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PHARMACOGNOSTIC, PHYTOCHEMICAL EVALUATION, AND ANTIOXIDANT ACTIVITY OF THE LEAVES OF *GREWIA BILAMELLATA* GAGNEP. (TILIACEAE)

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This research seeks to analyze the Pharmacognostic, Phytochemical evaluation, Total Phenolic, Flavonoid Content, and Antioxidant activity of the leaves of Grewia bilamellata Gagnep. (G. bilamellata). This Pharmacognostic study comprises microscopic observation, leaf characteristics, phytochemical and physicochemical analyses, and total phenolic and flavonoid content. Antioxidant activity was examined using 1,1-diphenyl-2-picrylhydrazyl (DPPH) and H_2O_2 assays. The leaf shows anomocytic stomata, covering, glandular and stellate type of trichomes, Total phenolic and flavonoid content turned greatest in ethanolic extract (EEGB) (90.69±2.86 mg GAE/g), and (26.21 ± 1.51 mg RE/g), respectively. EEGB showed the most intense 1,1-diphenyl-2-picrylhydrazyl (DPPH) and H_2O_2 scavenging activity, with IC_{50} 59.96 and 59.24 µg/ml. Total phenolic and flavonoid contents - DPPH and H_2O_2 scavenging assays were positively correlated. The Invitro Antioxidant results were also correlated with the Insilico method using xanthine oxidase (XOD) and superoxide dismutase (SOD) as targets. Microscopic and physicochemical parameters are valuable for authenticating drug identification and purity. G. bilamellata is a potential perspective source of antioxidants.

Keywords: *Grewia bilamellata Gagnep, Antioxidant, Pharmacognostic, Insilico*

INTRODUCTION

Plants have been used for medicinal purposes to treat diseases when there are no other effective therapeutic options. Traditionally, this knowledge has been passed down through legends, pictographs, and monographs[1]. The therapeutic effects of medicinal plants are caused by multiple secondary metabolites of varying composition[2]. Herbal medicines are a source of secondary metabolites with curative properties, owing to their antiquity. Several illnesses can be cured using remedies found in medicinal plants[3]. Several medicinal plants have not been extensively studied, and the results have not been compared with pharmacological and phytochemical tests used to treat various illnesses. Scientists are exploring the medicinal benefits of plants in humans[4]. According to the World Health Organization, approximately 80% of the global population relies on plants to treat various illnesses. Meanwhile, around 20-30% of all medicines available on the market are derived from natural sources[5]. To authenticate the ethnobotanical knowledge of herbal medicine, scientific research must be conducted to substantiate the traditional understanding of medicinal plants[6]. In pharmacognosy, the study of the physical, chemical, biological, and biochemical properties of drugs and the discovery of novel medicines has been undertaken[7]. Pharmacognostic evaluation helps screen commercial varieties, substitutes, adulterants, and other quality control of drugs[8]. It is a

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simple and reliable tool that helps obtain information about crude drugs' biochemical and physical properties[9]. The utilization of alternative medicine in prosperous countries has been impeded by a lack of proof of its application and stringent quality control regulations[10]. Therefore, it is essential to document the research on traditional medicines. This drawback makes it extremely important to ensure that the plants and parts of plants used as medicines are standardized. The standardization process can involve using several strategies and approaches, including pharmacognostic and phytochemical research, in a step-by-step manner.[11]. These steps and procedures help to identify and standardize plant materials. To ensure the reproducible quality of herbal medicine, characterization and quality assurance of the starting materials are essential steps.

In human tissue cells, imbalanced redox reactions produce free radicals, also known as reactive oxygen species (ROS), including superoxide, peroxide, singlet oxygen, hydroxyl radicals, and nitric oxide[12]. Food habits and lifestyle processes may also contribute to ROS overproduction. High ROS concentrations can oxidize biomolecules in the human body, leading to various endogenous diseases. Antioxidants maintain a balanced metabolic process by inhibiting ROS detoxification chains[13]. Most cells have a subtle system for removing the ROS generated by metabolism to prevent damage caused by oxygen free radicals[14&15]. The natural Antioxidant enzyme superoxide dismutase (SOD) plays a fundamental role in controlling cellular ROS damage during these processes. The enzyme converts superoxide (O2•) to hydrogen peroxide (H_2O_2), which is then converted into harmless water (H_2O). Hypoxanthine and xanthine are oxidatively hydroxylated by xanthine oxidase (XOD), which reduces the oxygen in xanthine to produce reactive oxygen. Excess XOD activity increases H_2O_2 and O2•, which increase oxidative stress damage from ROS in the enzymatic process. In addition, XOD can treat gout, which often occurs due to oxidative damage. Inhibition of XOD is beneficial for treating some diseases, such as gout, owing to its role in oxidative tissue damage.

Based on these two closely related enzymes, SOD and XOD can be screened for Antioxidant compounds in *G. bilamellata* to prevent oxidative damage. SOD and XOD are currently not being tested as target enzymes for the Antioxidant activity of the isolated phytoconstituents of *G. bilamellata*. In recent years, considerable attention has been paid to the natural antioxidants derived from traditional plant sources.[16&17].

With over 321 described species, *Grewia* is the largest and most diverse flowering genus in the family Tiliaceae. In East Africa, the bark of *G. bilamellata* is used to treat intestinal infestations and syphilis, while root infusion has been used to treat anemia, chest pain, snakebites, colds, diarrhea, and infertility in women. The leaves of the plant were used to treat helminthiasis, antidiabetic, febrifuge, and as anticonvulsant[18]. Till now, four neo-lignans have been isolated from the leaves and stems, namely 8-O-4’-neolignanguaia-cylglycerol-β-coniferyl ether(threo), 8-O-4’-neolignanguaia-cylglycerol-β-coniferyl ether (erythro), nitidanin, and bilagrewin; two coumarinolignans have been isolated, namely, Cleomiscosin D and Grewin; a quinone derivative, 2,6-dimethoxy-1-acetonylquinol; two triterpene derivatives, namely 3α,20-lupandiol and 2α ,3β-dihydroxyolean-12-en-28-oic acid; and a sterol derivative, daucosterol, has been isolated from stem bark[19-21]. This plant has been reported to have antimalarial[20] and anticancer activity[21].

Therefore, the present study aimed to conduct pharmacological studies on *Grewia bilamellata* for proper identification and standardization and to determine the Antioxidant potential of various extracts of *G. bilamellata* using in vitro and *in silico* methods. The studies mentioned above were conducted for the first time at this plant.

