

Pharmacognostic, Phytochemical, GC-MS and *In-silico* Pharmacological Evaluation of *Cleome gynandra* L. Flowers

Gowthaman P.¹; Dr. R. Thiruvengatasubramaniam²; Dr. B. Sangameswaran²

¹Postgraduate Research Scholar, Department of Pharmacognosy, SSM College of Pharmacy, Jambai, Erode, Tamil Nadu, India

²SSM College of Pharmacy, the Tamil Nadu Dr.M.G.R. Medical University, Jambai, Erode, Tamil Nadu, India

Publication Date: 2026/10/01

Abstract: The present study was undertaken to investigate the pharmacognostic, physicochemical, phytochemical, GC–MS and *in-silico* pharmacological profiles of *Cleome gynandra* L. flowers. The flowers were authenticated and subjected to macroscopic and powder microscopic evaluation, which revealed characteristic diagnostic features including tetramerous flowers, prominent stamens, multicellular trichomes, anther tissues, xylem vessels, astrosclereids, calcium oxalate crystals, pollen grains and oil droplets. Physicochemical evaluation revealed acceptable ash, moisture, foaming, and extractive values. These parameters support the suitability of the flowers for quality assessment and standardization. Successive Soxhlet extraction with solvents of increasing polarity showed a comparatively higher extractive response with polar solvents, suggesting the predominance of polar and water-soluble constituents. Preliminary phytochemical screening revealed the presence of diverse classes of primary and secondary metabolites, including alkaloids, glycosides, flavonoids, tannins, carbohydrates and proteins, with variations observed among the different extracts. GC–MS analysis of the aqueous extract identified Stigmasterol, β -sitosterol and a Lupeol derivative, which were selected for further *in-silico* studies. ADME profiling provided preliminary information on their drug-likeness and pharmacokinetic properties. Molecular docking against antioxidant-related targets, NOX (PDB: 2CDU) and SOD1 (PDB: 2C9V), showed favourable predicted binding interactions, with Lupeol showing the strongest predicted affinity. Similarly, docking against antidiabetic related targets, α -amylase (PDB: 1OSE) and α -glucosidase (PDB: 1UOK) demonstrated favourable interactions, particularly for Lupeol and β -sitosterol. Overall, Lupeol emerged as the most promising candidate among the investigated phytoconstituents. These findings provide preliminary *In-silico* evidence supporting the potential pharmacological relevance of *Cleome gynandra* L. flowers and warrant further experimental validation through *in-vitro* and *in-vivo* Studies.

Keywords: *Cleome gynandra* L. Pharmacognosy, Phytochemistry, GC-MS, *In-silico* Pharmacology, Molecular Docking.

How to Cite: Gowthaman P.; Dr. R. Thiruvengatasubramaniam; Dr. B. Sangameswaran (2026) Pharmacognostic, Phytochemical, GC-MS and *In-silico* Pharmacological Evaluation of *Cleome gynandra* L. Flowers. *International Journal of Innovative Science and Research Technology*, 11 (9), 2261-2277. <https://doi.org/10.38124/ijisrt/26sep1054>

I. INTRODUCTION

Herbs and herbal medicines have long been recognized as important sources of therapeutic agents and continue to play a significant role in traditional and modern healthcare systems. Herbal materials may be used as whole plants or specific plant parts and are commonly prepared as powders, extracts, decoctions, infusions, tinctures, oils, and other formulations. Their medicinal value is largely associated with the diverse phytoconstituents present in plant materials. Appropriate identification, preparation, quality evaluation, and scientific validation are therefore essential for their effective therapeutic application [1–3].

Oxidative stress occurs when the generation of reactive oxygen species (ROS) exceeds the protective capacity of endogenous antioxidant mechanisms, resulting in oxidative damage to cellular macromolecules [4-7]. Antioxidants can neutralize free radicals and retard oxidative reactions, thereby contributing to protection against oxidative damage. Plants are valuable sources of natural antioxidants, particularly phenolic compounds and other secondary metabolites. The antioxidant potential of herbal materials can be evaluated using several *in-vitro* methods, including DPPH, ABTS, FRAP, nitric oxide scavenging, reducing power, and other complementary assays [8-10,32]. Previous studies have also reported antioxidant potential in *Cleome gynandra* L. leaves [22,31].

Diabetes mellitus is a chronic metabolic disorder characterized by persistent hyperglycaemia resulting from impaired insulin secretion, impaired insulin action, or a combination of both. Medicinal plants have attracted considerable attention as potential sources of antidiabetic agents because their phytochemicals may influence glucose metabolism through multiple mechanisms, including inhibition of carbohydrate-digesting enzymes and modulation of oxidative stress-related pathways. Inhibition of carbohydrate-hydrolysing enzymes such as α -amylase and α -glucosidase is an established approach for reducing the rapid release and absorption of glucose following carbohydrate consumption [11-13,34]. Previous investigations have also reported antidiabetic activity associated with *Cleome gynandra* L. [33].

Cleome gynandra L., belonging to the family Cleomaceae, is a traditionally utilized leafy vegetable and medicinal plant distributed predominantly in tropical and subtropical regions. Its occurrence, taxonomy, morphology, traditional utilization, phytochemical composition, and pharmacological properties have been documented in several studies [17,19,23,24,27,28]. Different parts of *Cleome gynandra* L. have been investigated for various biological activities, including antioxidant, anti-inflammatory, antimicrobial, analgesic, hepatoprotective, and antidiabetic effects [27-31,33,35-38]. These reports indicate the pharmacological relevance of the species and provide a scientific basis for further systematic investigation.

Gas chromatography–Mass spectrometry (GC-MS) is a valuable analytical technique for the qualitative characterization and identification of volatile and semi-volatile phytoconstituents present in herbal extracts. GC-MS profiling can provide useful chemical information and assist in the identification and selection of potentially bioactive constituents for subsequent pharmacological investigations. Preliminary phytochemical screening further provides important information regarding the major classes of secondary metabolites present in plant extracts and can support the interpretation of their biological activities [40,41].

In-silico approaches, particularly molecular docking, provide useful computational methods for investigating the possible interaction of phytoconstituents with selected biological targets. Molecular docking can assist in predicting binding orientations, interaction patterns, and relative binding affinities of selected compounds and may therefore support target-based screening and mechanistic investigations [14-16]. Computational assessment of antioxidant-related phytochemicals and their interactions with relevant molecular targets has also been reported in recent studies [42]. Similarly, the integration of in-vitro antidiabetic evaluation with molecular docking has been employed to investigate the possible molecular interactions of plant-derived constituents with therapeutic targets [44].

➤ Plant Profile

Cleome gynandra L. is commonly known as dog mustard, spider plant and is a widely distributed herb of tropical and subtropical regions. In India, it occurs in areas including Tamil Nadu, Andhra Pradesh, Maharashtra, Uttar Pradesh and West Bengal, and is commonly found in wastelands, roadsides and cultivated fields. The plant is adapted to warm climates and well-drained soils. The flowers are complete, bisexual and tetramerous, with four sepals, four petals, six prominently exerted stamens and a superior monocarpellary ovary elevated by a gynophore; these floral characteristics are consistent with the botanical descriptions reported for the species [18-26]. The flowers were the selected plant material for the present study in Fig.1.

➤ Taxonomical Classification

Kingdom: Plantae;

Clade: Tracheophytes → Angiosperms → Eudicots;

Order: Brassicales;

Family: Cleomaceae;

Genus: *Cleome*;

Species: *Cleome gynandra* L.



Fig 1 *Cleome gynandra* L. Whole Plant

➤ Aim

The present study aimed to establish pharmacognostic, physicochemical standards for *Cleome gynandra* L. flowers, identify phytochemical constituents using GC-MS analysis and evaluate selected phytoconstituents through ADME prediction and Molecular docking against antioxidant and antidiabetic molecular targets.

II. MATERIALS AND METHODS

➤ Identification, Collection and Authentication

The plant material was collected from Athanur and Vennandur, Rasipuram, Namakkal District, Tamil Nadu, India, during January 2026. The botanical identity was authenticated by Dr. P. Radha, Research Officer, Central Council for Research in Siddha (CCRS), Ministry of AYUSH, Government of India, Mettur, Certificate code: C130126240G on 13/01/2026. The authenticated Botanical name was recorded as *Cleome gynandra* L., family- Cleomaceae.

➤ Pharmacognostical Evaluation

Fresh flowers were examined for organoleptic and macroscopic characters including colour, odour, taste, size, shape and floral type. The external features were documented using a digital camera and stereomicroscope. For powder microscopy, shade-dried flowers were powdered, passed through sieve No. 60 and examined using glycerin and staining reagents including safranin, iodine and Sudan III. The prepared slides were observed under a trinocular microscope (Magnus MX21 iLED) attached with a Magnus Pro 3.7 digital camera under bright field light. Diagnostic photomicrographs were captured and documented at 100x and 400x magnifications. Pharmacognostical observations were interpreted with reference to published descriptions of *Cleome gynandra* L. [22,26,27].

➤ Physicochemical Evaluation

Physicochemical constants were determined using reported pharmacopoeial procedures [1,3,25]. Total ash, acid-insoluble ash, water-soluble ash, sulphated ash, loss on drying, foaming index and extractive values were evaluated. For alcohol- and water-soluble extractives, 5 g of powdered material was macerated with 100 mL solvent for 24 h, followed by filtration and evaporation of an aliquot to constant weight. The foaming index was determined from the persistent foam produced by aqueous decoction.

➤ Successive Extraction

Shade-dried and coarsely powdered flowers (50 g) were subjected to successive Soxhlet extraction using solvents of increasing polarity: petroleum ether, chloroform, ethyl acetate, ethanol and water. The percentage yield, colour and consistency of each extract were recorded and the extracts were subjected to preliminary phytochemical screening. The use of successive solvent extraction was consistent with the solvent-screening approach described in the dissertation bibliography [39].

➤ Preliminary Phytochemical Screening

The extracts were screened qualitatively for alkaloids, glycosides, cardiac glycosides, flavonoids, carbohydrates, tannins/phenolic compounds and proteins using standard colour and precipitation reactions. The observations were recorded as positive (+) or negative (–) reactions. The qualitative screening procedures were based on standard preliminary phytochemical testing methodology [40].

