery, the Protein Data Bank (RCSB PDB) provides 3D protein structures, PubChem supplies compound data in formats such as SMILES, SDF, or MOL2, Chem3D Pro 12.0 is used for molecular modelling and structure optimization, and AutoDock is applied to perform molecular docking for predicting drug–target interactions.

2.1.3. Plant Materials

Mature seeds of *CMS* were collected from healthy, disease-free fruits harvested during the peak season. The plant material was authenticated and thoroughly cleaned, dried, and stored under suitable conditions for further analysis.

2.1.3.1. Selection and Collection

The gathering of *CMS* seeds for research is informed by literature that highlights the seeds' rich phytochemical composition, which includes proteins, fatty acids, and antioxidants. The specific selection of plant parts for investigation is guided by their purported medical advantages and traditional applications. The farmers and the closest harvesting fields in Mathabhanga (Lat: 26.344081; Long: 89.218525), Coochbehar, 736146, are the primary sources of the plant and seeds (Jahan, Farhana, et al., 2023).

2.1.3.2. Identification and Authentication

At the Central National Herbarium, Botanical Survey of India (BSI), Howrah, West Bengal 711103, a certified botanist verified the CMS seeds and placed a voucher specimen with specimen number BCDA/PS—01 for future use. The CNH/Tec.II/2025/45 is the authentication number (Karthigeyan, K., et al., 2022).

2.1.3.3. Plant Processing

Pumpkin seeds (*CMS*) were gathered at Mathabhanga, Dist. Coochbehar, and West Bengal, India (Lat: 26.344081; Long: 89.218525). After that, the seeds were removed and left to dry in the shade. Grind the seeds into a fine powder when they have thoroughly dried (Sharma, Ashok, et al., 2013).

2.1.3.4. Preparation of Extracts

A crucial stage in the pharmacological and phytochemical analyses of therapeutic plants is extraction. It entails separating bioactive substances from plant parts like seeds, bark, roots, and leaves. The polarity of the target chemicals determines the choice of solvent. Non-polar solvents (such as hexane, chloroform, and petroleum ether) extract non-polar materials like lipids and essential oils, while polar solvents (such as water, ethanol, and methanol) remove polar chemicals like flavonoids and tannins (Muchirah, Peninah Njoki, et al., 2018).

The *CMS* seeds were extracted using a standard extraction technique, such as repeated maceration. A new powder was made from dried seeds. The extraction process involved a series of macerations. 50 mL of N-Hexane and 10g of dry powder are combined in a glass beaker, which is then left in a dark room for 36 hours. Whatman filter paper was used to filter the macerated portion, which was subsequently evaporated in a water bath at 40°C and refrigerated at 4ºC. The result was a viscous extract. From non-polar to polar solvents, the same process was used for the remaining solvents (N-Hexane > Petroleum Ether > Ethanol > Methanol > Distilled Water) (Mehmood, Ansar, et al., 2022).

2.1.3.5. Chemicals and Reagents

Gallic acid, Folin-Ciacalteu's Phenol Reagent, sodium bicarbonate, and distilled water were utilized in the Total Phenolic Content (TPC) assay (Maiti, Milan Kumar, et al., 2024).Quercetin, Methanol, Aluminum Chloride, Potassium Acetate, and Distilled Water were utilized in the Total Flavonoid Content (TFC) test (Shraim, Amjad M., et al., 2021).Ascorbic acid, methanol, DPPH, and distilled water were utilized in the DPPH study (Marinova, G., & Batchvarov, V. et al., 2011).Ascorbic acid, methanol, ferrous sulphate, ferrozine, and distilled water were utilized in the Metal Chelation Assay (Gulcin, İ., & Alwasel, S. H. et al., 2022).

2.1.3.6. Equipment

The experimental work was conducted using a digital analytical balance (Model: AUW220D, Shimadzu, Japan) for accurate weighing of samples; a laboratory incubator (Model: BOD-150, Remi, India) for maintaining

controlled environmental conditions; and a UV–Visible spectrophotometer (Model: UV-1800, Shimadzu, Japan) for spectrophotometric measurements.

2.1.3.7. Glass Apparatus

General laboratory glassware and consumables used in the study included a stainless-steel spatula, borosilicate beakers of varying capacities, glass stirring rods, test tubes, and glass funnels (Borosil, India). Filtration was performed using Whatman No. 1 filter paper (GE Healthcare, UK).

2.2. Methodology

2.2.1. In-silico study

A component–target–disease network was constructed to elucidate the synergistic interactions and potential mechanisms of bioactive compounds from CMS. The selected compounds and smile code of all selected compound use in docking are given below: edoheptulosan(C1[C@@H]2[C@H]([C@H]([C@@H]([C@](O1)(O2)CO)O)O)O),isosorbide(C1[C@H]([C@@H]2[C@H](O1)[C@H](CO2)O)O),chlorogenic-acid (C1[C@H]([C@H]([C@@H](C[C@@]1(C(=O)O)O)OC(=O)/C=C/C2=CC(=C(C=C2)O)O)O)O)and cianidanol(C1[C@@H] ([C@H](OC2=CC(=CC(=C21)O)O) C3=CC(=C(C=C3)O)O)O) were analysed using GeneCards and other target prediction databases to identify potential disease-associated targets. The network was developed to examine the relationships of these compounds with inflammation, cancer, and T2DM, thereby providing insights into their possible therapeutic roles (Zhai, Yiyan, et al., 2025).

2.2.2. Network Pharmacology

2.2.2.1. Screening of active compounds and targets

The active ingredients and pertinent targets for Inflammation, Cancer, and Type II diabetes mellitus (T2DM) were screened using the GeneCards and Analysis Platform. According to Lee et al. (2018), they are important markers for assessing absorption, distribution, metabolism, and excretion (ADME) characteristics. These ADMEs' SMILES files are gathered from PubChem and their targets are then obtained by importing the screened active components into SuperPred databases. After being filtered, targets with a probability higher than 0 were gathered into a CSV file (Patil, Vishal S., et al., 2020).

2.2.2.2. Collection of targets

Targets for Inflammation, Cancer, and T2DM were collected from databases. Both targets were found in GeneCards by using keywords like “Inflammation,” "T2DM," and "Cancer." Create the disease target database by integrating disease targets from the database. The target database and the targets' intersection are entered into Venny 2.1.0 to create a Venn diagram. By collecting and classifying the disease targets and active substances, the intersection of the targets of both diseases and targets are found (Ye, Haowen, et al., 2022).

2.2.2.3. Calculation of protein-protein interaction networks

"Homo sapiens" was chosen as the organism type, and the intersection of targets was uploaded to the STRING database. The network's unconnected nodes were then concealed. Following that, Cytoscape 3.10.1 was used to import and analyse the data file in CSV format. Ultimately, a network graph of protein-protein interactions (PPIs) was created and arranged according to "degree" values (Farooq, Qurat ul Ain, et al., 2020).

2.2.2.4. KEGG pathway enrichment analysis

Target intersections were uploaded to ShinyGo 0.77, the species was set to "Homo sapiens," and enrichment analyses of the Kyoto Encyclopaedia of Genes and Genomes (KEGG) were done. The KEGG enrichment analysis data were plotted. Once relationships between target proteins and signaling pathways had been established, Cytoscape 3.10.1 was used to create their network diagrams (Wixon, J., & Kell, D. et al., 2000).[16].

2.2.2.5. Construction of active genes and targeting disease pathways

Based on enrichment analysis, the top 20 KEGG pathways most relevant to both disorders are selected. To illustrate the connections between the shared genes and targets related to the disorders, a network was constructed in Cytoscape 3.10.1; additional pathways are also involved. A node's "degree"—the number of edges that connect it—indicates its significance and influence on the network. Furthermore, drawing from the pertinent research, a representative key for both diseases can be found among the top twenty pathways that display a strong correlation with both disorders (Yousef, M., Ülgen, E., & Sezerman, O. U. et al., 2021).

2.2.3. Molecular docking

2.2.3.1. Collection of ligands and proteins

The Protein Data Bank (PDB) and PubChem databases provide the four-dimensional structures of receptor proteins and their related ligands. Additionally, Chem3D Pro 12.0 reduces the ligands' energy (Burley, Stephen K., et al., 2022).

2.2.3.2. Docking

The affinity and binding mechanisms of drugs with particular targets are investigated using AutoDock. A text document file is used to automate the docking procedure, which is scheduled to run 400 times. A specific command is used to simulate Command Prompt, and the output is a log file in the form of a text document. The value less than -1.2 kcal/mol. or less than -5 kJ/mol. was generally regarded as an acceptable binding energy for docking studies; tighter binding was suggested by lower binding energies. Consequently, given the docking data, the conformation with the lowest binding energy can be chosen. Lastly, the docking findings are analysed and visualized using Discovery Studio 2017: Both two-dimensional interaction plots between the chemical and the target and three-dimensional structural diagrams of the binding sites are produced using Discovery Studio 2017 (Satpute, U. M., & Rohane, S. H. et al., 2021).

2.2.4. Phytochemical Analysis

The technique of determining and describing the bioactive chemical components found in plants is known as phytochemical analysis. Alkaloids, flavonoids, tannins, saponins, glycosides, phenols, terpenoids, and steroids are examples of these naturally occurring substances, also referred to as phytochemicals. Usually, there are two phases to the analysis: quantitative estimation and preliminary qualitative screening (Borecka, M., & Karaś, M. et al., 2025 ; Yuan, Tiantian, et al., 2022 & Agidew, M. G. et al., 2022)).To determine if certain kinds of phytochemicals are present or absent, qualitative assays employ particular chemical reagents. For instance, alkaloids are tested with Dragendorff's reagent, phenolics with ferric chloride, and saponins using foam tests[22].Using methods such as UV-visible spectrophotometry, gravimetric analysis, or chromatographic procedures, quantitative analysis determines the precise quantity of phytochemicals (Sahoo, Chita Ranjan, et al., 2024).Drug discovery is supported by phytochemical analysis, which aids in comprehending the therapeutic potential of medicinal plants. Additionally, it is essential for standardizing herbal medications to guarantee their safety and consistency from batch to batch. Additionally, it offers scientific support for the conventional applications of therapeutic herbs in Ayurvedic and folk medicine (Wang, Hongting, et al., 2023).