MATERIALS AND METHODS

Collection and authentication of plant

Whole *G. bilamellata* plants were collected from the Seshachalam Forest region, Chittoor District, Andhra Pradesh. Plant material was authenticated by the Department of Botany, Sri Venkateswara University, Tirupati, Andhra Pradesh (Voucher No:0428).

Macroscopic evaluation

Macroscopic leaf parameters were also observed. Leaves were evaluated according to their shape, indentations, base, texture, venation, apex, and petioles[22].

Microscopical Analysis
Study of Transverse Section

Fresh *G. bilamellata* leaves were used for microscopic sectioning. Transverse sections were obtained by passing a razor blade through the midrib and lamina. Sections were placed in glycerin and stained with phloroglucinol and concentrated hydrochloric acid to observe the lignin-containing tissue.[23].

Powdered drug microscopy

Glycerin was used to mount the powdered leaves onto a glass slide for microscopic analysis. A Sony DSC WX200 digital camera captured photographs of magnified cellular structures[24].

Quantitative Microscopy

Standard procedures were applied to analyze leaf constants, including the palisade ratio, stomatal number, stomatal index, vein termination number, and vein islet number[25].

Preparation of Extracts

The leaves were washed with water, separated, and shade-dried to remove dirt and other foreign matter. A coarse powder was prepared from the dried plant material and passed through a No. 14. After drying, the powdered material was placed in the thimble tube of a Soxhlet apparatus and heated for six hours for extraction with various solvents, such as petroleum ether, ethyl acetate, and ethanol. For further study, the dried extract samples were kept in a refrigerator at a low temperature after being filtered, hot, and dried by rotary vacuum evaporation.

Qualitative phytochemical tests

Phytochemical analysis of various *G. bilamellata* leaf extracts, viz., PEGB, EAGB, and EEGB, was performed using standard methods[24].

Physiochemical analysis

Physiochemical parameters, including ash value, moisture content, foreign matter, fluorescence analysis, and extractive value, were determined according to standard procedures[24, 26].

Quantitative Phytochemical Analysis
Total Phenolic Content Estimation

This method was based on Singleton and Rossi[27] Folin–Ciocalteu reagent and was used to determine the total phenolic content of various *G. bilamellata* extracts by a colorimetric assay (n = 3). 1ml of plant extract, 1 ml of Folin-Ciocalteu reagent, and 1 ml of saturated sodium carbonate (35%) was mixed and incubated at room temperature in the dark for 90 min. After incubation, absorbance was measured at 725 nm against a blank. The results were expressed as mg of gallic acid equivalents (GAE) per gram of dry plant extract, using gallic acid as a standard.

Estimation of total flavonoid content

The method was based on Zhishen J *et al.,*[28]. The colorimetric assay determined the total flavonoid content of various extracts of *G. bilamellata* (n= 3). Mix 2 ml sample, 3 ml distilled water, and 500 µL of $NaNO_2$ (5%, w/v). After 6 min of incubation, AlCl3 solution (10%, w/v, 500 µL) was added, and the mixture solution was then combined with 4 mL of 4% sodium hydroxide solution and incubated for 15 min. The absorbance was measured at 510 nm against a blank. Using rutin equivalents (RE) as the indicator, we determined the flavonoid content in grams of dry extract per gram of flavonoid equivalents (FE).

In-vitro Antioxidant activity
DPPH and H_2O_2 radical scavenging activity

Various *G. bilamellata* extracts were subjected to DPPH[29] and H_2O_2[30] assays, as previously described. Each experiment was performed in triplicate, and the results were reported as IC_{50} values. In particular, ascorbic acid (standard drug) was used at each known concentration (20-100 µg/ml) of extracts, and ascorbic acid (standard drug) was used. The mixture was incubated at an appropriate temperature, time duration, and absorbance at a specific wavelength (DPPH: 37 °C, 30 min, 517 nm; H2O2:37 °C, 10 min, 230 nm wavelength.

In-silico Pharmacokinetic Analysis

Drug Likeliness and ADMET Analysis

Lipinski's rule of five evaluated the drug-likenesses of the compounds according to their structural and physicochemical properties using DruLito software[31]. The absorption, distribution, metabolism, excretion, and toxicity (ADMET) scores, which represent the pharmacokinetic properties of compounds, were predicted using the ADMETLab server [32].

Molecular Docking

Based on previous studies[33&34] molecular docking was used to explore the interaction mechanisms between the ligands and selected targets. XOD (PDB 1FIQ) and copper/zinc SOD (PDB 1CBJ) were used as targets for the simulations [35, 36]. The RSCB Protein Data Bank provided the crystallized structures, and PubChem provided 3D structural information on the ligands. The XOD and SOD structures were optimized, and the grid box parameters are listed in Table 1. To measure the docking parameters and stability of enzymes and ligands, Autodock Vina was executed, and root-mean-square deviations (RMSDs) were evaluated.

Table 1: Grid box coordinators used in Autodock Vina for Molecular Docking.

Centre	**x**	**y**	**z**
SOD (PDB ID: 1CBJ)	3.919000	19.127625	43.770675
XOD (PDB ID: 1FIQ)	30.401321	52.871509	98.007472
Size	**x**	**y**	**z**
	10	10	10
Exhaustiveness	8		