➤ GC-MS Analysis

The aqueous extract was subjected to Gas chromatography–Mass spectrometry (GC–MS) analysis for the separation and identification of its volatile and semi-volatile phytochemical constituents. The obtained chromatogram was evaluated based on the retention time, peak area, peak height, molecular mass, and mass spectral fragmentation pattern of the detected compounds. The compounds were identified by comparing their mass spectra with those available in standard spectral databases. The GC–MS profiling revealed the presence of several phytochemical constituents, among which the major and biologically relevant compounds were selected for further *in-silico* investigation. The identified compounds were considered potential bioactive constituents that may contribute to the antioxidant and antidiabetic properties of the aqueous extract. Accordingly, the selected compounds were subjected to molecular docking studies against appropriate protein targets associated with antioxidant and antidiabetic activities. This approach was employed to predict the binding interactions, binding affinity, and possible molecular mechanisms of the identified phytoconstituents with the respective target proteins.

The GC–MS analysis was performed using a Gas Chromatography–Mass Spectrometry (GC–MS) system equipped with an HP-5MS capillary column (30 m × 0.25 mm i.d., 0.25 µm film thickness). Helium (99.99% purity) was used as the carrier gas at a constant flow rate of 1.0 mL/min. The sample was injected at a volume of 1 µL in split mode with a split ratio of 10:1, and the injector temperature was maintained at 250 °C. The oven temperature was initially set at 60 °C and held for 2 min, followed by a temperature increase at a rate of 10 °C/min to 280 °C, which was subsequently held for 10 min. The mass spectrometer was operated in electron ionization (EI) mode at an ionization energy of 70 eV. The ion source temperature was maintained at 230 °C, and mass spectra were acquired over a scan range of m/z 40–600. The obtained mass spectra were used for the identification of the detected compounds by comparison with appropriate spectral libraries and/or authentic reference standards, where applicable. [41].

➤ In-silico Pharmacological Studies

In-silico antioxidant docking, Apocynin and Quercetin were used as reference compounds and NOX (PDB: 2CDU) and SOD1 (PDB: 2C9V) were selected as target proteins. *In-silico* antidiabetic docking, Acarbose and Metformin were used as reference compounds and α -amylase (PDB: 1OSE) and α -glucosidase (PDB: 1UOK) were selected as targets. The phytoconstituents identified through GC–MS analysis were selected for subsequent *in-silico* evaluation of their

potential antioxidant and antidiabetic activities. The computational workflow was based on the molecular-docking methodology described in the project references [14-16]. The antioxidant target selection and interpretation were supported by the project references on NADPH oxidase inhibition and SOD1-related quercetin interactions, while the antidiabetic docking rationale was supported by the cited *in-silico* antidiabetic study [42-44].

Docking-compatible protein and ligand structures were prepared by removing unwanted molecules, adding hydrogens and assigning charges. The active site was defined using the co-crystallized ligand or reported active-site information. Multiple poses were generated and evaluated using docking score and binding interactions. The pose with the lowest predicted binding energy and favourable interactions was considered the best-scoring conformation. Lower docking energy was interpreted as stronger predicted binding affinity, consistent with the general molecular-docking [14-16].

ADME profiling included molecular weight, lipophilicity, water solubility, gastrointestinal absorption, blood-brain barrier permeability and Lipinski's Rule of Five. The Rule of Five parameters recorded in the dissertation included hydrogen-bond donors, hydrogen-bond acceptors, rotatable bonds and Lipinski violation status. The computational drug-discovery context was based on the *in-silico* pharmacology and molecular docking [14-16].

➤ Software and Tools Used

Various computational tools and software platforms were employed for ligand preparation, protein structure

retrieval, molecular docking, structural visualization, molecular interaction analysis, and pharmacokinetic evaluation. ChemSketch was used for ligand structure drawing and SMILES generation. The three-dimensional structures of the target proteins were retrieved from the Protein Data Bank (PDB). UCSF Chimera 1.18 was utilized for protein structure preparation and molecular docking studies. PyMOL was employed for three-dimensional visualization and structural inspection of the protein–ligand complexes, while ChimeraX was used to visualize and analyze hydrogen-bond interactions. BIOVIA Discovery Studio Visualizer 2025 was used for detailed two-dimensional and three-dimensional analysis of protein–ligand interactions. The drug-likeness and physicochemical properties of the selected ligands were predicted using SwissADME, whereas ADMETlab 3.0 was employed for comprehensive prediction of absorption, distribution, metabolism, excretion, and toxicity (ADMET) properties.

III. RESULTS AND DISCUSSIONS

➤ Macroscopic and Powder Microscopic Evaluation

The flowers were loosely arranged on elongated terminal racemes. The flowers were complete, bisexual and pedicellate, with four green narrow sepals and four white petals arranged in a cross-like manner. The androecium consisted of six long, prominently exerted stamens with a distinct androphore. The gynoecium consisted of a superior monocarpellary ovary with numerous ovules and a distinct gynophore, giving the flower a characteristic gynandrophore. These are all the diagnostic botanical characters *Cleome gynandra* L. flowers in Fig.2.

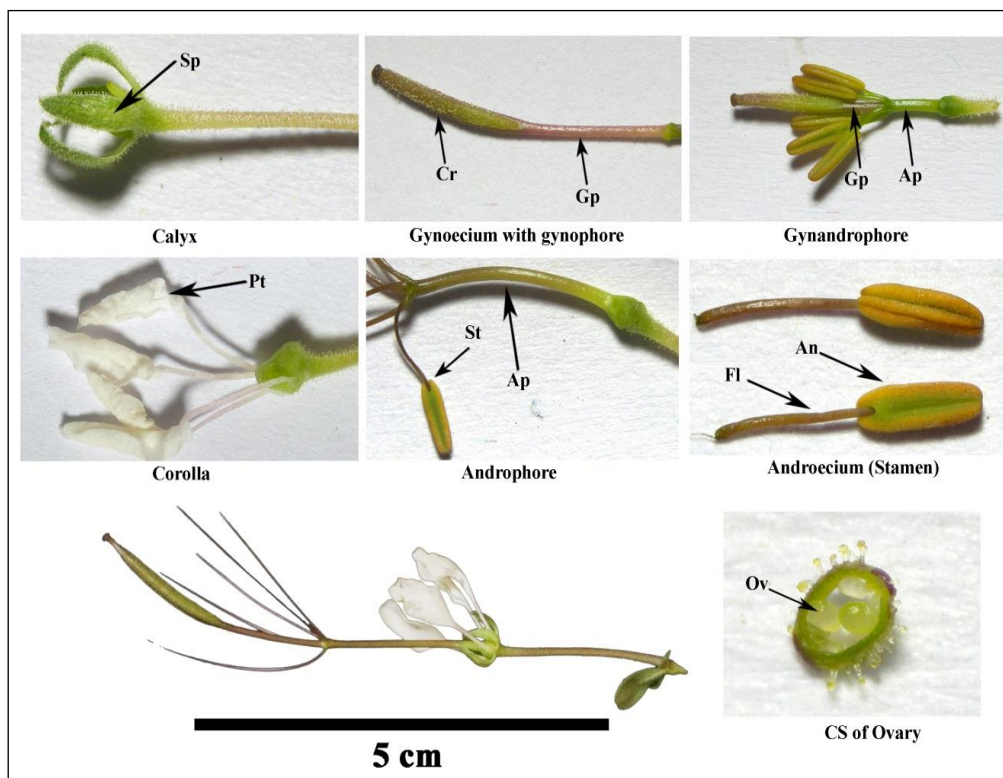


Fig 2 Floral Parts of *Cleome gynandra* L.

(Sp: Sepal, Pt: Petal, St: Stamen, Ap: Androphore, Cr: Carpel, Gp: Gynophore, An: Anther, Fl: Filament, Ov: Ovule)

Powder microscopy revealed diagnostic fragments of anther wall layers, a chlorenchyma layer of the gynoecium, the tip of anther connective tissue with central vascular

strands, multicellular globular trichomes, xylem vessels with annular and spiral thickening, astrosclereids with radiating arms, calcium oxalate crystals, oil droplets, pollen grains and dicotyledonous embryo. These features can serve as useful microscopic markers for identification and standardization of the crude drug in Fig.3.

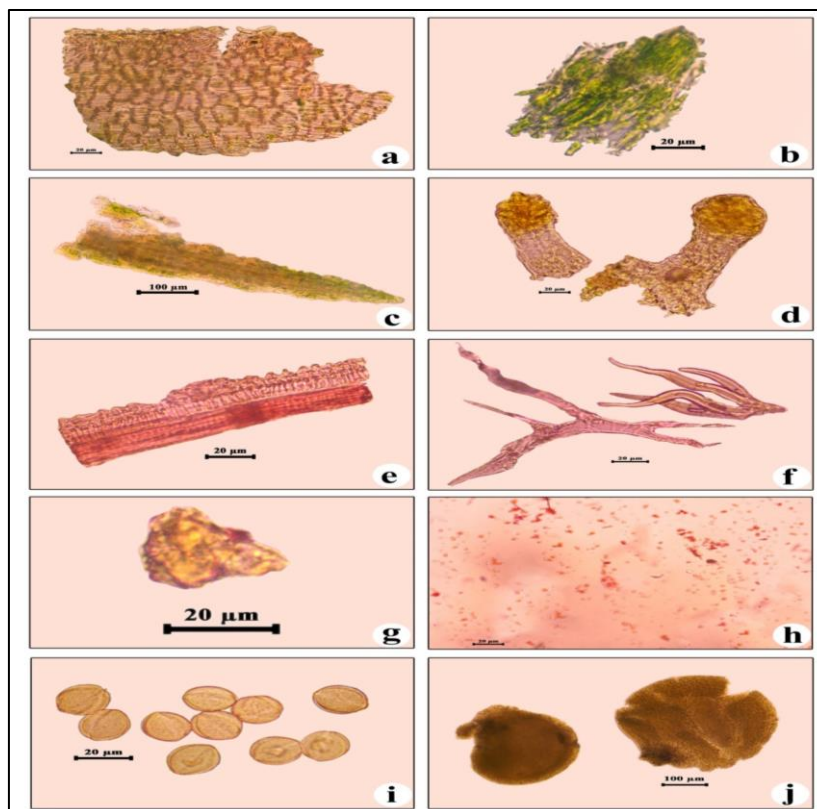


Fig 3 Powder Microscopy of *Cleome gynandra* L., Flower:

a. Fragment of Anther wall layers, b. Fragment of Chlorenchyma layer of gynoecium, c. Tip of Anther connective, d. Multicellular globular trichome, e. Bundle of xylem strands, f. Astrosclereids, g. Calcium oxalate crystal, h. Oil droplets, i. Pollen grains.

➤ Physicochemical Parameters

The physicochemical evaluation generated reproducible preliminary quality-control parameters for the

Cleome gynandra L. flower powder. The determined physicochemical characteristics provided valuable information regarding the quality, purity, identity, and consistency of the powdered plant material. These parameters are useful for assessing the suitability of the crude drug for further extraction and phytochemical investigations and may serve as important baseline data for the standardization and quality control of *Cleome gynandra* L. flower powder in Table.1.