2.2.4.1. Preliminary Phytochemical Assessment

N-hexane, petroleum ether, ethanol, methanol, and distilled water are among the solvents used to extract *CMS* seeds that have been ground into a powder. The extract is subjected to standard qualitative techniques for testing various phytochemical groups (Hagos, Mulu, et al., 2022 & Kulczyński, B.,et al.,2020).PPA of the plant extract was carried out using standard qualitative methods. Alkaloids were detected by Wagner's test, in which 2 mL of extract was treated with Wagner's reagent (iodine solution prepared by dissolving 1.27 g of I_2 and 2 g of KI in 100 mL distilled water), yielding a reddish-brown precipitate, and by Mayer's test, where the addition of Mayer's reagent (potassium mercuric iodide, prepared from 1.36 g $HgCl_2$ and 5 g KI in 100 mL water) produced a creamy precipitate. Flavonoids were identified through the alkaline reagent test, in which the extract was treated with 10% NaOH (aq.), producing a yellow colour that disappeared upon addition of dilute HCl, and by the Shinoda test, where Mg turnings with conc. HCl induced a pink–red coloration due to flavonoid–Mg^{2+} complex formation. Tannins and phenols were detected using the ferric chloride test, where 2 mL of extract was mixed with 5% $FeCl_3$ solution, producing a greenish or blue–black colour (formation of phenolate-Fe^{3+} complex). Saponins were identified by the foam test, where 2 mL of extract was shaken vigorously with 5 mL of distilled water, resulting in stable froth, and by the emulsion test with olive oil, where a stable emulsion indicated saponins.

For steroids and terpenoids, the Salkowski test was performed by mixing the extract with 2 mL of chloroform ($CHCl_3$) followed by conc. H_2SO_4 (H_2SO_4, 98%); a reddish-brown interface indicated steroids, whereas a golden-yellow colour confirmed terpenoids. Cardiac glycosides were detected by the Keller–Killiani test, in which the extract was treated with 2 mL of glacial acetic acid (CH_3COOH), one drop of $FeCl_3$ solution, and conc. H_2SO_4; the appearance of a reddish-brown ring confirmed glycosides. Reducing sugars were detected by Benedict's test, where 2 mL of extract was heated with 2 mL Benedict's reagent (alkaline solution of $CuSO_4$, sodium citrate, and Na_2CO_3); a brick-red precipitate of cuprous oxide (Cu_2O) indicated the presence of reducing sugars.

2.3. Total Phenolic Content (TPC)

The concentration of phenolic chemicals, which are well-known for their potent antioxidant qualities, in a sample is referred to as its TPC. These substances, which are frequently present in plants, are essential for scavenging free radicals and shielding cells from oxidative damage. A crucial first step in assessing the possible health advantages of natural goods, particularly in functional meals and nutraceuticals, is measuring TPC. The Folin-Ciacalteu's reagent is frequently utilized in TPC assays because of its sensitivity and dependability.

Determining the TPC content of *CMS* seed powder has aids in evaluating its potential as a natural antioxidant source (Shi, Linghong, et al., 2022).

2.3.1. Preparation of Standard and Samples

The Folin-Ciacalteu's colorimetric method, a commonly used and dependable technique, was used to measure the total phenolic content of *CMS* seed powder. A detailed description of the process is provided below.

2.3.1.1. Preparation of Standard

2 mg of gallic acid were precisely weighed and diluted in 2 mL of distilled water to create a stock solution (1 mg/ mL) to create the gallic acid standard solution. Distilled water was then used to further dilute 1 mL to 100 mL. Four different test tubes were then pipetted with 1 mL of this diluted solution. 100 µg/mL, 150 µg/mL, 200 µg/mL, and 250 µg/mL were the final concentrations obtained by adding 10 mL, 7.5 mL, 5 mL, and 4 mL of distilled water, respectively, to the first, second, third, and fourth test tubes. Folin-Ciacalteu's phenol reagent (0.4 mL) was added to each of them after 0.4 mL was put into fresh, separate test tubes. After adding an extra 4 mL of distilled water to each test tube, the tubes were left in a dark room for five minutes. After this incubation, 4 mL of a 7% sodium bicarbonate solution was added, then distilled water was added to each test tube until the final volume reached 10 mL. A UV-Visible spectrophotometer was then used to test each solution's absorbance at 730 nm, using distilled water as the blank (Genwali, G.R., et al., 2013).

2.3.1.2. Preparation of Samples

To measure the TPC, the same process was used to prepare 5 different solvent extract with different concentrated sample solutions (100 µg/ml, 150 µg/ml, 200 µg/ml, and 250 µg/ml) as follow for preparation of the standard.

2.4. Total Flavonoid Content (TFC)

The quantity of flavonoids, which are significant secondary metabolites from plants with anti-inflammatory, antioxidant, and cardioprotective qualities, that are present in a sample is referred to as its TFC. These substances support the general health advantages of many natural products by aiding in the neutralization of free radicals. When evaluating the therapeutic potential of plant-based products, TFC evaluation is crucial. Flavonoids are important bioactivity markers in studies on natural antioxidants. The usefulness of CMS seed powder as a functional food element is shown by measuring TFC in the powder (Elisha, Ishaku Leo, et al., 2016).

2.4.1. Preparation of Standard and Samples

The aluminium chloride colorimetric method, a straightforward and efficient technique, was used to assess the total flavonoid content of CMS seed powder. The steps are described below.

2.4.1.1. Preparation of Standard

A stock concentration of 1 mg/mL was obtained by precisely weighing 2 mg of quercetin and dissolving it in 2 mL of methanol to create the quercetin standard solution. Distilled water was used to dilute 1 mL of this solution to 100 mL. Four different test tubes were then pipetted with 1 mL of this diluted solution. The final concentrations were 100 µg/mL, 150 µg/mL, 200 µg/mL, and 250 µg/mL after adding 10 mL, 7.5 mL, 5 mL, and 4 mL of distilled water to the first, second, third, and fourth test tubes, respectively, to reach the required concentrations. 4 mL were taken from each of them and put into fresh test tubes. 0.2 mL of a 10% aluminium chloride solution and 0.2 mL of a 1 M potassium acetate solution were added to each test tube. 5.6 mL of distilled water was added to get the volume up to 10 mL. After that, each test tube was left in a dark spot for half an hour. Following incubation, a UV-Visible spectrophotometer was used to measure each solution's absorbance at 415 nm, using methanol as the blank (Dmitrienko, S. G., et al., 2012).

2.4.1.2. Preparation of Samples

Different concentrated sample solutions (100 µg/ml, 150 µg/ml, 200 µg/ml, and 250 µg/ml) were prepared using the same method as the standard to measure the TFC.

2.5. In Vitro Antioxidant Study

To assess a natural compound's capacity to scavenge free radicals in a controlled laboratory setting, in vitro antioxidant studies are crucial. The capacity of plant extracts or chemicals to counteract oxidative agents that can harm cells is determined in part by these investigations. DPPH and Metal Chelation assays are common assays that shed light on various antioxidant mechanisms. Finding promising natural antioxidants for use in food, medicine, and cosmetics requires the use of such analysis. CMS seed powder's functional and therapeutic potential can be evaluated by examining its in vitro antioxidant activity (Kotha, Raghavendhar R., et al.,2022).

2.5.1. DPPH Assay

One popular in vitro technique for assessing plant extracts' capacity to scavenge free radicals is the DPPH (2,2-diphenyl-1-picrylhydrazyl) assay. The DPPH assay is used to evaluate the antioxidant activity of CMS seed powder in the following manner.

2.5.1.1. Preparation of Standard

Two milligrams of ascorbic acid were precisely weighed and dissolved in 2 mL of methanol to achieve a concentration of 1 mg/mL to create the ascorbic acid standard solution. Distilled water was used to further dilute 1 mL of this solution to 100 mL. Four different test tubes were then pipetted with 1 mL of the diluted solution. 100 µg/mL, 150 µg/mL, 200 µg/mL, and 250 µg/mL were the final concentrations obtained by adding 10 mL, 7.5 mL, 5 mL, and 4 mL of distilled water, respectively, to the first, second, third, and fourth test tubes. 3 mL of each of them was moved into fresh, separate test tubes, and then 3 mL of DPPH solution was added. After that, 4 mL of distilled water was added to each test tube until the volume reached 10 mL. For half an hour, every test tube was kept in an incubator. Following incubation, a UV-Visible spectrophotometer was used to measure each solution's absorbance at 517 nm, using methanol as the blank (Zeng, W., et al., 2005).

2.5.1.2. Preparation of Samples

Similar steps were taken to prepare the standard and several concentrated sample solutions (100 µg/ml, 150 µg/ml, 200 µg/ml, and 250 µg/ml) for measuring the DPPH.

2.6. Metal Chelation Assay

The metal chelation assay assesses a sample's capacity to chelate ferrous ions (Fe^{2+}), which inhibits Fenton processes that produce dangerous free radicals. The following procedure is used to evaluate *CMS* seed powder's metal chelating activity:

2.6.1. Preparation of Standard

The ascorbic acid standard solution was made by precisely weighing 15 mg of ascorbic acid and dissolving it in 15 mL of methanol until the concentration was 1 mg/mL. Five test tubes were used to prepare the various concentrations. To achieve a concentration of 100 µg/mL, 1 mL of the ascorbic acid solution and 9 mL of distilled water were combined in the first test tube. Similarly, to reach final concentrations of 200 µg/mL, 300 µg/mL, 400 µg/mL, and 500 µg/mL, 2 mL, 3 mL, 4 mL, and 5 mL of the stock solution were added to the second, third, fourth, and fifth test tubes, respectively, with the corresponding amounts of distilled water adjusted to 8 mL, 7 mL, 6 mL, and 5 mL. 2 mL of a 0.5 mM ferrozine solution and 1 mL of a 0.3 mM ferrous sulphate solution were put into each of these test tubes. To guarantee adequate mixing, each test tube was vortexed separately before being incubated for half an hour. Following incubation, a UV-Visible spectrophotometer was used to detect each sample's absorbance at 562 nm, using methanol as the blank (Pisoschi, Aurelia Magdalena, et al., 2014).