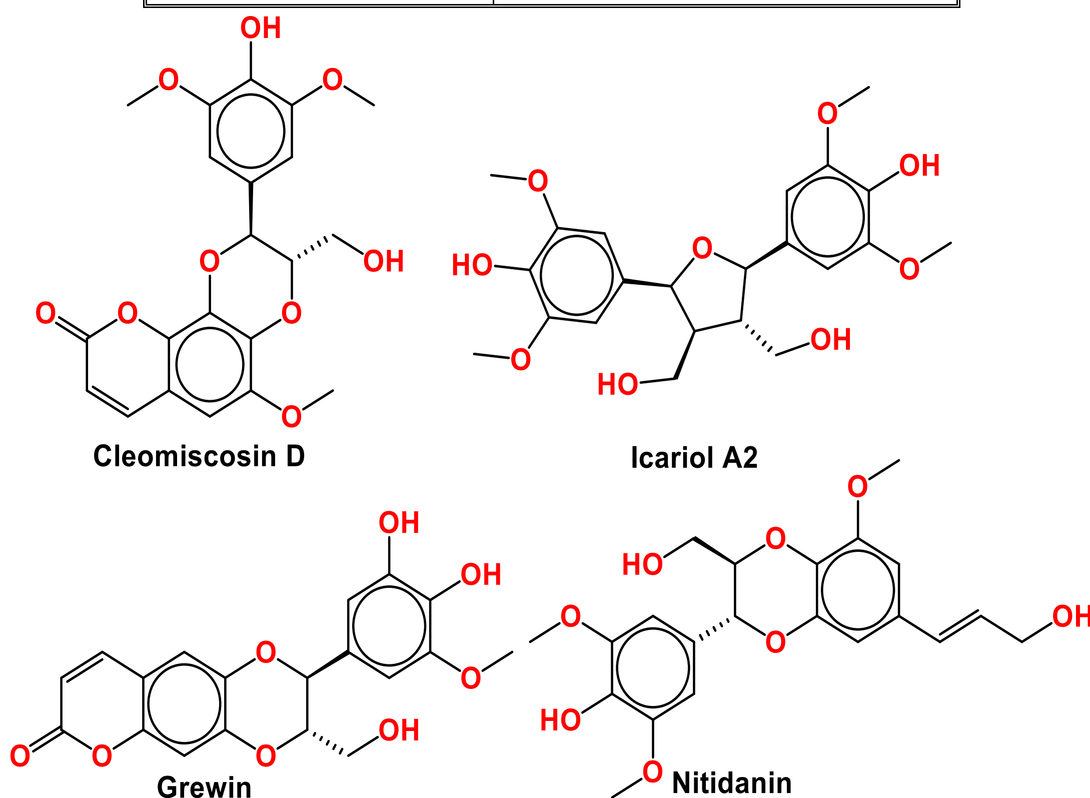

Fig. 1: 2D representation of phytoconstituents isolated from *G. bilamellata.*

Statistical Analysis

Data are presented as the mean ± standard deviation (SD) based on triplicate experiments. There is a plot depicting the percentage of scavenging activity. The IC_{50} values were derived by plotting the scavenging rates against the concentration gradient in triplicate. Statistical significance was set at $P < 0.05$.

RESULTS AND DISCUSSION

Macroscopic characters of the leaf of G. bilamellata

Table 2 provides a detailed examination of the morphology and characteristics of *the G. bilamellata* leaves.

Table 2: Macroscopic characters of *G. bilamellata.*

Characters	Leaf Morphology
Leaf	Simple, Alternate
Shape	Circular
Size	1.5-4 cm
Texture	Rough
Apex	Rounded
Margin	Toothed
Base	Symmetrical
Surface	Dorsal: Green Ventral: Pale Green
Color	Green
Venation	Reticulate
Taste	Characteristic
Odour	Characteristic
Petiole	Petiolate

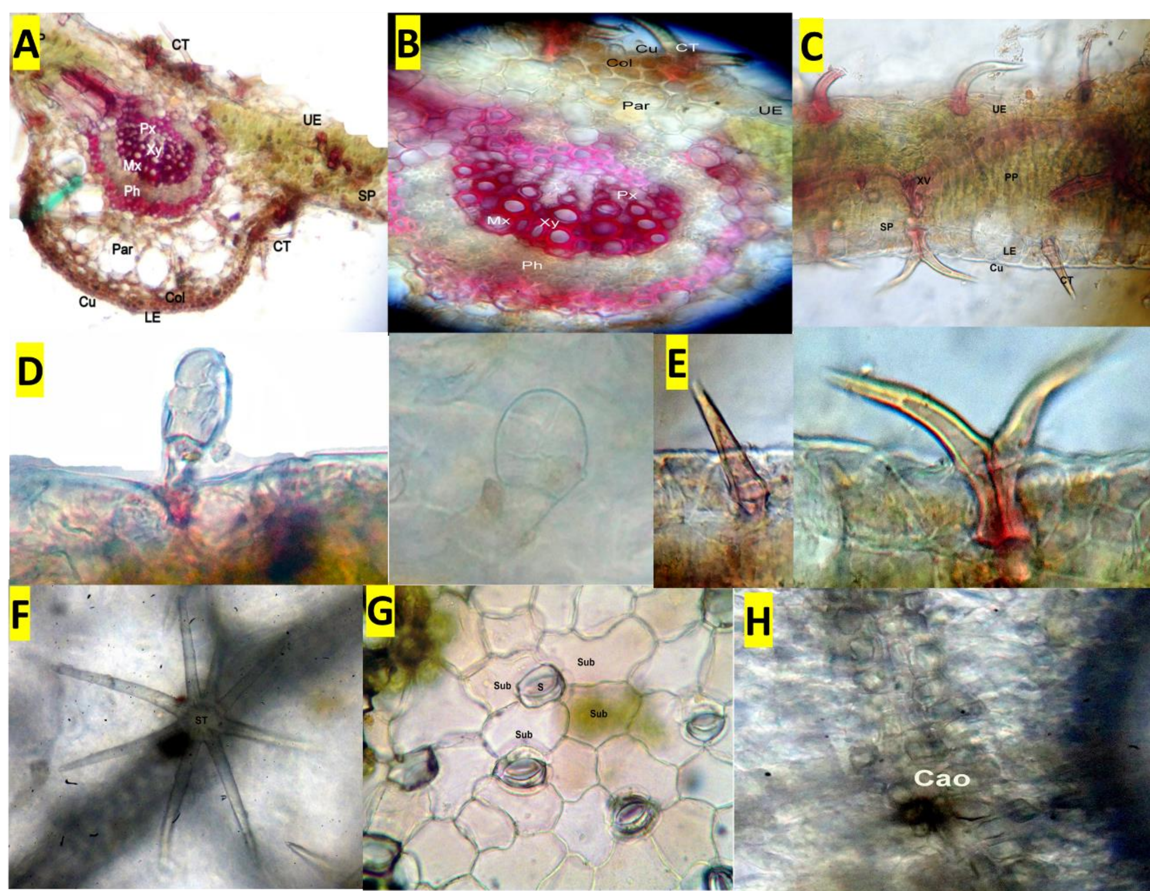

Fig. 2: Microscopiacal charcterstics of *G. bilamellata*. (A) Phloroglucinol and Conc. HCl stained TS of the leaf (B) Vascular bundles arrangements in the TS of the midrib of the leaf, (C) TS of Lamina portion, (D, E) Leaf showed covering and glandular trichomes, (F) Stellate hairs, (G) Anomocytic Stomata, (H) Calcium oxalate crystals.