Table 1 The Percentage of Physiochemical Characters of *Cleome gynandra* L. Flowers

S. No.	Physicochemical parameter	Indication / Purpose	Observed value
1	Total ash	Total inorganic matter	6.9% w/w
2	Acid-insoluble ash	Siliceous matter, sand, and earthy dirt	2.3% w/w
3	Water-soluble ash	Water-soluble minerals & inorganic salts	4.6% w/w
4	Sulphated ash	Free oxides converted to stable metal sulphates	7.3% w/w
5	Loss on drying	Moisture content and volatile matter	9.03% w/w
6	Foaming index	Saponin contents	100
7	Water-soluble extractive	Water-soluble phytoconstituents (glycosides, tannins, mucilage)	9.8% w/w
8	Ethanol-soluble extractive	Alcohol-soluble phytoconstituents (alkaloids, resins, flavonoids)	11% w/w

➤ Successive Extractive Values

Successive Soxhlet extraction showed a marked difference in extractive yield among solvents in Fig.4. Water produced the highest yield. The comparatively high aqueous

yield indicates substantial extraction of polar and water-soluble constituents from the *Cleome gynandra* L. flower powder in Table.2.

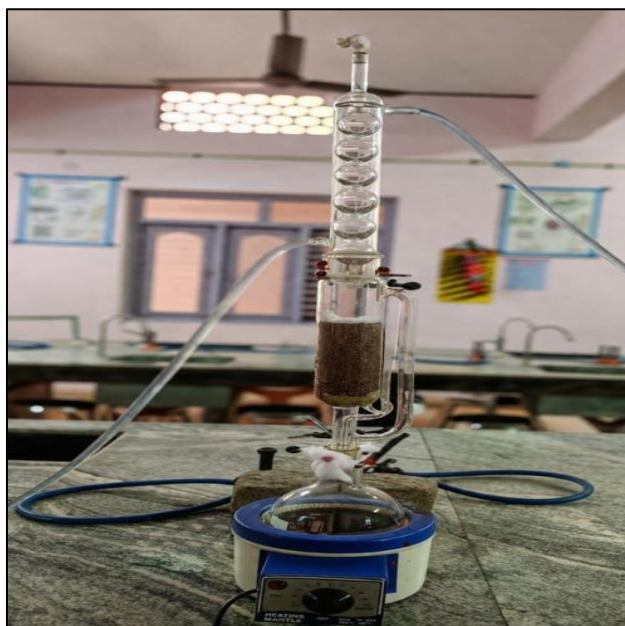


Fig 4 Soxhlet Extraction

Table 2 Successive Extractive Values of *Cleome gynandra* L. Flowers

S. No.	Extract	Nature and Colour	Percentage yield
1	Petroleum ether	Greenish brown, semisolid	6.06%
2	Chloroform	Greenish brown, semisolid	2.09%
3	Ethyl acetate	Greenish brown, semisolid	2.52%
4	Ethanol	Greenish brown, semisolid	8.90%
5	Water	Greenish brown, semisolid	26.06%

➤ Preliminary Phytochemical Screening

Qualitative screening showed that alkaloids were detected by all four alkaloid tests in all five extracts. Flavonoids were consistently positive in the petroleum ether, chloroform, ethyl acetate, ethanol and water extracts by Shinoda and alkaline reagent tests. Glycosides and cardiac glycosides showed solvent-dependent reactions. Carbohydrate, tannin/phenolic and protein reactions also varied among the extracts, demonstrating that the solvent system influenced the phytochemical profile. The water extract exhibited a broad phytochemical profile, showing positive reactions in 21 out of the 24 qualitative tests

performed. Notably, it showed positive reactions for alkaloids, glycosides, several cardiac glycoside tests, flavonoids, carbohydrates, tannins and proteins. The chloroform extract also showed 21 positive reactions, although the pattern of detected constituents differed between the two extracts. These findings demonstrate that the extraction solvent markedly influenced the qualitative phytochemical profile of *Cleome gynandra* L. flowers. The observed phytochemical diversity is broadly consistent with the reported phytochemical composition of *Cleome gynandra* L. in Table.3.

Table 3 Phytochemical Screening of the Extracts of *Cleome gynandra* L. Flowers

S.No.	Phytochemical group / test	Petroleum ether	Chloroform	Ethyl acetate	Ethanol	Water
1.	Alkaloids – Dragendorff	+	+	+	+	+
2.	Alkaloids – Mayer	+	+	+	+	+
3.	Alkaloids – Wagner	+	+	+	+	+
4.	Alkaloids – Hager	+	+	+	+	+
5.	Glycosides – Borntrager	–	+	+	+	+
6.	Modified Borntrager	–	+	–	–	+
7.	Cardiac glycosides – Keller-Kiliani	+	+	–	–	+
8.	Cardiac glycosides – Legal	–	+	–	+	+
9.	Cardiac glycosides – Raymond	–	+	–	–	+
10.	Cardiac glycosides – Baljet	–	+	–	–	–
11.	Cardiac glycosides – Kedde	+	+	–	–	+
12.	Flavonoids – Shinoda	+	+	+	+	+
13.	Flavonoids – Alkaline reagent	+	+	+	+	+
14.	Flavonoids – Zinc-HCl	+	+	–	+	–

15.	Carbohydrates – Molisch	+	+	–	+	+
16.	Carbohydrates – Barfoed	–	–	–	–	–
17.	Carbohydrates – Seliwanoff	–	+	+	+	+
18.	Carbohydrates – Pentose	–	+	+	+	+
19.	Carbohydrates – Fehling's	+	+	–	–	+
20.	Tannins – Ferric chloride	–	–	–	+	+
21.	Tannins – Phenazone	–	–	–	+	+
22.	Tannins – Gelatin	–	+	–	+	+
23.	Proteins – Biuret	–	+	+	–	+
24.	Proteins – Xanthoprotein	–	+	+	+	+

Passes the test = +

Fails the test = -

➤ GC-MS Profiling

GC-MS analysis of the aqueous extract produced 42 identified chromatographic peaks in Fig.5. The profile included low-molecular-weight oxygenated and nitrogen-containing compounds as well as sterols and triterpenoid

derivatives. Among the identified constituents, Stigmasterol, β -sitosterol and Lupeol were considered important for subsequent computational evaluation like *In-silico* pharmacological evaluation in Table.4.

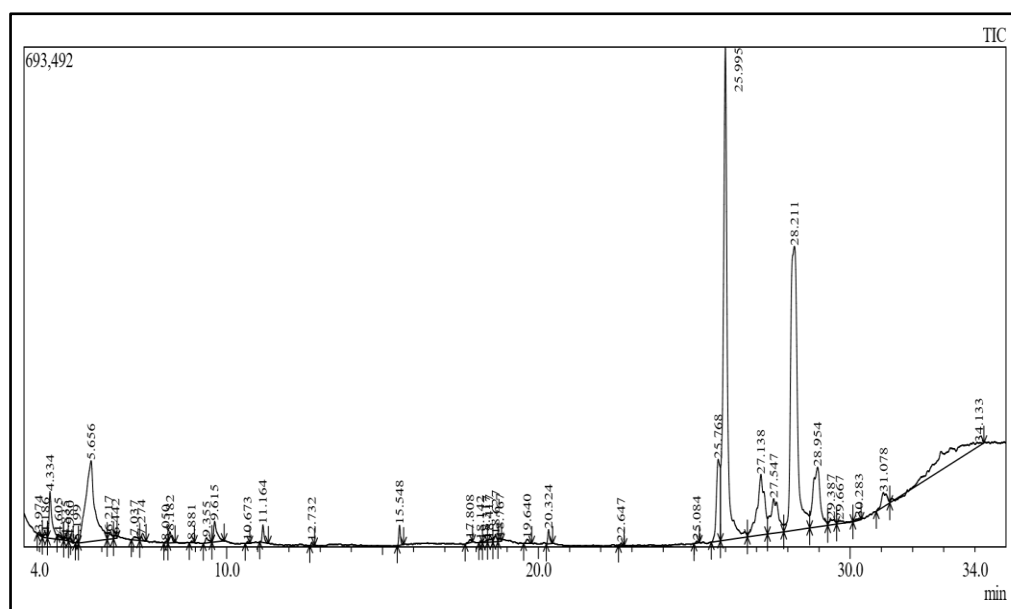


Fig 5 Chromatogram GC-MS Analysis of Water Extract

Table 4 GC-MS Identified Compounds of Water Extract

Peak #	R. Time	Area	Height	Identified compound
1	3.974	13001	3379	2-Hexanone, 3-methyl-
2	4.186	31392	4335	cis-2-Allylpyrrolidin-5-ol
3	4.334	467421	64288	1,2-Cyclopentanedione
4	4.605	16046	2262	5-Aminoisoxazole
5	4.835	15108	4153	L-Glutamine tert-butyl ester
6	4.980	18190	5223	Cyclodecylamine
7	5.199	7630	2214	Formic acid, TMS derivative
8	5.656	2314638	111818	Glycerin
9	6.217	79854	8971	Butyric acid, neopentyl ester
10	6.442	23537	3788	Hexyl trifluoroacetate
11	7.037	45263	4149	2(3H)-Furanone, 5-butylidihydro-
12	7.274	22462	5932	Nonane, 3,7-dimethyl-
13	8.050	11496	2044	Pentane, 3-methyl-
14	8.182	90569	15171	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6
15	8.881	8716	2496	2-(Benzylmethylamino)ethanol N-oxide
16	9.355	54906	7132	Isoamyl nitrite
17	9.615	225099	28574	4-Vinylphenol
18	10.673	7743	2469	Propargyl alcohol, trifluoroacetate