2.6.2. Preparation of Samples

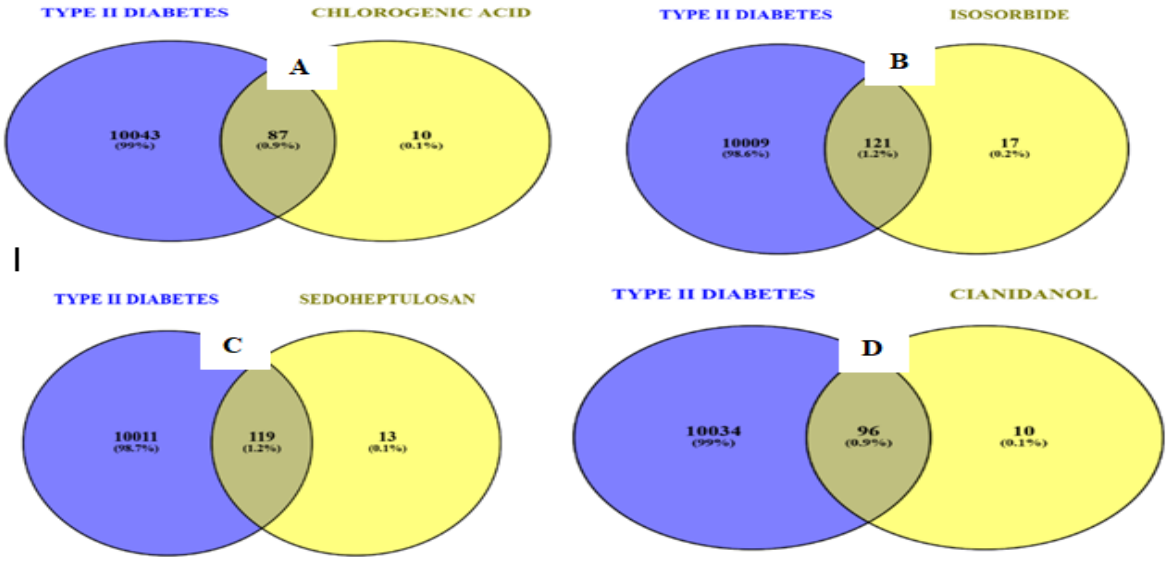
The same process was used to prepare the standard and various concentrated sample solutions (100 µg/ml, 150 µg/ml, 200 µg/ml, and 250 µg/ml) for detecting metal chelation.

III. Results and Discussion

3.1. Network pharmacology

3.1.1. Collection of targets

In the context of type 2 diabetes mellitus (T2DM), four phytoconstituents—Chlorogenic acid (87 targets), Isosorbide (121 targets), Sedoheptulosan (119 targets), and Cianidanol (96 targets)—were found to overlap with 10,145 T2DM-related targets, yielding 87, 121, 119, and 112 shared targets, respectively.



Fig,2. – Targets for T2DM (A. Chlorogenic Acid, B. Isosorbide, C. Sedoheptulosan, D. Cianidanol)

The intersection of 18,963 cancer-associated targets with four therapeutic candidates—Chlorogenic acid (95 targets), Isosorbide (137 targets), Sedoheptulosan (130 targets), and Cianidanol (104 targets)—resulted in 95, 137, 130, and 104 shared targets, respectively.

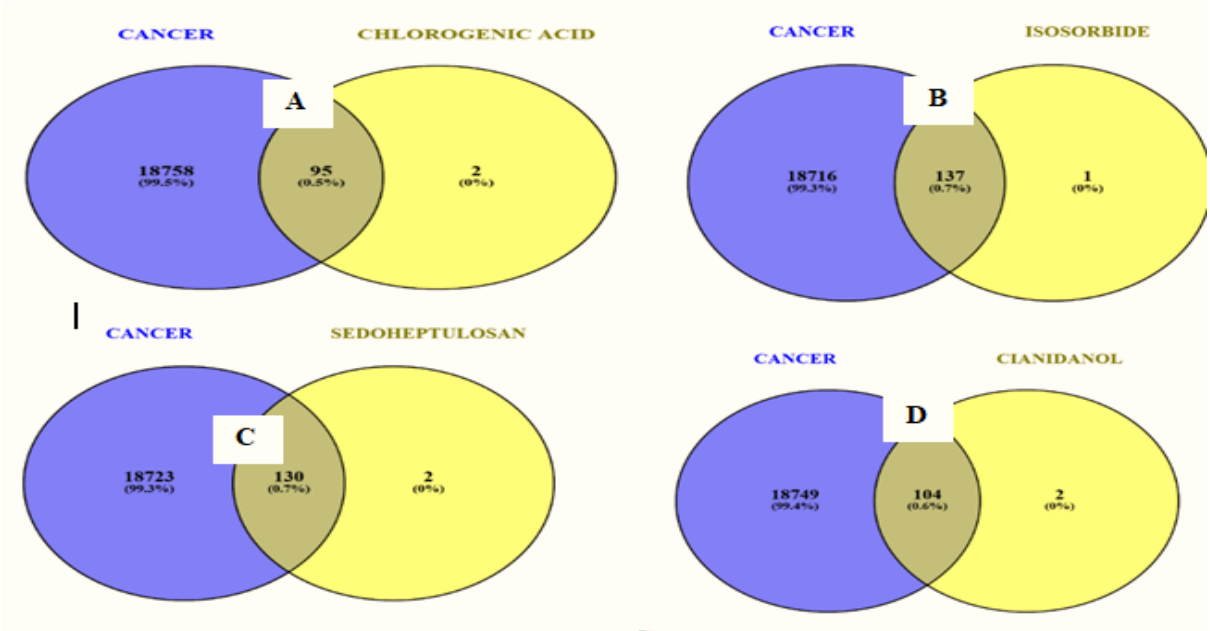


Fig.3. – Targets for Cancer (A. Chlorogenic Acid, B. Isosorbide, C. Sedoheptulosan, D. Cianidanol)

The intersection of 11,524 inflammation-associated targets with four therapeutic candidates—Chlorogenic acid (91 targets), Isosorbide (129 targets), Sedoheptulosan (126 targets), and Cianidanol (99 targets)—resulted in 91, 129, 126, and 99 shared targets, respectively.

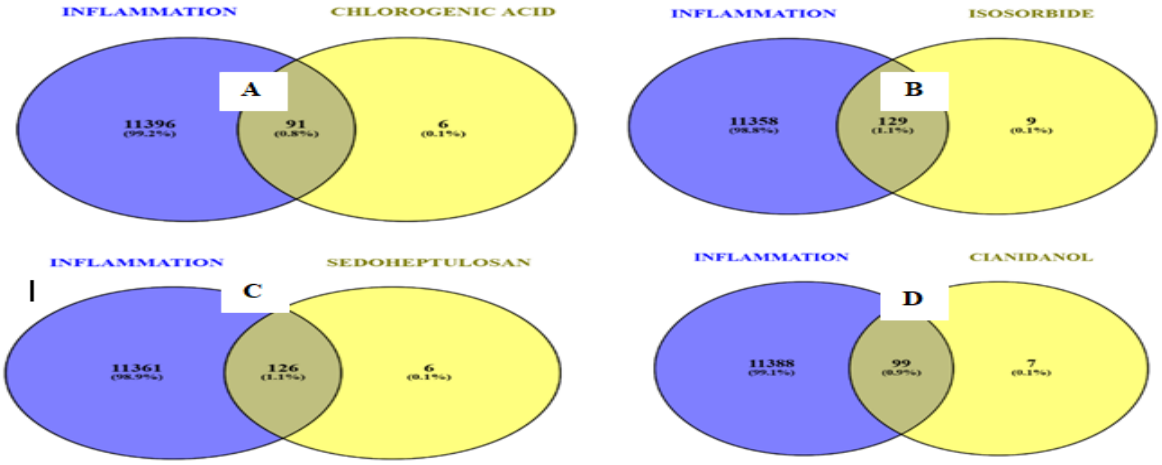


Fig.4. – Targets for Inflammation (A. Chlorogenic Acid, B. Isosorbide, C. Sedoheptulosan, D. Cianidanol)

The information on these intersection targets is then entered into the Cystoscope 3.10.3 program to investigate the possible mechanism of the treatment impact of four distinct medications. The outcome demonstrated that various components and targets mediate the four-drug molecules' effects on diabetes and cancer. Additionally, the program was used to examine the connections among the proteins, illnesses, and medication compounds. The findings showed that the four-drug compounds and the proteins PIK3CB (PI3K catalytic subunit; drives tumor growth and survival via PI3K/AKT signaling), PIK3R1(PI3K regulatory subunit; mutations alter PI3K activity and promote oncogenesis), MAPK1(ERK2 kinase; mediates MAPK/ERK pathway, enhancing proliferation and metastasis), PTGER2 (Prostaglandin E2 receptor; promotes inflammation-driven tumor progression), PRKACA (PKA catalytic subunit; dysregulation enhances cell proliferation and survival), and PRKCA (PKCα isoform; involved in tumor invasion, angiogenesis, and drug resistance) are associated with Cancer, PIK3CB (PI3K catalytic subunit; critical for insulin signaling and glucose uptake), MAPK1(ERK2 kinase; contributes to insulin resistance and β-cell dysfunction), CACNAB1(Calcium channel subunit; regulates insulin secretion from pancreatic β-cells), PIK3R1(PI3K regulatory subunit; mutations impair insulin sensitivity and glucose metabolism), and PRKCD (PKCδ isoform; mediates β-cell apoptosis and insulin resistance under hyperglycemia) are associated with T2DM, and PRKACA (PKA catalytic subunit; modulates cytokine production and immune cell activity), PRKCD(PKCδ isoform; regulates inflammatory signaling and apoptosis of immune cells), PRKCA(PKCα isoform; promotes NF-κB activation and pro-inflammatory cytokine release), PIK3R1(PI3K regulatory subunit; controls immune cell survival and inflammatory responses), PTGER2 (Prostaglandin E2 receptor; mediates PGE2-driven inflammation and immune modulation), and ALOX12 (Lipoxygenase enzyme; generates pro-inflammatory lipid mediators (12-HETE) are associated with Inflammation.