Transverse Section of leaf

The Transverse Section of the leaf shows a well-developed upper and lower epidermis, covered with a thin cuticle and composed of thin-walled rectangular cells **(Fig. 1 A, B).** Anomocytic stomata were observed in the upper and lower epidermis **(Fig. 2G**). More stomata were observed on the abaxial surface than on the adaxial surface. The lamina portion of the leaf comprises palisade and spongy mesophyll tissues interposed between the upper and lower epidermis. The spongy parenchyma is loosely arranged **(Fig. 2C).** The epidermis of both regions contains simple uniseriate and multicellular covering trichomes, glandular trichomes with a unicellular stalk, and multicellular head **(Fig. 2E**). The collenchymatous tissue extends below the parenchymatous tissue. A bowl-shaped structure containing phloem and thick-walled xylem elements formed the midrib vascular strands **(Fig. 2B).** The abaxial surface of the leaf has stellate hairs and prismatic-shaped calcium oxalate crystals, as shown in the **fig. 2H.**

Powder Microscopy

The leaf powder was green. It revealed stellate hairs **(Fig. 3A**), a multicellular head with unicellular stalk glandular trichomes **(Fig. 3B**), multicellular, uniseriate covering trichomes **(Fig. 3C**), prismatic calcium oxalate crystals **(Fig. 3D),** anomocytic stomata **(Fig. 3E),** and annular xylem vessels **(fig. 3F).**

Quantitative Microscopy

Table 3 shows the leaf constants for *G. bilamellata* based on the various parameters investigated.

Physicochemical Parameters

Table 4 summarizes the physicochemical properties of *G. bilamellata* leaves. The results are presented as the average of three separate measurements rounded to the nearest standard deviation (SD).

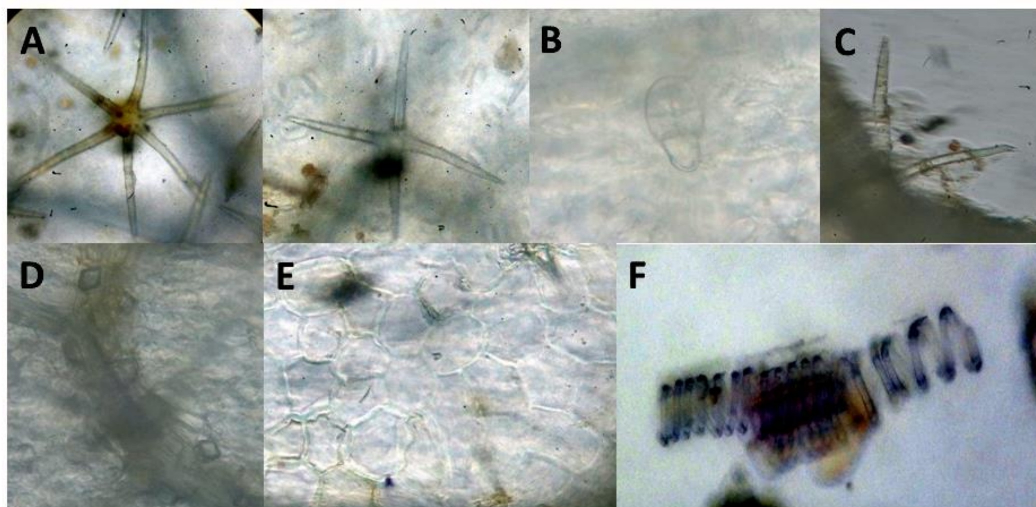

Fig. 3: Powder Microscopy of Leaf of *G. bilamellata*. (A) Stellate Hairs, (B) Multicellular Head with uniseriate glandular trichomes, (C) multicellular uniseriate covering trichomes, (D) prismatic calcium oxalate crystals, (E) anomocytic stomata, and (F) spiral-shaped xylem vessels.

Table 3: Leaf constants of *G. bilamellata* leaf.

Parameters	Range (Per Sq.mm)
Stomatal number	27.27 - 37.5
Stomatal Index	12.2-16.25
Vein Islet number	18.31 - 26.31
Vein Termination number	20.27 - 26.22
Palisade ratio	12.2 - 16

Table 4: Physico-chemical parameters of leaves of *G. bilamellata.*

WHO parameters	Values (% w/w)
Foreign Matter	2.11 ± 0.821
Moisture Content	8.72 ± 1.52
Total Ash	9.11 ± 2.47
Acid Insoluble Ash	2.33 ± 0.66
Water Soluble Ash	1.45 ± 0.87
Pet. ether soluble extractive	2.5 ± 0.36
Ethyl acetate soluble extractive	4.98 ± 0.55
Ethanol soluble extractive	8.52 ± 1.73

Qualitative Phytochemical Analysis

The initial phytochemical screening of the PEGB, EAGB, and EEGB extracts was carried out, and the results are displayed in **Table 5.**

Quantitative Phytochemical Analysis

The Folin-Ciocalteu colorimetric method was used to evaluate the total phenolic content of various extracts of *G. bilamellata*. **Table 3** shows that the phenolic content of EEGB and EAGB was found to be 24.08±2.51 and 14.23 ± 0.52 mg GAE/g. Extracts differ in phenol content based on the polarity of the solvent used. There is evidence that plant extracts obtained using polar solvents contain higher concentrations of phenols than those obtained using nonpolar solvents [37].

The highest phenolic concentration was found in EEGB, which demonstrated the highest Antioxidant activity. Phenols possess Antioxidants that enable them to scavenge free radicals, such as reactive oxygen species, and protect against oxidation processes [38].

Flavonoids, which are secondary metabolites, have Antioxidant properties, and their strength depends on the number and position of the free hydroxyl groups. There was a 26.21 ± 1.51 and 12.22 ± 0.98 mg RE/g of flavonoids in EEGB and EAGB (**Table 6, Fig. 4**). The flavonoid content in EEGB was nearly twice that of EAGB. The findings from this study indicate the influence of solvent polarity on flavonoid concentrations in the extracts.[39].

Table 5: Preliminary phytochemical analysis of various extracts of *G. bilamellata.*

S. No	Phytochemical	Test Name	Results		
			PEGB	EAGB	EEGB
1	Alkaloids	Mayers	-	+	+
		Wagners	-	+	+
2	Flavonoids	Shinoda	-	+	+
		Alkaline	-	+	+
3	Tannins	$Fecl_3$	-	+	+
		Lead acetate	-	+	+
4	Steroids	Salkowski	+	-	+
		Liebermann- Burchard	+	-	+
5	Volatile oils	-	+	-	+
6	Saponins	Foam	-	-	+
7	Glycosides	-	-	-	+
8	Carbohydrates	Molisch	-	-	+
9	Proteins	Biuret	-	-	+
		Millons	-	-	+
10	Amino acids	Ninhydrin	-	-	+
11	Fixed oils	-	+	-	+

(+) **Present (-) Absent**

Table 6: Total Phenolic and flavonoid content of *G. bilamellata* extracts.