19	11.164	103616	23839	2-Methoxy-4-vinylphenol
20	12.732	14345	4378	Hexane-2,6-dicarbonitrile, 1-(formyloxymethyl)
21	15.548	103416	27522	Diethyl Phthalate
22	17.808	27387	4321	2-(1,2,3,6-Tetrahydro-1-pyridinyl)-1-propanol
23	18.142	7109	2509	s-Triazine, 2-amino-4-(piperidinomethyl)-4-pi
24	18.317	14071	2511	Phosphorodichloridic acid, hexyl ester
25	18.417	38149	3496	Benzamide, 2-methoxy-N-(2-phenylethyl)-N-t
26	18.627	57452	8742	s-Triazine, 2-amino-4-(piperidinomethyl)-4-pi
27	18.767	21070	3325	Ethane, isocyanato-
28	19.640	37653	5340	Oxalic acid, cyclohexyl isobutyl ester
29	20.324	83024	19489	n-Decanoic acid
30	22.647	12951	3299	Propanedioic acid, propyl-
31	25.084	10977	2786	Spiropentane, propyl-
32	25.768	974018	113595	Stigmasterol
33	25.995	5982675	683932	(3S,8S,9S,10R,13R,14S,17R)-17-((2R,5R)-5-
34	27.138	1398562	83493	24-Norursa-3,12-diene
35	27.547	853116	47646	Pregnane-11,20-dione, 3,21-dihydroxy-, (3.alpha., 5.beta)
36	28.211	5884879	393160	beta-Sitosterol
37	28.954	1314167	82371	Lupeol, trifluoroacetate
38	29.387	90679	7559	1-Cyclopropene-1-pentanol, .alpha.,.epsilon.,.e
39	29.667	52228	3981	trans-Carveol, O-(trifluoroacetyl)-
40	30.283	22809	2353	Benzamide, N-(2-piperidinylmethyl)-2,5-bis(2
41	31.078	236939	18549	cis-Z-.alpha.-Bisabolene epoxide
42	34.133	1659048	4201	Ergostan-6-one, 3,25-bis(acetyloxy)-5-hydroxy-

➤ Selected Compounds and ADME Profile

The selected reference compounds and GC-MS-derived constituents were subjected to SwissADME profiling. Quercetin and Apocynin showed zero Lipinski violations, while Acarbose showed three violations. Among the identified constituents, Stigmasterol and β -sitosterol each showed one violation, whereas Lupeol showed two

violations. The dissertation recorded high gastrointestinal absorption for Quercetin, Apocynin and Metformin, with SwissADME predicted low gastrointestinal absorption for sterols and lupeol because of their high lipophilicity and low absorption for Acarbose and the three selected phytoconstituents in Table.5.

Table 5 ADME Profiling of Standard and Isolated Compounds

Compound	Molecular weight	Lipinski violations	GI absorption	H-bond acceptors	H-bond donors	Rotatable bonds
Quercetin (standard- Antioxidant)	302.2 g/mol	0	High	7	5	1
Apocynin (standard- Antioxidant)	166.1 g/mol	0	High	3	1	2
Acarbose (standard-Antidiabetic)	645.6 g/mol	3	Low	19	14	13
Metformin (standard- Antidiabetic)	129.1 g/mol	0	High	2	3	2
Stigmasterol	412.9 g/mol	1	Low	1	1	5
β -sitosterol	414.1 g/mol	1	Low	1	1	6
Lupeol	426.729 g/mol	2	Low	5	0	4

➤ In-silico Pharmacological Evaluation:

• In-silico Antioxidant Docking

Docking was performed against NADPH oxidase [NOX (PDB: 2CDU)] and Superoxide dismutase [SOD1 (PDB: 2C9V)] in Fig.6-15, using Apocynin and Quercetin as reference compounds. All three selected phytoconstituents

demonstrated favourable predicted interactions with both antioxidant-related targets. Lupeol produced the lowest docking energies among the investigated ligands, indicating the strongest predicted affinity within the present computational comparison. The antioxidant-docking rationale is supported by the project references describing NADPH oxidase-related antioxidant phytochemicals and Quercetin/SOD1 interactions.

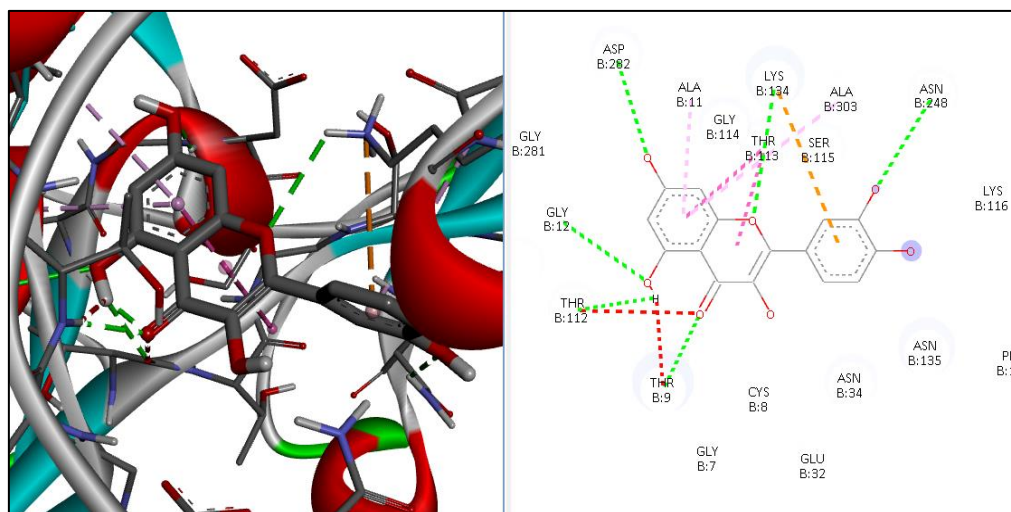
➤ *In-silico* Antioxidant Activity in Targeted Protein - NADPH Oxidase [NOX (PDB: 2CDU)]• *QUERCETIN* with NADPH Oxidase [NOX (PDB: 2CDU)]

Fig 6 3D and 2D Docking Pose of Quercetin with 2CDU

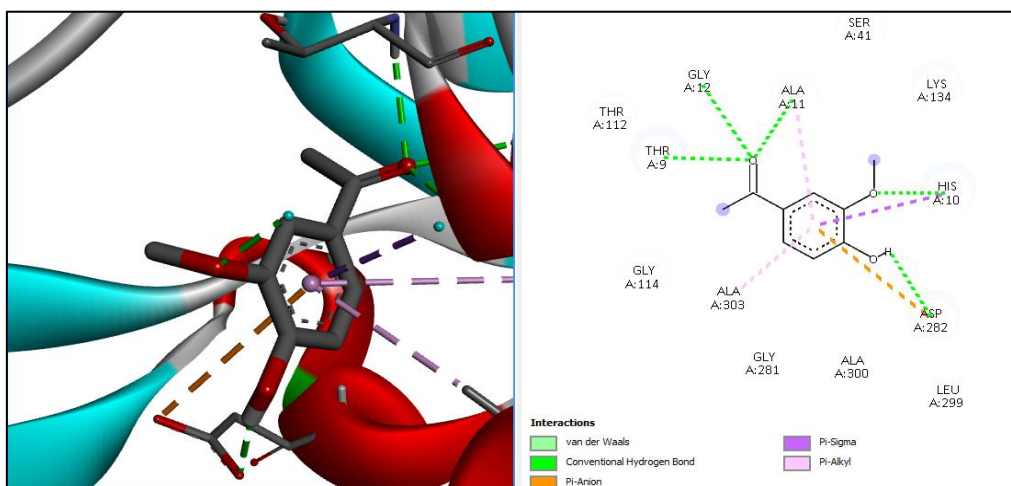
• *APOCYNIN* with NADPH Oxidase [NOX (PDB: 2CDU)]

Fig 7 3D and 2D Docking Pose of Apocynin with 2CDU

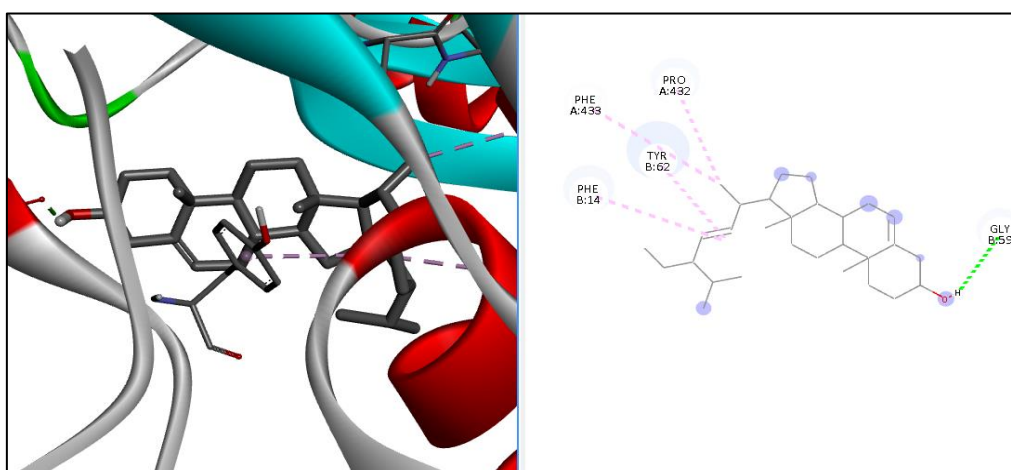
• *STIGMASTEROL* with NADPH Oxidase [NOX (PDB: 2CDU)]

Fig 8 3D and 2D Docking Pose of Stigmasterol with 2CDU

- β -SITOSTEROL with NADPH Oxidase [NOX (PDB: 2CDU)]

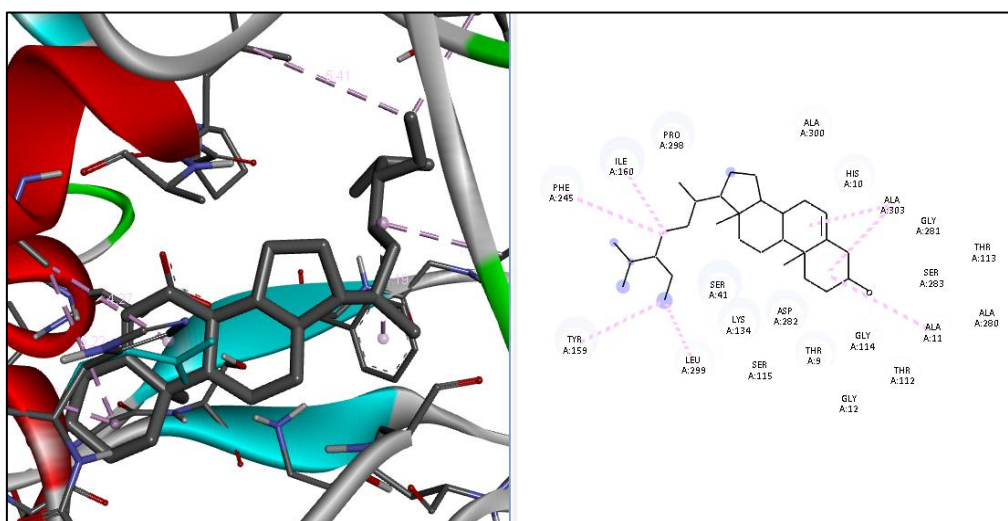


Fig 9 3D and 2D Docking Pose of β -Sitosterol with 2CDU

- LUPEOL with NADPH Oxidase [NOX (PDB: 2CDU)]