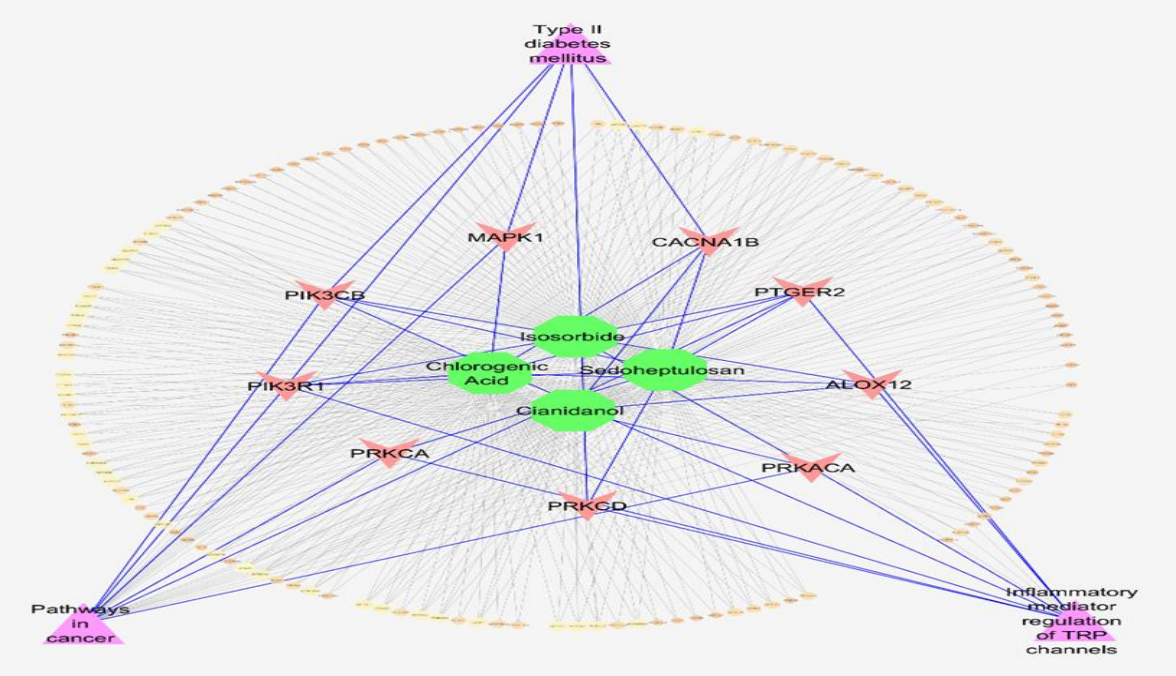


Fig.5. - Network of T2DM, Cancer, and Inflammation, and four different bioactive molecules.

The compound–target–pathway network revealed a multi-targeting mechanism underlying the therapeutic potential of the selected phytoconstituents. Core compounds such as Chlorogenic acid, Isosorbide, Sedoheptulosan, and Cianidanol (green nodes) were associated with several key signaling proteins **(**PIK3CB, PIK3R1, MAPK1, PRKCA, PRKACA, PRKCD, CACNA1B, PTGER2, and ALOX12, red nodes). These targets were further linked to critical disease-related pathways, including Type II diabetes mellitus, pathways in cancer, and inflammatory mediator regulation of TRP channels (purple nodes). Notably, PIK3R1 and PIK3CB emerged as central hubs, reflecting their involvement in the PI3K/AKT signaling axis, which is crucial in both glucose homeostasis and tumor progression. MAPK1 was also enriched, indicating its dual role in insulin resistance and cancer cell proliferation. Similarly, ALOX12 and PTGER2 were strongly associated with inflammatory processes, highlighting their contribution to the chronic inflammation observed in diabetes and cancer. Protein kinases (PRKACA, PRKCA, and PRKCD) displayed cross-talk across all three pathways, suggesting their importance as regulatory nodes bridging metabolic, oncogenic, and inflammatory signaling.

Overall, the integrative network underscores the multi-target and multi-pathway nature of phytochemicals, providing mechanistic insight into their potential role in modulating complex diseases such as diabetes, cancer, and inflammation.

3.1.2.KEGG enrichment analyses –

A KEGG enrichment study was carried out using the ShinyGO 0.77 database. Numerous pathways were implicated in these targets, indicating that the anti-diabetic and anti-cancer actions of the three-drug compounds may be mediated by many pathways. For additional examination, the top 20 enriched targets were chosen.

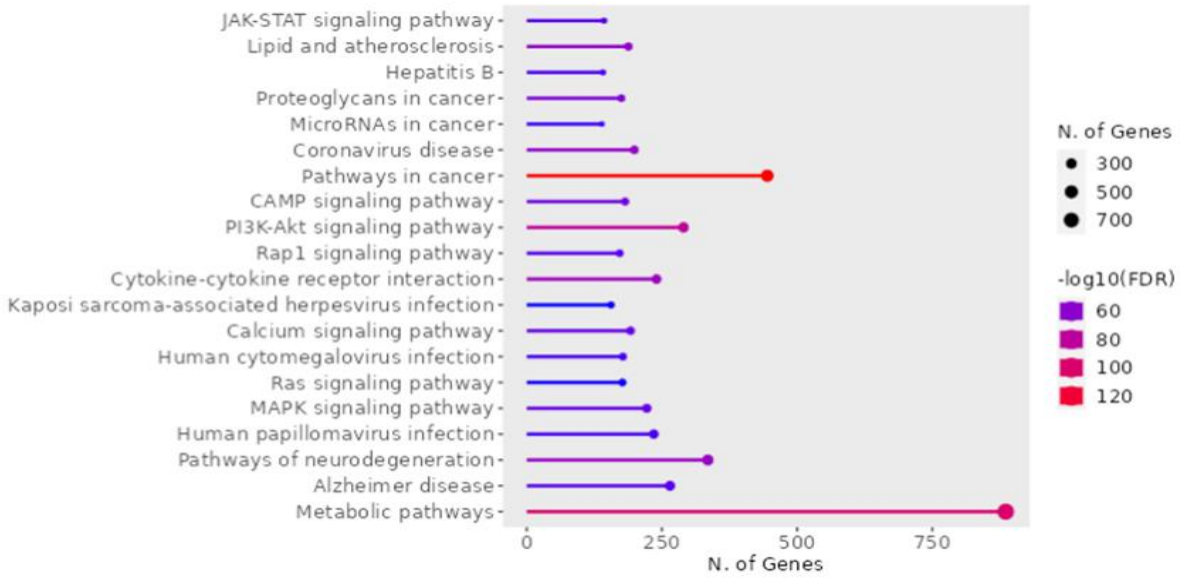


Fig.6. – Top 20 KEGG Enrichment Analysis

KEGG enrichment analysis revealed significant involvement of targets in metabolic pathways **(**highest gene enrichment over 750 genes), followed by pathways in cancer, PI3K-Akt, MAPK, Ras, and cAMP signaling pathways reflect thecross-talk of target genes between oncogenic signaling and infection-related processes. Additional associations with cytokine interactions, calcium signaling, viral infections, and neurodegenerationindicate the multi-functional roles of these targets in metabolism, cancer, inflammation, and infectious diseases**.**

3.2. Molecular docking –

Molecular docking between the drug molecules and the targeted proteins was done to confirm the findings of network pharmacology and ascertain the binding location and binding affinity. The targeted proteins and all four medication molecules have a strong bond. The table lists the drug compounds' and protein targets' binding details.

The proteins for Inflammation and Cancer were mostly common, so that the docking was done at once, and for T2DM, two major proteins were found, and the docking was done. The binding energy (Kcal/mol) was given below the tables (Table 1 and Table 2). 3D and 2D images are given below.

Table 1: Real docking binding energy and binding data for important anti-diabetic targets

Drug Molecules	Key Targets	Key Amino Acid Residues	Binding Energy (Kcal/mol)
Chlorogenic Acid	PIK3R1	7PG5	-9.2
	PIK3CB	Not Available	Not Available
Isosorbide	PIK3R1	7PG5	-5.8
	PIK3CB	Not Available	Not Available
Sedoheptulosan	PIK3R1	7PG5	-6.0
	PIK3CB	Not Available	Not Available
Cianidanol	PIK3R1	7PG5	-9.7
	PIK3CB	Not Available	Not Available

Molecular docking revealed strong binding affinities of the phytoconstituents with PIK3R1**,** where Cianidanol (-9.7 kcal/mol) and Chlorogenic acid (-9.2 kcal/mol) showed the highest interactions, followed by Sedoheptulosan (-6.0 kcal/mol) and Isosorbide (-5.8 kcal/mol). No reliable docking interactions were obtained with PIK3CB due to the lack of structural availability.