Name of the Extract	Total Phenolic Content (mg GAE /g)	Total flavonoid content (mg RE/g dry extract)
EAGB	14.23 ± 0.52	12.22 ± 0.98
EEGB	24.08±2.51	26.21 ± 1.51

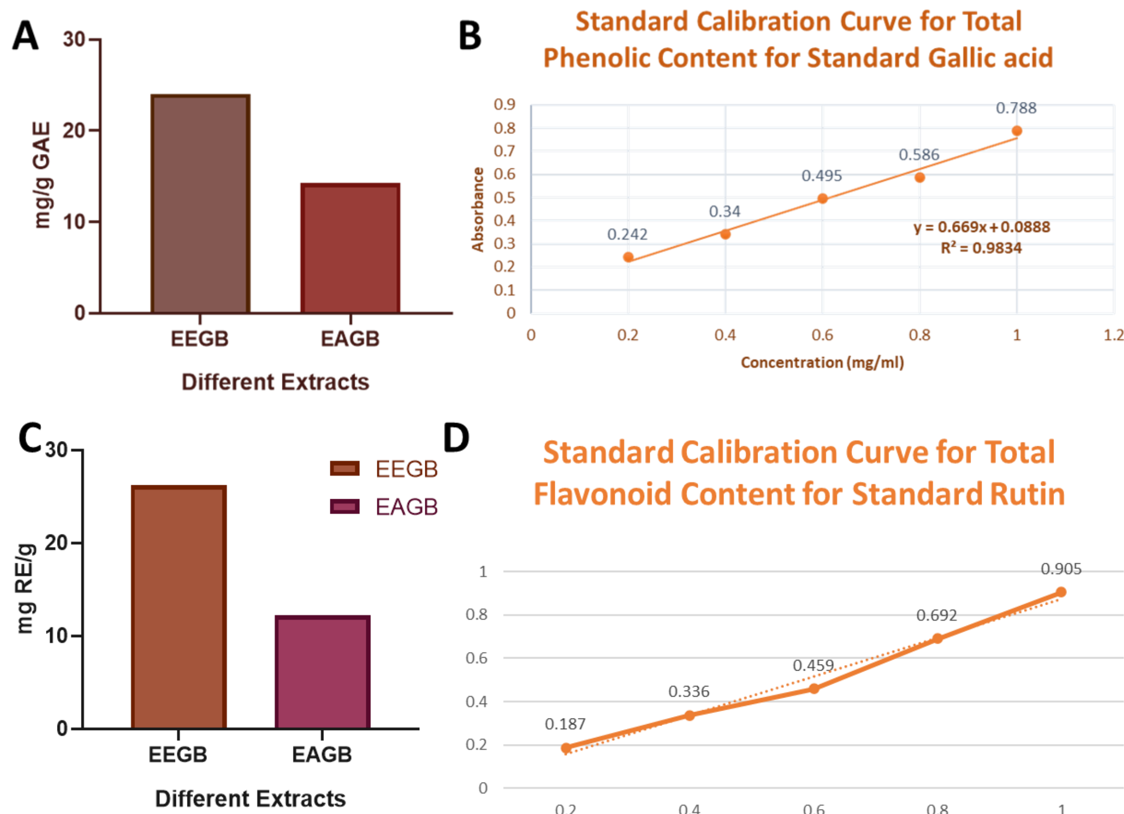

Fig. 4: (A) Quantitative estimation of total phenolic content (TPC) in EEGB and EAGB
(B) Standard calibration curve for total phenolic content for standard gallic acid
(C) Quantitative estimation of total flavonoid content (TFC) in EEGB and EAGB
(D) Standard calibration curve for total flavonoid content for standard rutin.

Invitro Antioxidant Activity

The Antioxidant potentials of EEGB, EAGB, and PEGB were evaluated and compared using two representative assays (DPPH and H_2O_2) in parallel because of the different scavenging modes of ROS and the complexity of natural phytochemicals. The EEGB of *G. bilamellata* exhibits significant DPPH scavenging potential (IC_{50} = 59.96 g/mL; **Table 7).** Figure 5A shows the free radical scavenging activity percentage determined using the DPPH Method. EEGB showed better H_2O_2 free radical scavenging activity, with an IC_{50} of 59.24, as shown in Fig. 5B. Due to higher concentrations of phenolics and flavonoids in EEGB, there was more significant activity than the other extracts in donating electrons to hydrogen peroxide.

Table 7: Scavenging activity of *G. bilamellata* extracts against DPPH free radicals and H_2O_2 radicals according to their dose.

Method	Treatment	% Scavenging activity					IC_{50}
		20 µg/ml	40 µg/ml	60 µg/ml	80 µg/ml	100 µg/ml	
DPPH Free radical Scavenging activity	EEGB	28.22±1.52	38.55±2.84	49.26±0.55	62.18±3.11	71.88±2.62	59.96
	EAGB	18.55±0.81	24.62±1.78	32.81±2.11	44.16±3.62	56.94±1.62	87.64
	PEGB	12.22±0.42	16.15±2.58	23.54±1.22	29.66±0.82	31.22±3.52	141.08
	Ascorbic acid	32.52±0.144	48.22±2.41	61.52±1.89	73.52±0.85	86.21±2.51	50.99
H_2O_2 Free radical Scavenging activity	EEGB	31.22±1.52	42.81±0.821	56.31±3.47	68.36±2.65	73.91±0.152	59.24
	EAGB	16.22±1.62	26.62±3.96	34.21±2.52	48.91±1.58	61.22±1.22	81.619
	PEGB	10.58±2.22	14.52±1.51	26.32±2.85	34.52±0.08	41.84±1.08	118.06
	Ascorbic acid	38.52±3.84	53.26±2.78	62.85±1.62	78.22±1.47	90.22±1.58	50.11