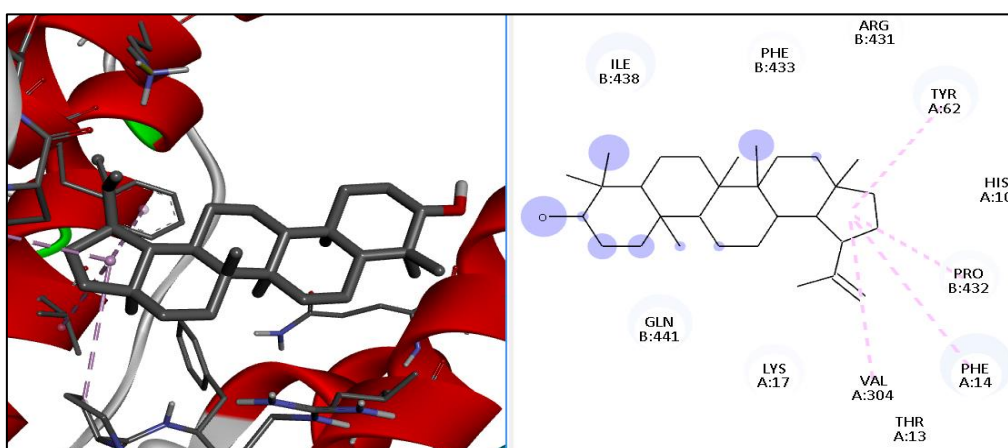


Fig 10 3D and 2D Docking Pose of Lupeol with 2CDU

- In-silico Antioxidant Activity in Targeted Protein- Superoxide Dismutase [SOD1 (PDB: 2C9V)]

- QUERCETIN with Superoxide dismutase [SOD1 (PDB: 2C9V)]

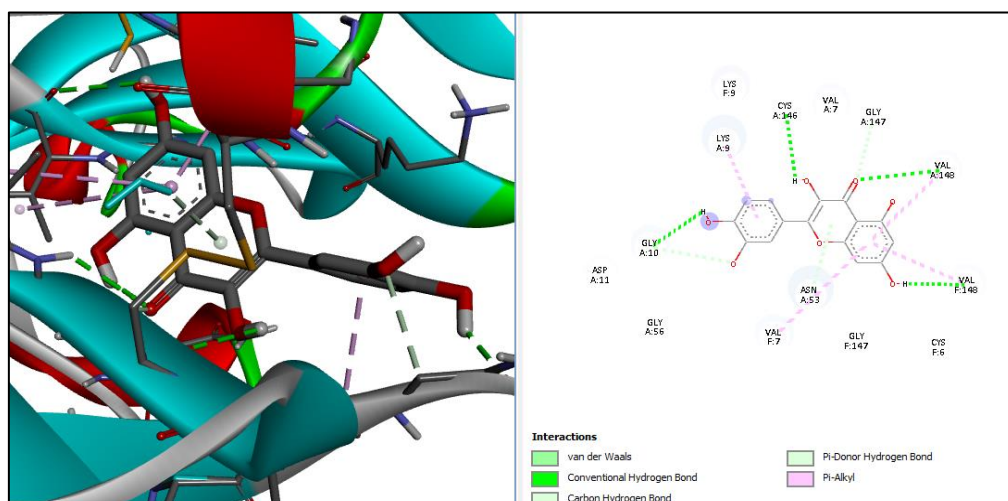


Fig 11 3D and 2D Docking Pose of Quercetin with 2C9V

• *APOCYNIN with Superoxide Dismutase [SOD1 (PDB: 2C9V)]*

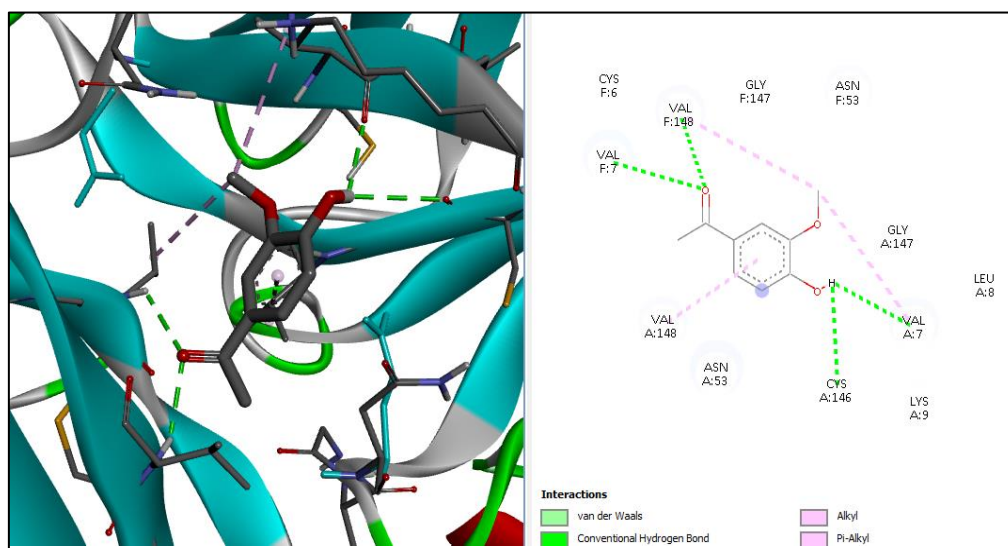


Fig 12 3D and 2D Docking Pose of Apocynin with 2C9V

• *STIGMASTEROL with Superoxide Dismutase [SOD1 (PDB: 2C9V)]*

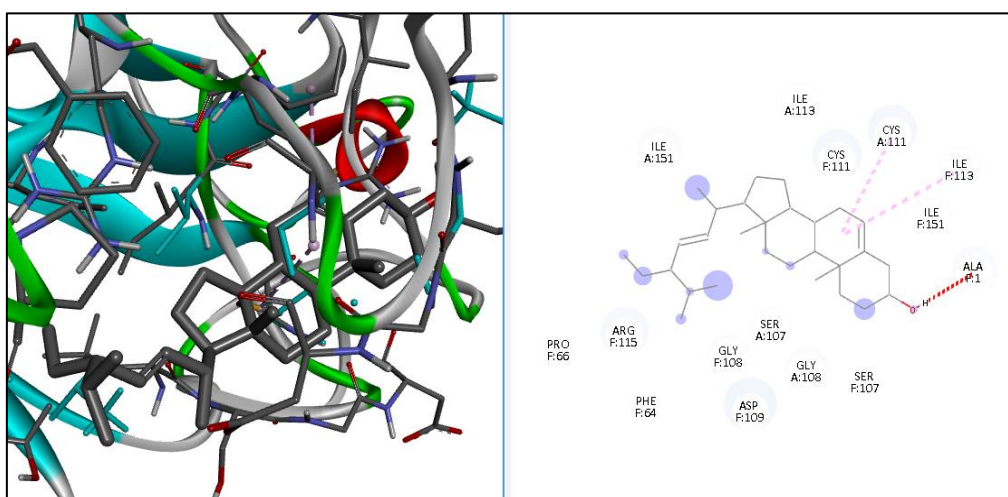


Fig 13 3D and 2D Docking Pose of Stigmasterol with 2C9V

• *β -SITOSTEROL with Superoxide Dismutase [SOD1 (PDB: 2C9V)]*

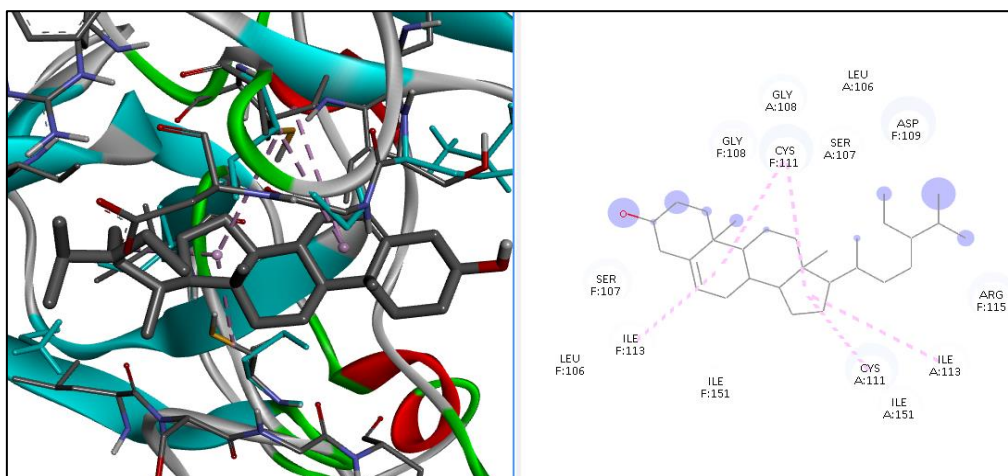


Fig 14 3D and 2D Docking Pose of β -Sitosterol with 2C9V

• *LUPEOL with Superoxide Dismutase [SOD1 (PDB: 2C9V)]*

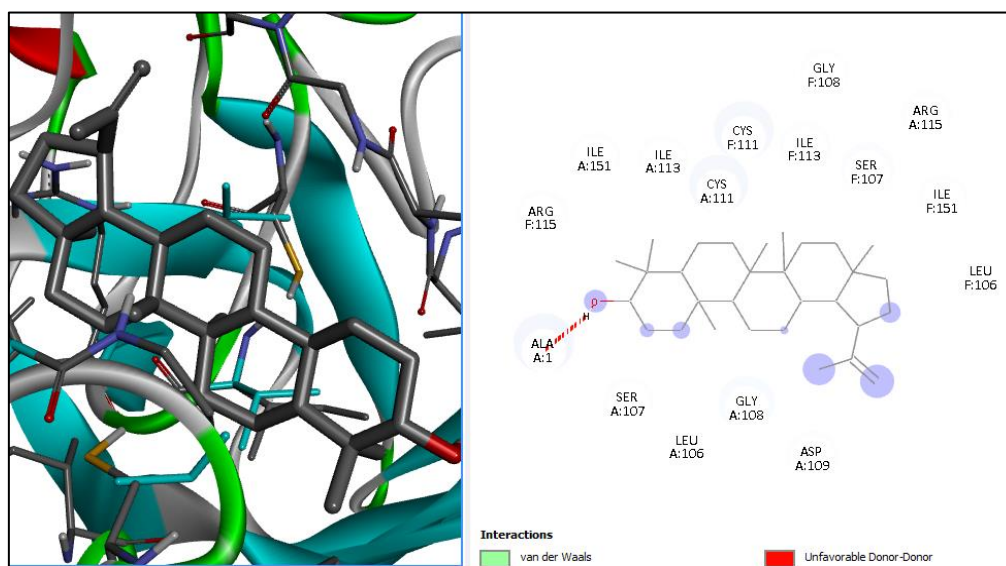


Fig 15 3D and 2D docking Pose of Lupeol with 2C9V

Table 6 Comparison of Docking Score for *In-silico* Antioxidant Activity

Ligand	PubChem CID	NADPH oxidase [NOX (PDB: 2CDU)]	Superoxide dismutase [SOD1 (PDB: 2C9V)]
Quercetin	5280343	−9.0 kcal/mol	−8.8 kcal/mol
Apocynin	2214	−6.7 kcal/mol	−6.4 kcal/mol
Stigmasterol	5280794	−9.7 kcal/mol	−8.6 kcal/mol
β-sitosterol	222284	−9.7 kcal/mol	−8.5 kcal/mol
Lupeol	259846	−10.5 kcal/mol	−9.2 kcal/mol

• *In-silico Antidiabetic Docking*

The antidiabetic docking analysis used α -amylase (PDB: 1OSE) and α -glucosidase (PDB: 1UOK) in Fig.16-25,

with Acarbose and Metformin as reference compounds. Stigmasterol, β -sitosterol and Lupeol showed favourable predicted binding energies against both enzymes.