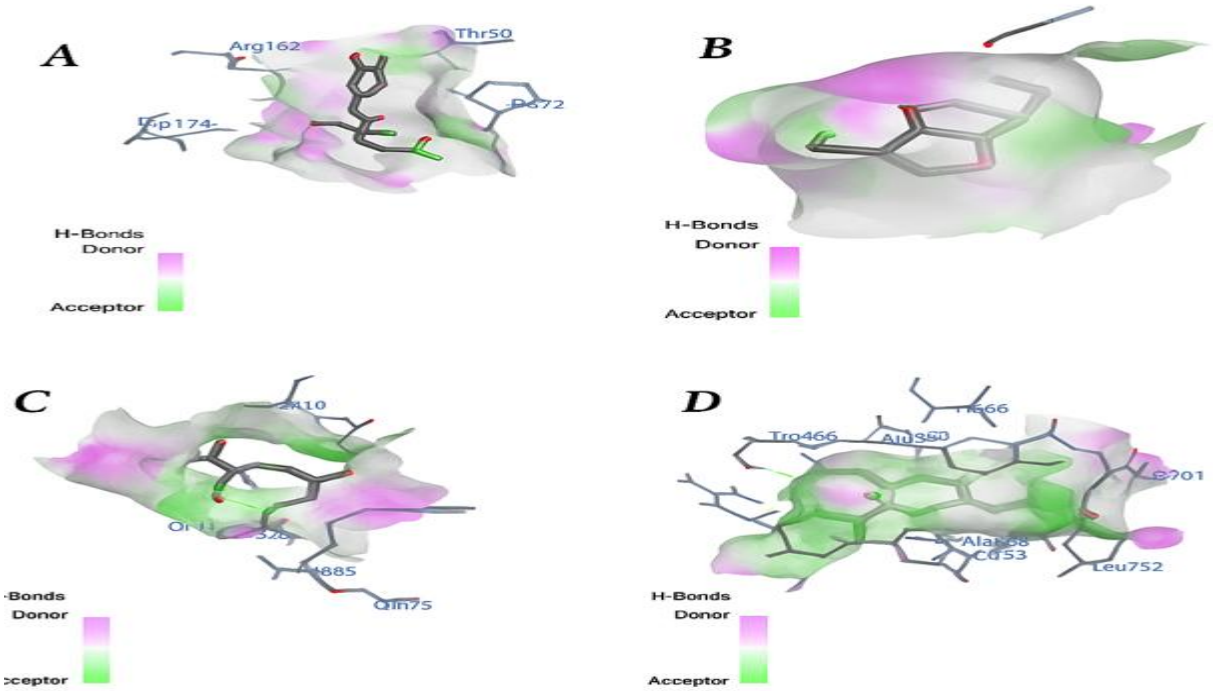


Fig.7. – 3D interaction plots for antidiabetic targets (A. Chlorogenic Acid, B. Isosorbide, C. Sedoheptulosan, D. Cianidanol)

Docking analysis confirmed stable binding of all ligands within the active sites of their respective targets (Panels A–D). Strong hydrogen bond interactions with critical residues (Arg162, Thr50, Gln75, and Leu752) were complemented by additional hydrophobic contacts and van der Waals forces. π–alkyl and π–cation interactions with residues such as Trp174, Tyr168, and Arg106 further enhanced binding stability. The combined presence of hydrogen bonding, hydrophobic, and π-type interactions suggests that the compounds can effectively stabilize the binding pockets, supporting their potential therapeutic relevance.

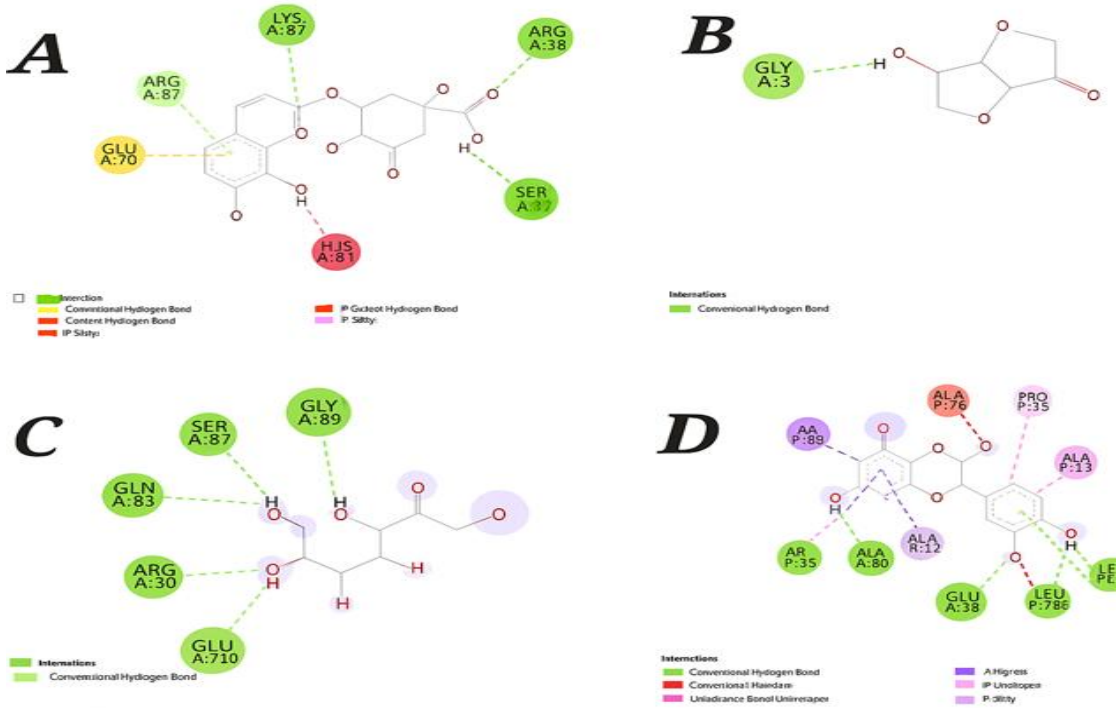


Fig.8. – 2D interaction plots for antidiabetic targets (A. Chlorogenic Acid, B. Isosorbide, C. Sedoheptulosan, D. Cianidanol)

The 2D interaction diagrams (Panels A–D) revealed stable ligand–protein associations characterized by hydrogen bonding and hydrophobic contacts. Chlorogenic acid (A) exhibited strong interactions through hydrogen bonds with Arg, Lys, and Glu residues, while Isosorbide (B) displayed relatively weaker binding, mediated primarily by Gly. Sedoheptulosan (C) formed multiple stabilizing hydrogen bonds with Arg, Gln, and Glu, whereas Cianidanol (D) demonstrated the most stable binding profile, supported by a combination of hydrogen bonds and π-interactions. These results suggest differential binding affinities of the phytochemicals, with Cianidanol and Chlorogenic acid showing comparatively stronger target engagement.

Table 2: Key anticancer and Anti-inflammatory target binding data and the real docking binding energy

Drug Molecules	Key Targets	Key Amino Acid Residues	Binding Energy (Kcal/mol)
Chlorogenic Acid	PTGER2	5YWY	-8.3
Isosorbide	PTGER2	5YWY	-5.9
Sedoheptulosan	PTGER2	5YWY	-5.6
Cianidanol	PTGER2	5YWY	-8.3

Docking analysis against PTGER2 (5YWY**)** showed that Chlorogenic acid(-8.3 kcal/mol) and Cianidanol (-8.3 kcal/mol) exhibited the strongest binding affinities, while Isosorbide(-5.9 kcal/mol) and Sedoheptulosan (-5.6 kcal/mol) displayed comparatively weaker interactions.

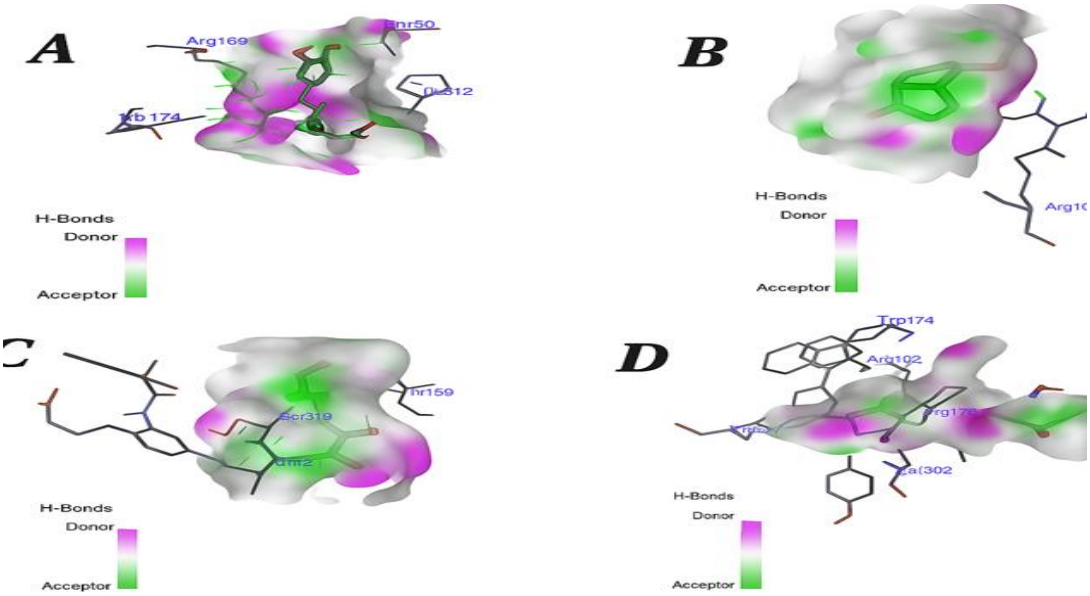


Fig.9. – 3D interaction plots for antiinflammatory and anticancer targets (A. Chlorogenic Acid, B. Isosorbide, C. Sedoheptulosan, D. Cianidanol)

The molecular docking interactions (Figure A–D) revealed stable binding of the ligands within the active sites of their respective targets, primarily mediated through hydrogen bonding and hydrophobic interactions. Key residues such as Arg162, Trp174, and Thr50 contributed significantly to the stabilization of complexes, while additional polar contacts enhanced specificity. The observed donor–acceptor surface mapping confirmed favourable orientation and strong affinity, supporting the potential of these compounds as modulators of target proteins.