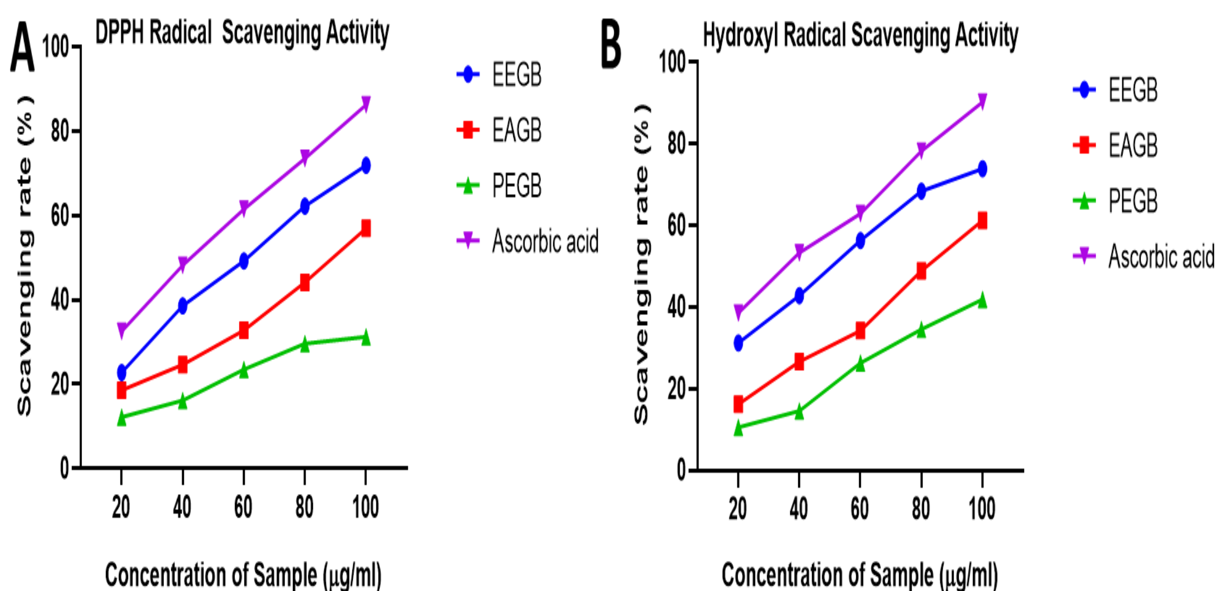

Fig. 5: Antioxidant activity of various extracts of *G. bilamellata* using DPPH and H_2O_2 methods.

Insilico Pharmacokinetics

Drug Likeliness

Drug likeness is the fundamental evaluation of drug candidates in the drug discovery and development stages. This measure ties a substance's physical and chemical characteristics with its biopharmaceutical characteristics in the human body, particularly its effect on oral bioavailability. The DruLito program was used to study the physicochemical characteristics of the nine selected active chemicals[32]. **Table 8** shows that all selected phytocompounds obey Lipinski's rule of five.

ADMET Analysis

The ADMET attributes of the ligands were studied using admetSAR (http://lmmd.ecust.edu.cn/admetsar2/) and Protox-II (https://tox-new.charite.de/protox_II/) web servers. The predicted ADMET properties of the selected phytocompounds are listed in **Table 9**.

Table 8: Drug Likeliness of the Selected Phytoconstituents from *G. bilamellata.*

Sr. No.	Title	MW	logp	Alogp	HBA	HBD	TPSA	AMR	Lipinski's Rule Violation
1	Cleomiscosin D	397.97	1.61	-1.571	9	0	72.45	112.84	No
2	Icariol A2	411.99	-0.222	-2.892	3	0	46.15	62.97	No
3	Grewin	357.97	1.478	-1.137	8	0	53.99	101.15	No
4	Nitidanin	381.97	1.324	-1.376	8	0	46.15	114.57	No

Table 9: ADMET analysis of Selected Phytoconstituents from *G. bilamellata.*

Phytocompounds	ADMETSAR							PROTOX-II					
	HIA	CaCO2	BBB	CYP1A2	CYP2C19	CYP2C9	CYP2D6	LD50 (mg/kg)	Hepatotoxicity	Carcinogenicity	Immunotoxicity	Mutagenicity	Cytotoxicity
Nitidanin	0.9753	0.6881	0.5932	0.8383	0.5269	0.7991	0.8238	1190	Active	Inactive	Active	Inactive	Inactive
Cleomiscosin D	0.9390	0.6283	0.8052	0.9270	0.8518	0.7504	0.8725	550	Inactive	Inactive	Active	Inactive	Inactive
Grewin	0.8991	0.6553	0.9	0.8545	0.7948	0.7512	0.8910	3000	Inactive	Inactive	Active	Inactive	Inactive
Icariol A2	0.9704	0.6356	0.7159	0.5133	0.7748	0.64	0.8003	1500	Inactive	Inactive	Active	Inactive	Inactive

The desired drugs should be non-toxic and have good ADME properties. Drug likeness is a significant factor to consider during the initial pharmaceutical discovery and development phases. [40]. Using these descriptors, it is possible to determine whether a compound is absorbed, distributed, metabolized, or excreted, as well as if it is toxic. Although there are different in vitro methods for establishing ADMET profiles, in silico determination is a faster, cheaper, and life-saving method for determining ADMET profiles [41].

In addition to being non-toxic, ideal drug candidates should exhibit acceptable ADME characteristics. We examined ADME profiles based on ProTox-ii and admetSAR, including drug-likeness, partition coefficients, solubility, HIA, BBB, and toxicity profiles of the identified molecules **(Table 9)**[42].

Molecular Docking

Molecular docking analysis was performed on phytoconstituents isolated from *G. bilamlellata* using ascorbic acid as a reference to understand better the mechanism by which these ligands interact with SOD and XOD. The four compounds from *G. bilamellata* displayed different SOD and XOD binding levels, as shown in **Tables 10 and 11 (Figs. 6 and 7).**

Analysis of the molecular simulation outcomes of the identified phytomolecules against xanthin oxidase (1FIQ) illustrated that these compounds have varying affinities with the enzyme, which allow them to function as inhibitors, ranging between -7.5 kcal/mol (Nitidanin) and -6 kcal/mol (Icariol A2) (Table 4). Consequently, they display Antioxidant properties by restraining the liberation of reactive species. **Table 8** provides information on these interactions.

The high-affinity compound (nitidanin) showed various interactions. All the ligands displayed better binding energies than the reference ascorbic acid. The results revealed that these phytochemicals had better Antioxidant activity than the standard.

The order of binding energy based on the outcome of the molecular docking exploration of the chosen phytochemicals and SOD was Cleomiscosin D > Grewin> Nitidanin > Icariol A2 > Ascorbic acid **(Table 7).** These ligands showed better binding energies than the standard. Based on amino acid interactions, ASN A:86, GLU A:100, ALA B:346, and VAL A:342 are vital amino acids that play an essential role in the stability–protein complex of the ligand. None of the compounds formed electrostatic interactions. All the ligand interactions are depicted in **Fig 7**.