➤ *In-silico Antidiabetic Activity in Targeted Protein - α -Amylase (PDB: 1OSE)*

• *ACARBOSE with α -Amylase (PDB: 1OSE)*

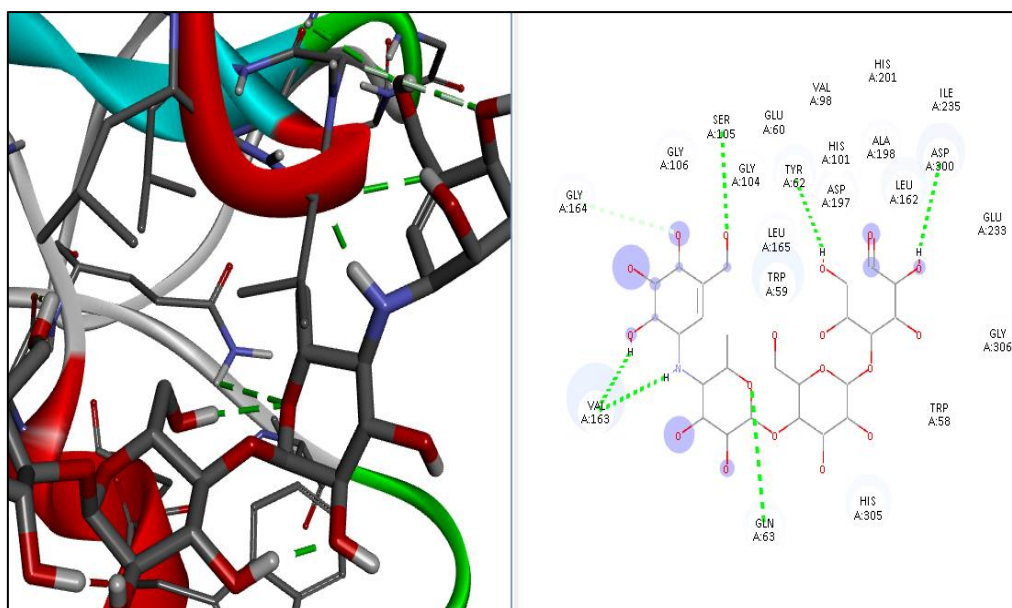


Fig 16 3D and 2D Docking Pose of Acarbose with 1OSE

- *METFORMIN with α -Amylase (PDB: 1OSE)*

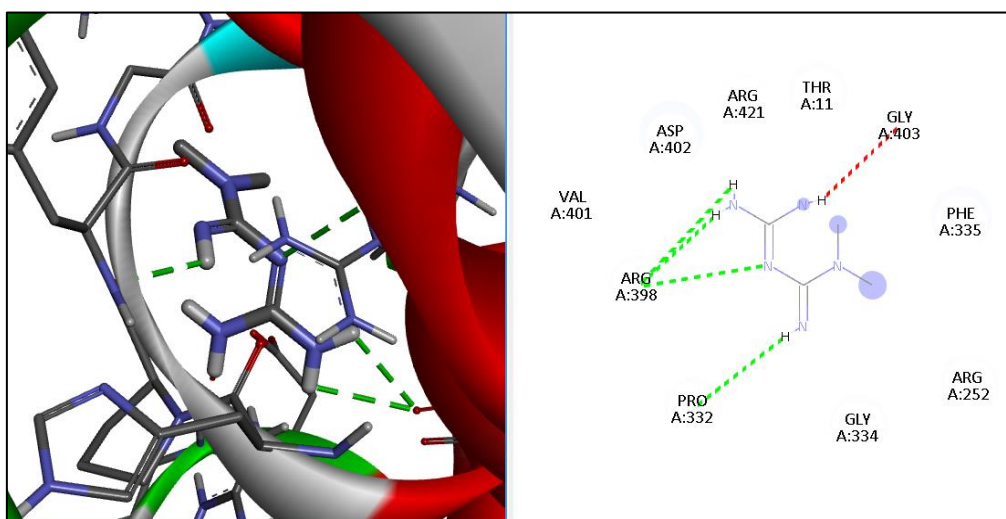


Fig 17 3D and 2D Docking Pose of Metformin with 1OSE

- *STIGMASTEROL with α -Amylase (PDB: 1OSE)*

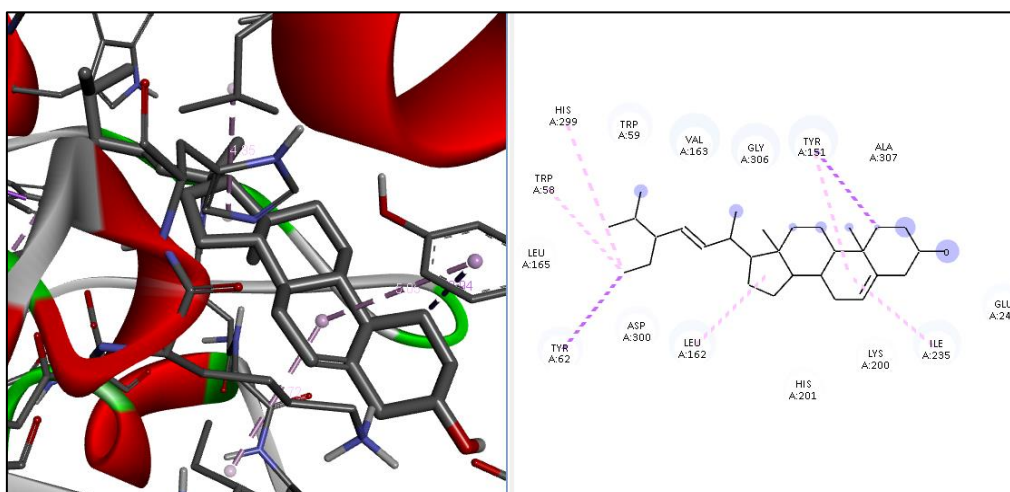


Fig 18 3D and 2D Docking Pose of Stigmasterol with 1OSE

- *β -SITOSTEROL with α -Amylase (PDB: 1OSE)*

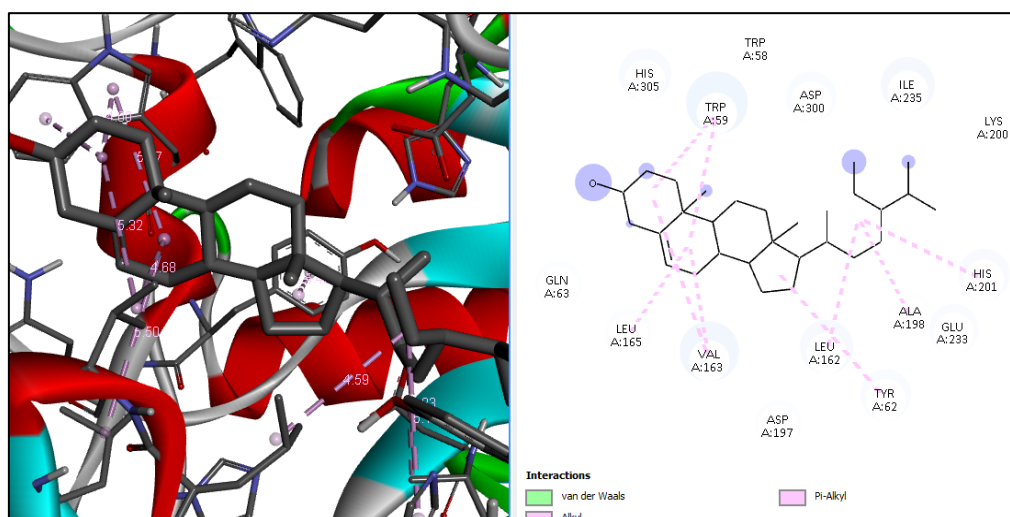


Fig 19 3D and 2D Docking Pose of β -Sitosterol with 1OSE

- *LUPEOL with α -Amylase (PDB: 1OSE)*

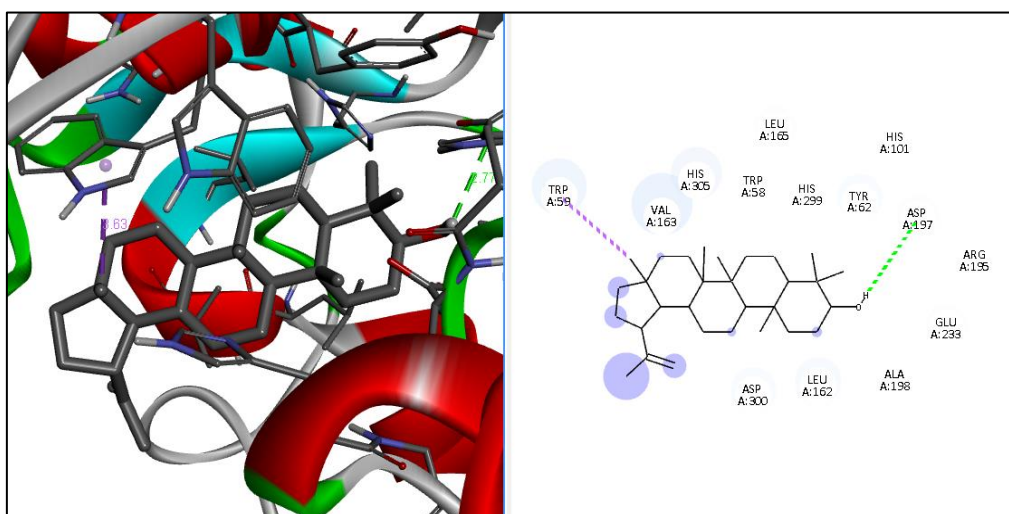


Fig 20 3D and 2D Docking Pose of Lupeol with 1OSE

➤ *In-silico Antidiabetic in Targeted Protein - α -Glucosidase (PDB: 1UOK)*

- *ACARBOSE with α -Glucosidase (PDB: 1UOK)*

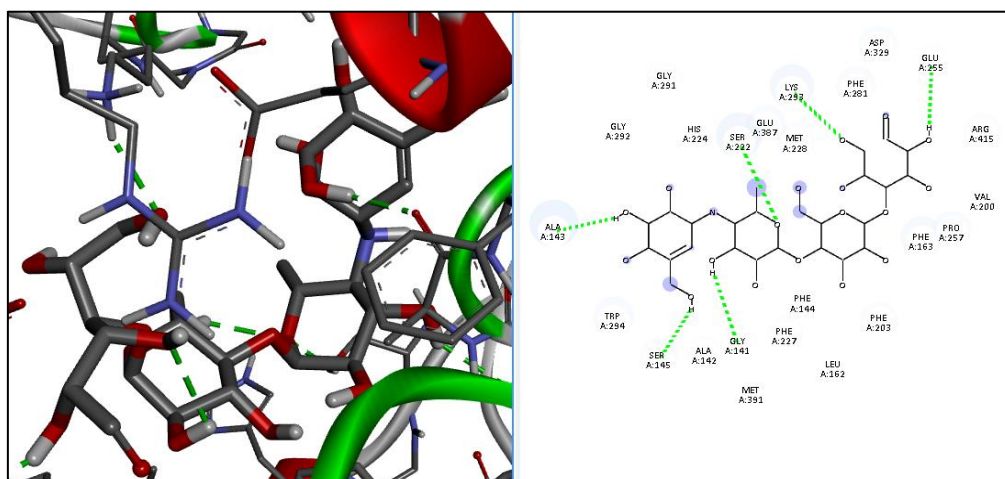


Fig 21 3D and 2D Docking Pose of Acarbose with 1UOK

- *METFORMIN with α -Glucosidase (PDB: 1UOK)*

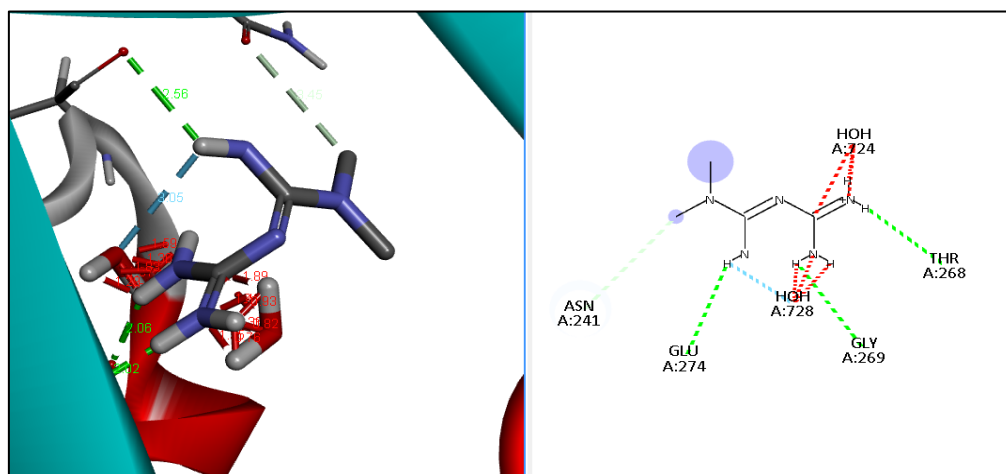


Fig 22 3D and 2D Docking Pose of Metformin with 1UOK

- *STIGMASTEROL with α -Glucosidase (PDB: 1UOK)*

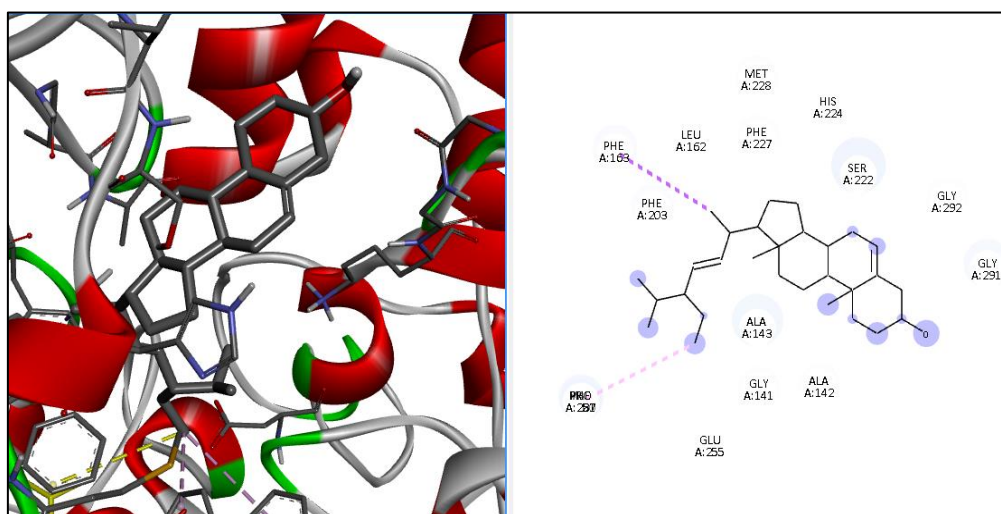
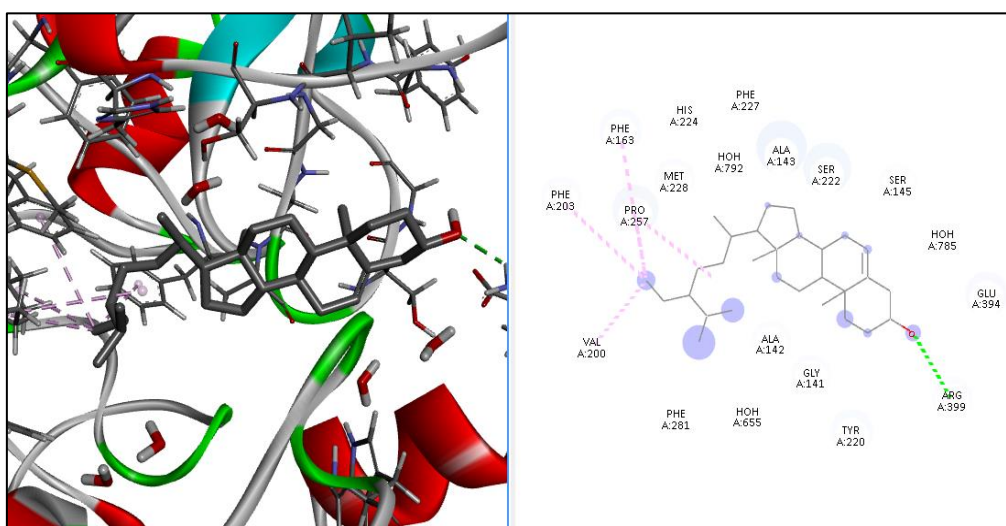


Fig 23 3D and 2D Docking Pose of Stigmasterol with 1UOK

- *B-SITOSTEROL with α -Glucosidase (PDB: 1UOK)*

Fig 24 3D and 2D Docking Pose of β -Sitosterol with 1UOK

- *LUPEOL with α -Glucosidase (PDB: 1UOK)*

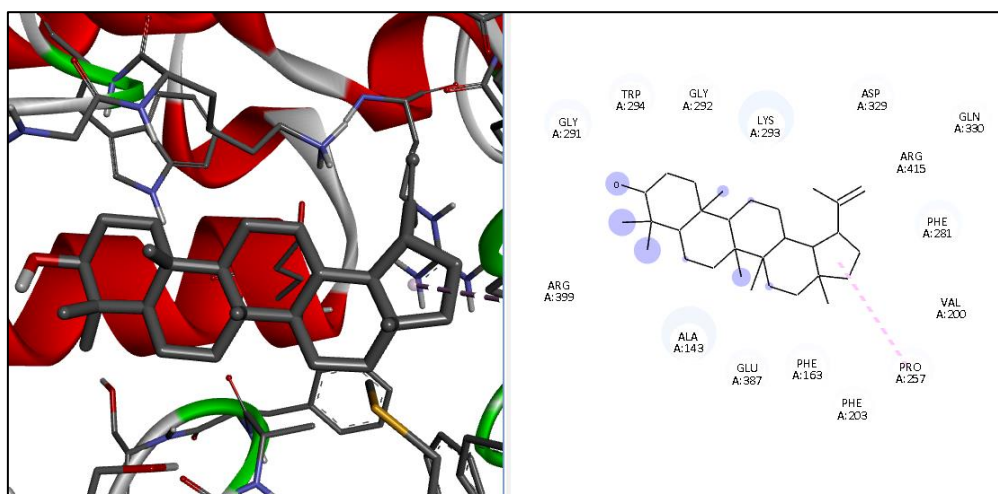


Fig 25 3D and 2D Docking Pose of Lupeol with 1UOK