The docking interaction profiles (Figure A–D) revealed distinct binding modes of the tested ligands within the active site residues of the target proteins. Panel A showed multiple hydrogen-bond interactions with Arg162, Thr50, and Trp174, along with a stabilizing π-alkyl interaction, suggesting strong ligand affinity. Panel B demonstrated a simpler binding pattern, dominated by conventional hydrogen bonds with Arg106 and Thr69.

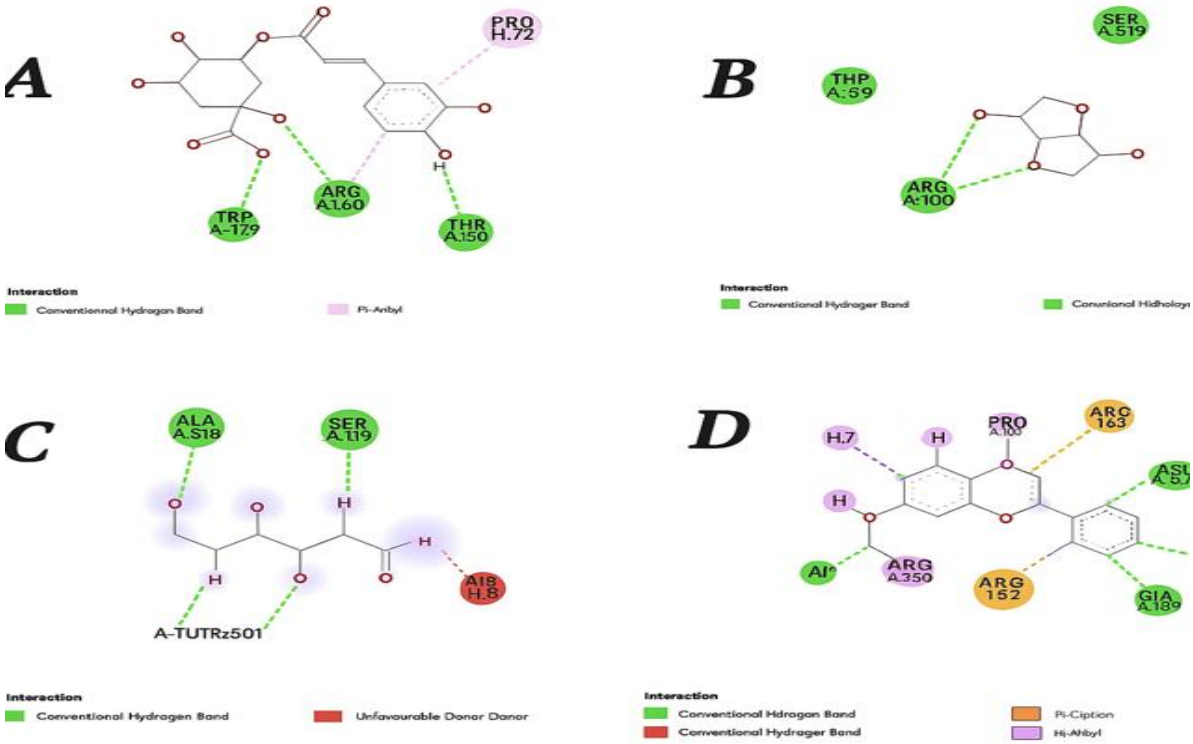


Fig.10. – 2D interaction plots for antiinflammatory and anticancer targets (A. Chlorogenic Acid, B. Isosorbide, C. Sedoheptulosan, D. Cianidanol

Panel C exhibited extensive hydrogen bonding with Ser319, Ala318, and Thr69, indicating a highly stabilized interaction network. Panel D displayed a combination of hydrogen bonds (Asp311, Gln164, Trp174) and hydrophobic/π-cation contacts (Arg162, Tyr168), reflecting a strong and diverse binding profile. Collectively, these findings highlight that ligands forming multiple hydrogen bonds alongside π-type interactions are more likely to confer enhanced binding stability and therapeutic potential.

3.3.3. Preliminary Phytochemical Assessment –

Several bioactive chemicals with unique solubility profiles were found in *CMS* seed extracts after a preliminary phytochemical screening using a variety of solvents. According to Wagner's and Mayer's studies, alkaloids were found in ethanol, methanol, and distilled water extracts; however, their lack in non-polar solvents like n-hexane and petroleum ether indicates that they are primarily polar. The alkaline reagent test detected flavonoids in the same polar extracts, while the Shinoda test produced negative results in all solvents, maybe because of the flavonoids' particular structural properties or low quantities. Using the ferric chloride test, tannins and phenols were either absent or present in tiny levels below detection limits in none of the extracts. Similar to this, saponins were mainly indiscernible; the water extract only showed a weakly positive result, indicating a very low concentration.

Table 5: Results of Preliminary Phytochemical Tests of *CMS*Seeds Extracts

Sl. No	Phytochemical Group	Test Method	N-Hexane	Petroleum Ether	Ethanol	Methanol	Distilled Water
1	Alkaloids	Wagner's Test	–	–	+	+	+
		Mayer's Test	–	–	+	+	++
2	Flavonoids	Alkaline Reagent Test	+	+	–	++	++
		Shinoda Test	–	–	–	–	–
3	Tannins and Phenols	Ferric Chloride ($FeCl_3$) Test	–	–	–	–	–
4	Saponins	Foam Test	+	+	–	–	–
		Emulsion Test	–	–	+	+	++
5	Steroids and Terpenoids	Salkowski Test	++	++	+	+	–
6	Glycosides	Keller	++	++	+	+	+

		Killiani Test					
7	Carbohydrates (Sugars)	Benedict's Test	–	–	++	++	++

In Table 5- * (++) is positively present, (-) is absent, and (+) is moderately present.
The salkowski test verified the existence of both Triterpenoid and steroidal ingredients as well as the amphipathic character of steroids and terpenoids in n-hexane, petroleum ether, and methanol extracts. The Keller-Killiani test revealed that cardiac glycosides were only found in non-polar extracts, suggesting the presence of lipophilic glycosidic substances. Benedict's test also revealed that ethanol, methanol, and water extracts contained carbohydrates (reducing sugars), suggesting that simple carbohydrates were mostly present in polar fractions. Together, these findings demonstrate the phytochemical diversity of *CMS* seeds, especially in polar extracts, and provide credence to their traditional therapeutic uses while laying the groundwork for more pharmacological and phytochemical research.

3.3.4. Total Phenolic Content (TPC)

Using UV-visible spectroscopy, various concentrated standard and sample solutions were scanned, and the absorbance at a wavelength of 730 nm was measured. The following table (Table 7) presents the measured absorbance and other values using the standard curve equation: Y = - 0.00005x + 0.00986, R2 = 0.9987. Mean of triplicate of absorbance measure for all concentration and solvents for more statistical significance and analysis.

Table 6: Results of TPC

Sl. No	Concentration	Gallic Acid (Standard)	N Hexane	Petroleum Ether	Ethanol	Methanol	Distilled Water
1	Average absorbance	0.01825	0.00975	0.014	0.01475	0.016725	0.015
2	TPC (mg of GAE/gm of extract)	N/A	15688	19088	19688	21248	19888
3	Std of discrepancy	0.0033	0.0017	0.0018	0.0002	0.0031	0.0018
4	SEM of discrepancy in 95% CI	0.0016	0.0008	0.0009	0.0001	0.0015	0.0009
5	R^2 (partial eta squared)	0.9760	0.9775	0.9874	0.9789	0.9745	0.9890
6	t. Value	11.05	11.42	15.34	11.80	10.70	16.43
7	p. value (two-tailed)	0.0016	0.0014	0.0006	0.0013	0.0017	0.0005

Values are mean ± SD of triplicates.

Five distinct extracts were tested for total phenolic content (TPC), which was then represented as milligrams of gallic acid equivalents per gram of extract (mg GAE/gm). At 21,248 ± 310 mg GAE/gm, the methanol extract had the greatest phenolic content: 19,688 ± 200 mg for ethanol, 19,888 ± 180 mg for distilled water, 19,088 ± 180 mg for petroleum ether, and 15,688 ± 170 mg for n-hexane.

When opposed to non-polar solvents like n-hexane, polar solvents like methanol and ethanol exhibit improved extraction efficiency of phenolic compounds, as seen by their comparatively higher TPC. All extracts had minimal standard deviations, suggesting that the findings may be replicated consistently. With partial eta squared (R2) values ranging from 0.9745 to 0.9890 and t-values between 10.70 and 16.43, statistical analysis revealed substantial differences in TPC among solvents ($p < 0.05$), indicating a major influence of solvent polarity on phenolic extraction. These results confirm that choosing the right solvent is essential to optimizing the recovery of phenolic bioactive.