Table 10: Interactions of Xanthine Oxidase (PDB ID:1FIQ) amino acid residues with ligands of *G. bilamellata* at the active site of the receptor.

Ligands	Binding Affinity, ΔG (Kcal/mol)	Amino acids involved and Distance (Å)		
		Hydrogen-Bond Interactions	Hydrophobic Interactions	Electrostatic Interactions
Nitidanin	-7.5	THR B:262 (4.21), ASN B:351 (4.40), ARG A:426 (6.87)	VAL A:342 (4.65), LEU A:74 (5.82), ASN B:351 (3.94), ALA B: 346 (3.92, 7.35), SER A:347 (3.64)	ARG A:426 (6.87)
Cleomiscosin D	-7.5	ASP B:360 (3.40)	PHE B:337 (5.19, 7.59), VAL A:342 (4.55), ALA B:346 (6.54), GLY B:350 (3.86), SER B:359 (4.17), THR B:354 (4.37)	-
Grewin	-7.1	SER B:347 (3.32), ARG B:394 (6.62)	ALA B: 346 (5.36), ASN B:351 (3.43)	GLU B:263 (5.37, 6.57), ARG B:426 (8.30, 8.52)
Icariol A2	-6	ILE B: 358 (3.93)	GLY B:350 (6.28), ILE B:266 (6.95), SER B:359 (3.68), VAL B:342 (5.11), LEU B:305 (5.45), ALA B:346 (3.84), PHE A:337 (5.80)	ARG A:426 (7.99), GLU B:263 (5.73)
Ascorbic acid	-6.1	SER B:347 (3.15)	-	-

Table 11: Interactions of Superoxide dismutase (PDB ID:2C9V) amino acid residues with ligands of *G. bilamellata* at the active site of the receptor.

Ligands	Binding Affinity, ΔG (Kcal/mol)	Amino acids involved and Distance (Å)	
		Hydrogen-Bond Interactions	Hydrophobic Interactions
Cleomiscosin D	-6.2	SER A:98 (3.55), GLU A:100 (4.09, 5.37), ASN A:80 (4.33)	VAL A:97 (3.55), SER A:98 (6.18), ILE A:99 (4.28, 4.89), LYS A:75 (5.05), PRO A:74 (5.15), GLY A:85 (4.33)
Grewin	-6.1	ASP A:96 (5.34), SER A:98 (3.30)	ILE A:99 (5.73), LYS A:75 (4.87, 6.10), PRO A:74 (5.03), ASN A:86 (4.86)
Nitidanin	-4.8	LEU A:84 (4.56)	ILE A:99 (5.83), LEU A:42 (5.01), PRO A:74 (4.43, 5.11), LYS A:75 (3.78), LEU A:126 (4.99)
Icariol A2	-4.8	-	PRO A:74 (4.01), ILE A:99 (4.26), LYS A:75 (5.06), SER A:98 (5.19), LEU A:42 (4.50), ASN A:86 (5.03)
Ascorbic acid	-4.4	ASN A:86 (4.22), GLU A:100 (3.81), SER A:98 (4.66), LEU A:84 (5.30)	

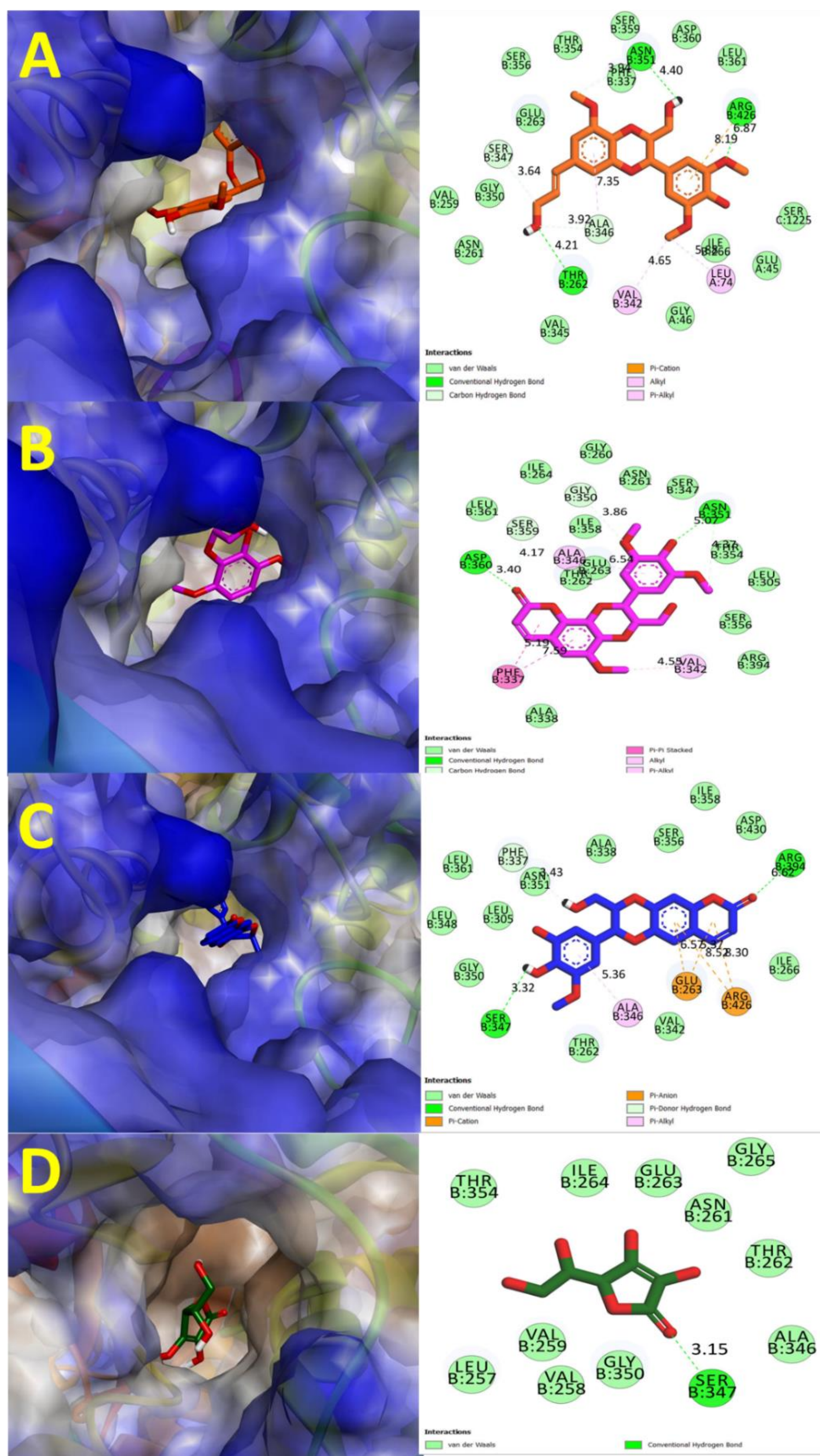

Fig. 6: Surface representation and 2D binding poses of various ligands from *G. bilamellata* to Xanthine Oxidase (PDB ID:1FIQ). (A) Nitidanin, (B) Cleomiscosin D, (C) Grewin, and (D) ascorbic acid.