Table 7 Comparison of Docking Score for *In-silico* Antidiabetic Activity

Ligand	PubChem CID	α -Amylase (1OSE)	α -Glucosidase (1UOK)
Acarbose	9811704	-7.4 kcal/mol	-7.1 kcal/mol
Metformin	4091	-5.1 kcal/mol	-5.3 kcal/mol
Stigmasterol	5280794	-9.0 kcal/mol	-8.2 kcal/mol
β -sitosterol	222284	-9.4 kcal/mol	-7.4 kcal/mol
Lupeol	259846	-9.4 kcal/mol	-9.0 kcal/mol

➤ Overall Discussions

The integrated results demonstrate that the flowers possess distinct pharmacognostical markers together with measurable physicochemical characteristics and a diverse phytochemical profile. The high aqueous extractive yield and the presence of flavonoids, alkaloids, glycosides and tannin/phenolic constituents support the chemical richness of the material, consistent with the phytochemical literature on *Cleome gynandra* L. GC-MS further revealed a complex chemical profile and identified sterol and triterpenoid constituents suitable for prioritization in computational studies like *In-silico* Pharmacological studies.

Within the docking analysis, lupeol consistently showed the most favourable predicted interactions across the antioxidant and antidiabetic targets examined. β -sitosterol also displayed strong predicted affinity against α -amylase, while stigmasterol showed favourable interactions with both antioxidant targets and the carbohydrate-digesting enzymes. These computational observations should be interpreted as hypothesis-generating evidence rather than proof of pharmacological efficacy and they require confirmation using biochemical, *in-vitro* and *in-vivo* studies.