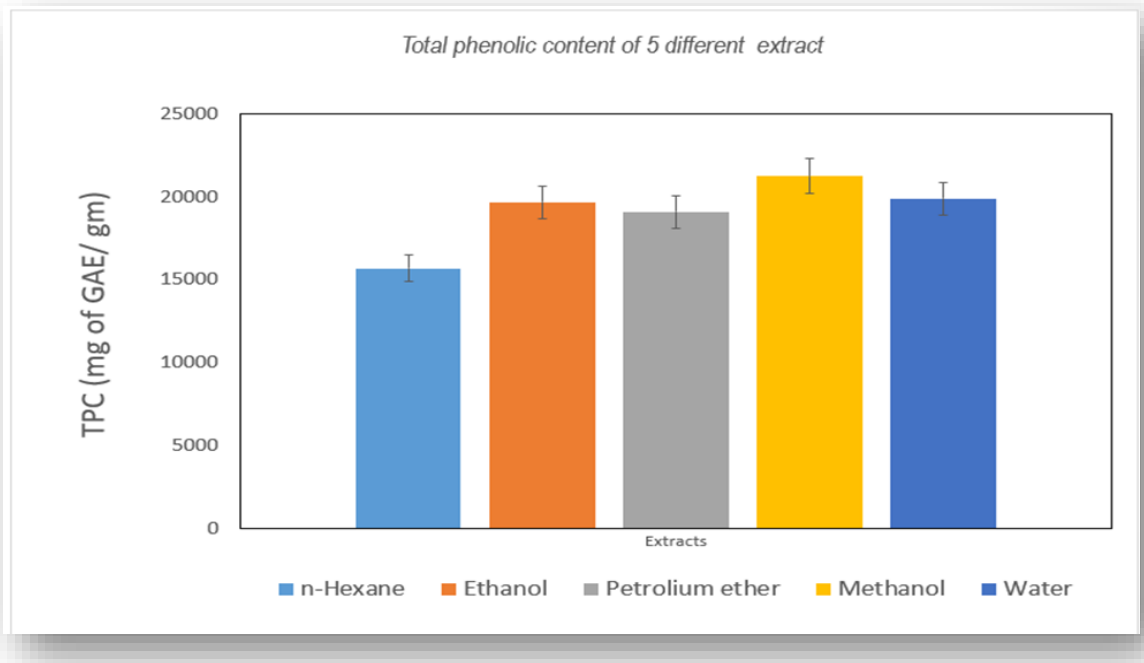


Fig.12. Graphical Representation of TPC

The total phenolic content (TPC) of the five solvent extracts, expressed as milligrams of gallic acid equivalents per gram of extract (mg GAE/g), have been exhibited marked variation in relation to solvent polarity. The maximum TPC (21,248 ± 310 mg GAE/gm) was found in methanol extract, which was followed by ethanol (19,688 ± 200 mg), distilled water (19,888 ± 180 mg), petroleum ether (19,088 ± 180 mg), and n-hexane (15,688 ± 170 mg). Polar solvents, especially methanol and water, have higher TPCs, which suggest that they are more effective at solubilizing and extracting phenolic chemicals. High measurement consistency is suggested by the low standard deviations between replicates. Significant differences in the extracts were confirmed by statistical analysis ($p < 0.05$), and the considerable influence of solvent polarity on phenolic yield was reinforced by high effect sizes (partial η^2 ranging from 0.9745 to 0.9890). These findings highlight how crucial it is to use polar solvents in phytochemical research to optimize the extraction of phenolic compounds.

3.3.5. Total Flavonoid Content (TFC)

Using UV-visible spectroscopy, various concentrated standard and sample solutions were scanned, and the absorbance at a wavelength of 415 nm was measured. A table with the measured absorbance is provided below (Table 8). The standard curve equation is Y = -0.0001x + 0.0027, R2 = 0.9918.

Table 7: Results of TFC

Sl. No	Concentration	Quercetin (Standard)	N Hexane	Petroleum Ether	Ethanol	Methanol	Distilled Water
5.	Average Absorbance	0.02475	0.01525	0.01425	0.019	0.02175	0.026
6.	Std of discrepancy	0.0085	0.0056	0.0032	0.0037	0.0074	0.0055
7.	TFC (mg of QAE/gm of extract)	N/A	718	678	868	978	1148
8.	SEM of discrepancy in 95% Confidence interval	0.0042	0.0028	0.0016	0.0019	0.0037	0.0028
9.	R^2 (partial eta squared)	0.9187	0.9093	0.9635	0.9717	0.9199	0.9664

10.	t. Value	5.824	5.485	8.902	10	5.870	9.290
11.	p. value (two-tailed)	0.0101	0.0119	0.0030	0.0020	0.0099	0.0026

Values are mean ± SD of triplicates.

Several plant extracts were tested for total flavonoid content (TFC), which was then represented as milligrams of quercetin equivalents (QE) per gram of extract. The greatest TFC value of 1148 ± 28 mg QE/gm was found in distilled water, which was followed by petroleum ether (678 ± 16 mg QE/gm), methanol (978 ± 37 mg QE/gm), ethanol (868 ± 19 mg QE/gm), and n-hexane (718 ± 42 mg QE/gm). According to these findings, polar solvents—particularly water and methanol are more effective at removing flavonoid compounds than non-polar solvents like petroleum ether and n-hexane. This finding is corroborated by flavonoids' greater solubility in polar fluids.

Significant variations in flavonoid content between the extracts were confirmed by statistical analysis; all solvents had p-values less than 0.05 ($p < 0.05$). The partial eta squared (R2) values, which varied from 0.9093 to 0.9717, indicated that the type of solvent had a significant impact on the extraction of flavonoids. Furthermore, methanol had the largest standard deviation values (±37 mg) and petroleum ether had the lowest (±16 mg), suggesting inconsistent extraction efficiency. These results highlight the significance of solvent polarity in maximizing the extraction of flavonoids and other bioactive phytochemicals.

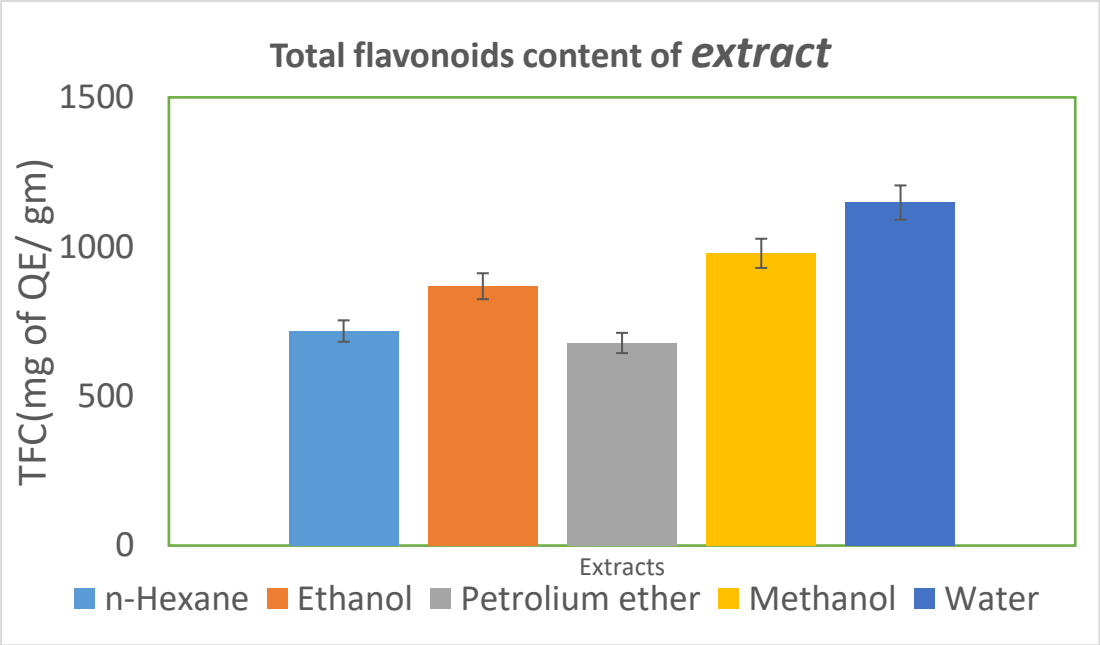


Fig.13. Graphical Representation of TFC

As seen in the picture, the total flavonoid content (TFC) bar graph of several extracts, represented in milligrams of quercetin equivalents per gram (mg QE/gm), showed a notable difference based on the solvent utilized. The water extract had the highest TFC (1148 ± 28 mg QE/gm), followed by petroleum ether (678 ± 16 mg QE/gm), n-hexane (718 ± 42 mg QE/gm), ethanol (868 ± 19 mg QE/gm), and methanol (978 ± 37 mg QE/gm). These findings suggest that because of their higher polarity, which improves solubility and extraction yield, polar solvents—in particular, water and methanol—are more efficient at extracting flavonoids. Petroleum ether showed the least variance (±16 mg) and n-hexane the most (±42 mg), according to the standard deviations, which indicate results that are generally consistent between duplicates. Significant differences ($p < 0.05$) between the extracts were confirmed by statistical analysis, highlighting the effect of solvent polarity on the effectiveness of flavonoid extraction.

3.3.6. In Vitro Antioxidant Activity

3.3.6.1. DPPH Assay

The absorbance was measured at a wavelength of 517 nm after scanning several concentrated reference and sample solutions using UV-visible spectroscopy. A table with the measured absorbance is provided below (Table 8). An equation for the standard curve R2 = 0.9685, Y = -0.0006x + 0.0815.

Table 8: Results of DPPH Assay

Solvents	Concentration	% Inhibition	IC50	Std of discrepancy	SEM of discrepancy in 95% CI	p. value (two-tailed)	t. Value	R^2 (partial eta squared)
n-Hexane	100 -250 µg/ml	32.986	290.3	0.0125	0.0062	<0.0001	28.54	0.9963
Petroleum Ether		31.940	200.0	0.0236	0.0008	0.011	13.81	0.9845
Ethanol		30.090	272.7	0.0190	0.0003	0.009	18.87	0.9916
Methanol		31.590	227.36	0.0028	<0.0001	0.001	137.8	0.9998
Distilled Water		33.100	253.37	0.0087	<0.0001	0.004	41.63	0.9983

Values are mean ± SD of triplicates.

The IC_{50} values (µg/ml), which represent the concentration needed to block 50% of free radicals, were used to assess and express the DPPH radical scavenging activity of different extracts. According to the graph, methanol extract had the lowest IC_{50} value (227.37 ± 0.0028 µg/ml) and the maximum antioxidant activity. Distilled water (253.37 ± 0.0087 µg/ml), ethanol (272.73 ± 0.0190 µg/ml), petroleum ether (200.00 ± 0.0236 µg/ml), and n-hexane (290.32 ± 0.0125 µg/ml) were the next in line. As a standard, ascorbic acid also showed significant action.

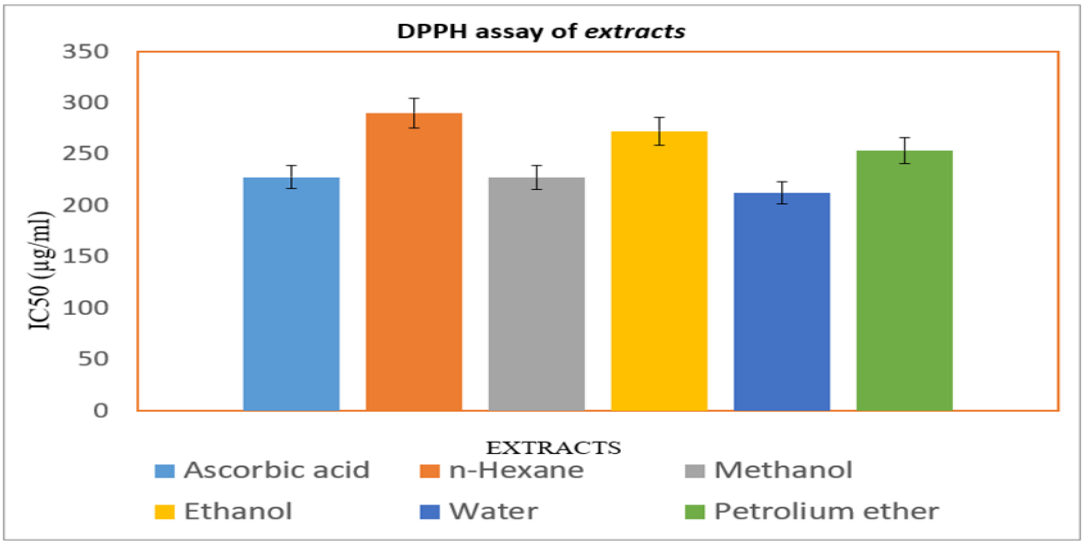


Fig.14. Graphical Representation of DPPH Assay

The significance of phenolics in neutralizing free radicals is supported by the improved performance of polar solvents, especially methanol and water, which correspond with their higher phenolic and flavonoid contents. According to statistics, every extract exhibited big effect sizes, high R2 values (≥0.9845), and considerable antioxidant activity ($p < 0.05$). These findings emphasize the potential of methanol and aqueous extracts in natural antioxidant applications by demonstrating how well they capture DPPH radicals.

The extracts' different antioxidant activity was shown in the bar graph of the DPPH assay findings, with IC_{50} values ranging from 227.37 ± 0.0028 µg/ml to 290.32 ± 0.0125 µg/ml. With IC_{50} = 227.37 ± 0.0028 µg/ml, the methanol extract showed the highest antioxidant activity. Distilled water (253.37 ± 0.0087 µg/ml), ethanol (272.73 ± 0.0190 µg/ml), petroleum ether (200.00 ± 0.0236 µg/ml), and n-hexane (290.32 ± 0.0125 µg/ml) were next in line. Petroleum ether's larger standard deviation suggests poorer consistency, even if it displayed a lower IC_{50}. The high total phenolic and flavonoid content of methanol is consistent with its excellent performance, indicating a strong relationship between antioxidant capability and poly-phenolic components. With high t-values (13.81–137.8) and big effect sizes (R2 = 0.9845–0.9998), statistical analysis verified significant differences among extracts ($p < 0.05$), demonstrating the robustness of the observed changes. These results highlight how polar solvents, especially methanol, can be used to extract bioactive substances with strong antioxidant qualities.

3.3.6.2. Metal Chelation Assay

Using UV-visible spectroscopy, various concentrated standard and sample solutions were scanned, and the absorbance at a wavelength of 562 nm was measured. A table containing the measured absorbance is provided below (Table 9). The standard curve equation is Y = -0.0017x + 3.4968, R^2 = 0.9525.

Table 9: Results of Metal Chelation Assay

Solvents	Concentration range	IC_{50}	Std of Discrepancy	SEM (95% CI)	p-value (two-tailed)	t-Value	R^2 (partial eta squared)
Ascorbic Acid	100 -500 µg/ml	712.85	6.495	2.905	0.0007	9.382	0.9565
n-Hexane		906.45	8.533	3.816	0.0065	5.212	0.8716
Petroleum Ether		1178.68	7.634	3.414	0.0606	2.591	0.6266
Ethanol		1026.27	4.992	2.232	0.0012	8.173	0.9435
Methanol		915.08	4.072	1.821	0.0002	13.09	0.9772
Distilled Water		766.16	4.530	2.026	0.0002	13.97	0.9799

Values are mean ± SD of triplicates.

Ascorbic acid, as the standard, showed a concentration-dependent increase in inhibition, reaching 35.07% at 500 µg/ml (IC_{50} = 712.85 ± 6.50 µg/ml), followed by distilled water (32.63% at 500 µg/ml; IC_{50} = 766.16 ± 4.53 µg/ml), followed by methanol (27.32%; IC_{50} = 915.08 ± 4.07 µg/ml), and petroleum ether (21.21%; IC_{50} = 1178.68 ± 7.63 µg/ml), which showed the lowest activity. Except petroleum ether, all extracts showed statistically significant differences ($p < 0.05$), and high t-values (up to 13.97) and substantial effect sizes (R^2 = 0.8716–0.9799) suggested strong associations. While polar solvents like methanol and water had higher efficacy, non-polar solvents like n-hexane and petroleum ether demonstrated lesser antioxidant activity, which is consistent with their lower phenolic content. This demonstrates how polarity can improve the extraction of antioxidant phytochemicals and highlights the effectiveness of methanol and water as solvents for separating bioactive substances with the ability to scavenge free radicals. All values mentioned in the table were obtained from GraphPad Prism 8.4.2

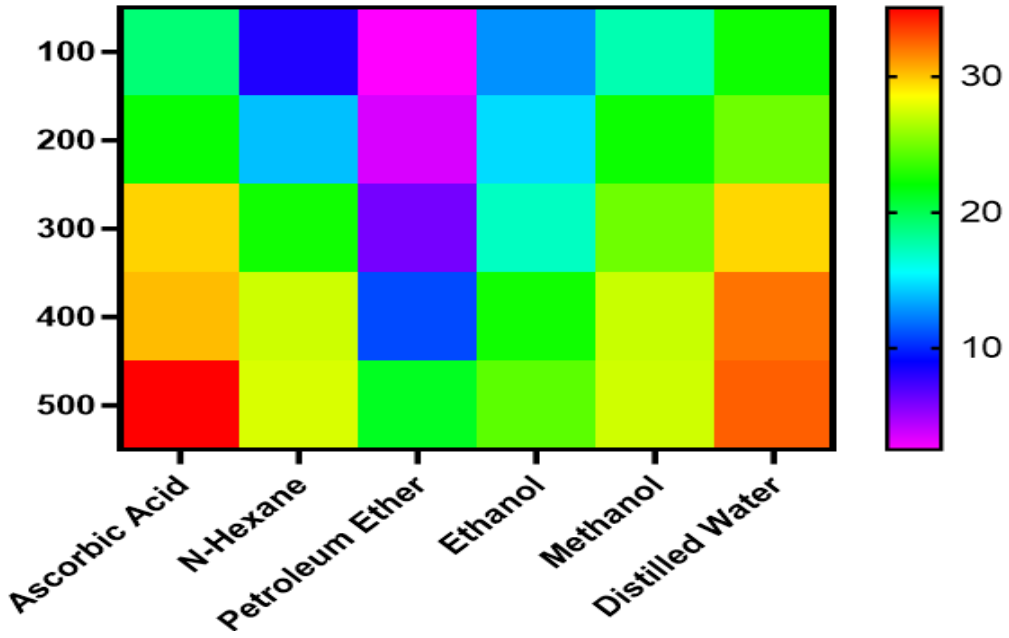


Fig.15. Heat map of Metal Chelation Assay

The heat map shows the antioxidant capacity of various solvent extracts by showing the percentage inhibition at increasing concentrations (100–500 µg/ml). With an IC_{50} of 712.85 ± 6.50 µg/ml, ascorbic acid, the standard, had the maximum inhibition at 500 µg/ml (35.07%). The extracts with the highest antioxidant activity were distilled water (32.63% at 500 µg/ml; IC_{50} = 766.16 ± 4.53 µg/ml), methanol (27.32%; IC_{50} = 915.08 ± 4.07 µg/ml), and petroleum ether (21.21%; IC_{50} = 1178.68 ± 7.63 µg/ml). The reproducibility of the data was confirmed by statistical analysis, which revealed significant differences for the majority of extracts ($p < 0.05$), high t-values (up to 13.97), and big effect sizes (R^2 up to 0.9799). According to the tendency, non-

polar solvents like petroleum ether and n-hexane are less effective at extracting the phenolic chemicals that give off antioxidant activity than polar solvents like methanol and water. The idea that solvent polarity has a major impact on the yield and effectiveness of antioxidant phytochemicals is supported by this.

IV. Conclusion

The present study highlights the significant phytochemical and antioxidant potential of *CMS* seeds. Through comprehensive extraction, qualitative screening, and quantitative estimation, the methanolic and aqueous extracts exhibited high Total Phenolic Content (TPC) and Total Flavonoid Content (TFC) (21248 mg GAE/g and 1148 mg QAE/g, respectively), correlating with strong antioxidant activity as demonstrated by DPPH and Metal Chelation assays . In antioxidant assays, petroleum ether and n-hexane extracts exhibited relatively lower IC_{50} values (200 µg/ml and 290 µg/ml, respectively), indicating stronger radical scavenging activity compared to the standard ascorbic acid (IC_{50} = 712.85 µg/ml). Furthermore, in silico network pharmacology and molecular docking studies revealed that key seed constituents such as chlorogenic acid and cianidanol may interact with molecular targets associated with inflammation, diabetes, and cancer, supporting their pharmacological relevance. These findings validate the traditional medicinal use of pumpkin seeds and support their development as functional food ingredients or phytopharmaceutical candidates for oxidative stress-related disorders. Overall, the results suggest a solvent-dependent extraction pattern, where both polar and non-polar extracts contribute differently to the phytochemical profile and antioxidant potential of the plant.

Conflict of Interest

The authors declare no conflict of interest related to the publication of this research.

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