IV. CONCLUSION

The present investigation established a systematic pharmacognostic, physicochemical, phytochemical, GC-MS and *in-silico* profile of *Cleome gynandra* L. flowers. The macroscopic and powder microscopic characteristics provide useful diagnostic markers for identification and standardization. The physicochemical results and successive extraction yields provide preliminary quality-control reference data, with water giving the highest extractive yield (26.06%). GC-MS analysis identified a complex mixture of constituents, including Stigmasterol, β -sitosterol and Lupeol. *In-silico* ADME and docking analyses indicated favourable predicted interactions of the selected phytoconstituents with antioxidant and antidiabetic-related targets, with Lupeol emerging as the most promising candidate. Further *in-vitro* antioxidant, α -amylase and α -glucosidase inhibition studies, followed by *in-vivo* pharmacological evaluation, toxicity assessment and mechanistic studies are required to establish therapeutic relevance.

REFERENCES

- [1]. Kokate CK, Purohit AP, Gokhale SB. Pharmacognosy. 55th ed. Pune: Nirali Prakashan; 2008. p.1.11.
- [2]. Kar A. Textbook of Pharmacognosy and Phytochemistry-1. Delhi: Ahuja Book House Pvt Ltd; p.36-38.
- [3]. Arumugam KR, Muruges N. Textbook of Pharmacognosy. 7th ed. 2004. p.21-23.
- [4]. Ames BN, Shigenaga MK, Hagen TM. Oxidants, antioxidants and the degenerative diseases of aging. Proceedings of the National Academy of Sciences. 1993;90:7915-7922.
- [5]. Halliwell B, Gutteridge JMC. Free Radicals in Biology and Medicine. 3rd ed. Oxford University Press; 1999.
- [6]. Droge W. Free radicals in the physiological control of cell function. Physiological Reviews. 2002;82:47-95.
- [7]. Valko M, Izakovic M, Mazur M, Rhodes CJ, Telser J. Role of oxygen radicals in DNA damage and cancer incidence. Molecular Cell Biochemistry. 2004;266:37-56.
- [8]. Pisochi AM, Negulescu GP. Methods for total antioxidants activity determination: A review. Biochemistry and Analytical Biochemistry. 2011;1:1-10.
- [9]. Prior RL, Wu X, Schaich K. Standardized methods for the determination of antioxidant capacity and phenolics of foods and dietary supplements. Journal of Agricultural and Food Chemistry. 2005;53:4290-4302.
- [10]. Pacher P, Beckman JS, Liaudet L. Nitric oxide and peroxynitrite in health and disease. Physiological Reviews. 2007;87:315-424.
- [11]. Chan CH, Ngho GC, Yusoff R. A brief review on anti-diabetic plants: Global distribution, active ingredients, extraction techniques and acting mechanisms. 2012. doi:10.4103/0973-7847.95854.
- [12]. Jung M, Park M, Lee HC, Kang YH, Kang ES, Kim SK. Antidiabetic Agents from Medicinal Plants. 2006.
- [13]. Patel DK, Kumar R, Laloo D, Hemalatha S. Diabetes mellitus: An overview on its pharmacological aspects and reported medicinal plants having antidiabetic activity. Asian Pacific Journal of Tropical Biomedicine. 2012;2(5):411-420.
- [14]. Ekins S, Mestres J, et al. In silico pharmacology for drug discovery: methods for virtual ligand screening and profiling. British Journal of Pharmacology. 2007;152(1).
- [15]. Poudyl B, et al. Molecular Docking technique. National Journal of Pharmaceutical Sciences. 2021;1(1):08-14.
- [16]. Tiwari S, Prakash K, et al. Unrevealing the complex interplay: molecular docking: A comprehensive review on current scenario, upcoming difficulties, forthcoming initiatives and viewpoints. International Journal of Chemistry Research. 2023;8(1):1-9.
- [17]. Shanmugam S, Rajendran K, Suresh B. Medicinally Important Plant *Cleome gynandra* L.: A Phytochemical and Pharmacological Review.

- Research Journal of Pharmacy and Technology. 2013;6(8):859-863.
- [18]. Mishra SS, Moharana SK, Dash MR. Review on *Cleome gynandra* L. International Journal of Research in Pharmacy and Chemistry. 2011;1(3):681-689.
- [19]. Bhattacharya S, Singh S, Kumar A. A Comprehensive Study on the Occurrence Records of the African Neglected and Underutilized Weed Species *Cleome gynandra* L. (cat's whiskers): validating the Eco geographical range expansion in West Bengal, India. NeBIO. 2019;10(1):1-7.
- [20]. Debnath A, Paul C, Debnath B. Eight new additions of plant species to the flora of foot Himalayan state Tripura, North East India: distributional range extension, geographic map, and their less-known ethnomedicines. NeBIO. 2017;8(4):1-8.
- [21]. Mishra SS, Moharana SK, Dash MR. Review on *Cleome gynandra* L. International Journal of Research in Pharmacy and Chemistry. 2018;8(2):1-8.
- [22]. Anbazhagi K, Kadavul G, Suguna L, Petrus AJA. Studies on the pharmacognostical and in vitro antioxidant potential of *Cleome gynandra* Linn. Leaves. Natural Product Radiance. 2009;8(2):151-157.
- [23]. Shilla O, Dinssa FF, Omondi EO, Winkelmann T, Abukutsa-Onyango MO. *Cleome gynandra* L. origin, taxonomy and morphology: A review. African Journal of Agricultural Research. 2019;14(32):1568-1583.
- [24]. Chweya JA, Mnzava NA. *Cleome gynandra* L.: Promoting the conservation and use of underutilized crops. IPGRI, Rome; 1997.
- [25]. The Ayurvedic Pharmacopoeia of India. Part I, Volume I. Ministry of Health and Family Welfare, Government of India; 1989.
- [26]. Saravanan R, Natesan R, Ramalingam S. Pharmacognostic Profiling of *Cleome gynandra* L.— A Traditional Folk Medicine. JPNR. 2022;3746-3753.
- [27]. Adhikari PP, Paul SB. Medicinally important plant *Cleome gynandra*: a phytochemical and pharmacological explanation. Asian Journal of Pharmaceutical and Clinical Research. 2018;11(1):21-29.
- [28]. Khuntia A, Martorell M, Ilango K, Bungau SG, Radu AF, Behl T, Sharifi-Rad J. Theoretical evaluation of *Cleome* species' bioactive compounds and therapeutic potential: A literature review. Biomedicine & Pharmacotherapy. 2022;151:113161.
- [29]. Narendhirakannan RT, Kandaswamy M, Subramanian S. Anti-inflammatory activity of *Cleome gynandra* L. on hematological and cellular constituents in adjuvant-induced arthritic rats. Indian J Med Food. 2005;8(1):93-99.
- [30]. Narendhirakannan RT, Subramanian S, Kandaswamy M. Anti-inflammatory and lysosomal stability actions of *Cleome gynandra* L. studied in adjuvant induced arthritic rats. Food and Chemical Toxicology. 2007;45(6):1001-1012.
- [31]. Sowunmi LI, Afolayan AJ. Phytochemical constituents and antioxidant properties of acetone extract of *Cleome gynandra* (L.) growing in the Eastern Cape, South Africa. African Journal of Traditional, Complementary and Alternative Medicines. 2015;12(3):1-8.
- [32]. Pandey KB, Rizvi SI. Plant polyphenols dietary antioxidants in human health and disease. Indian Journal of Clinical Biochemistry. 2009;24(3):270-278.
- [33]. Ravichandra B, Ram PS, Saritha C, Shankaraiah P. Anti diabetic and anti-dyslipidemia activities of *Cleome gynandra* in alloxan induced diabetic rats.
- [34]. Choudhury S, Sharan RN, Khanna VK. Phytochemicals with anti-hyperglycemic activity: Evidence from Indian medicinal plants. Indian Journal of Natural Products and Resources. 2012;3(2):145-152.
- [35]. Sivakumar KK, Rajasekaran A, Ponnilarasan I, Somasundaram A, Sivasakthi R. Antiulcer and analgesic activity of the ethanol extract of *Cleome gynandra* Linn leaves. Research Journal of Pharmacy and Technology. 2010;3(3):766-769.
- [36]. Imanirampa L, Alele PE. Antifungal activity of *Cleome gynandra* L. aerial parts for topical treatment of *Tinea capitis*: an in-vitro evaluation. BMC Complementary and Alternative Medicine. 2016;16:1-8.
- [37]. Narendhirakannan RT, Subramanian S, Kandaswamy M. Antifungal potential of phytochemical-rich extracts of *Cleome gynandra* L. against pathogenic fungi. Indian Journal of Experimental Biology. 2005;43(9):789-793.
- [38]. Sridhar N, Kiran BV, Sasidhar DT, Kanthal LK. In-vitro antimicrobial screening of methanolic extracts of *Cleome chelidonii* and *Cleome gynandra*. Bangladesh Journal of Pharmacology. 2014;9(2):161-166.
- [39]. Abeysinghe Pushpa D, Weeraddana Chaminda De S. Screening of Petroleum ether, Chloroform, Ethyl acetate, Ethanol and Water Extracts of Medicinal Plant, *Avicennia marina* for Antibacterial Activity against Antibiotic Resistant Bacteria Species, *Staphylococcus* and *Proteus*. JPBMS. 2011-11(18).
- [40]. Shaikh JR, Patil MK. Qualitative tests for preliminary phytochemical screening: An overview. International Journal of Chemical Studies. 2020;8(2):603-608.
- [41]. Sathe KP, et al. A review on Gas Chromatography-Mass Spectrometry (GC-MS). World Journal of Pharmaceutical Research. 2021;10(3):741-763.
- [42]. Mhya DH, Jakwa AG, Agbo J. *In Silico* Analysis of Antioxidant Phytochemicals with Potential NADPH Oxidase Inhibitory Effect. Journal of Health Science and Medical Research. 2023;41(2):e2022912.
- [43]. Bhatia NK, Modi P, Sharma S, Deep S. Quercetin and Baicalein Act as Potent Anti-amyloidogenic and Fibril Destabilizing Agents for SOD1 Fibrils. ACS Chemical Neuroscience. 2020;11(8):1129-1138.
- [44]. Lenh Vo Van, Pham EC, Nguyen CV, Nguyen NT, Le TVT, Truong TN. In vitro and in vivo antidiabetic activity, isolation of flavonoids, and *in silico* molecular docking of stem extract of *Merremia tridentata* (L.). Biomedicine & Pharmacotherapy. 2022;146: 112611.