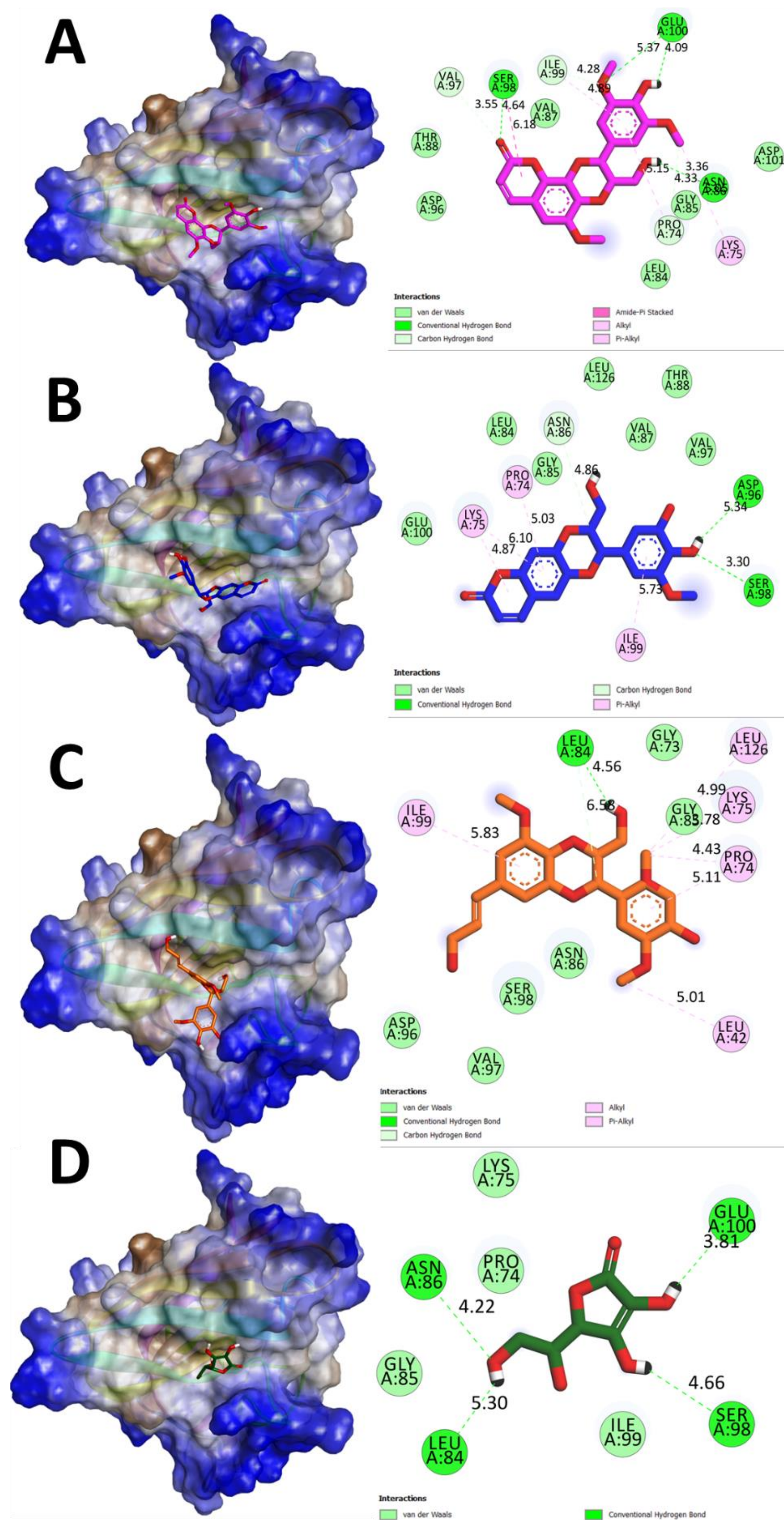

Fig. 7: Surface representation and 2D binding poses of various ligands from *G. bilamellata* to Superoxide dismutase (PDB ID:2C9V) (A) Cleomiscosin D, (B) Grewin, (C) Nitidanin and (D) Ascorbic acid.

This investigation has determined that morphological, physicochemical, and anatomical characteristics are indispensable for taxonomic data and the demarcation of genera and species. These details willow the recognition of the species from its co-species and prohibit the plant from being corrupted. It has been suggested that polyphenols, flavonoids, and other phytoconstituents of *G. bilamellata* may contribute to its Antioxidant activity. Based on *Insilico* studies, the reported phytoconstituents, Cleomiscosin D, Grewin, and Nitidanin, have been shown to have better Antioxidant properties against SOD and XOD. These results confirm that the ethnomedicinal use of *G. bilamellata* might be a resource for scavenging free radicals.

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نشرة العلوم الصيدليـــة
جامعة أسيوط

تقييم عقاقيرى و كيميائي، والنشاط المضاد للأكسدة لأوراق نبات جرويا بيلاميلاتا جاجنب (زيزفونية)

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يهدف هذا البحث إلى تحليل التقييم العقاقيرى و الكيميائي، والمحتوى الفينولى و الفلافونويد الكلي، والنشاط المضاد للأكسدة لأوراق نبات جرويا بيلاميلاتا جاجنب. تشتمل هذه الدراسة العقاقيرية على الملاحظة المجهرية، وخصائص الأوراق، والتحليلات الكيميائية و الكيميائية الفيزيائية، والمحتوى الكلي من الفينول والفلافونويد. تم فحص النشاط المضاد للأكسدة باستخدام ١،١ diphenyl-2-picrylhydrazyl- (DPPH) و H2O2. تُظهر الورقة ثغورًا شاذة ، و شعيرات غدية ونجمية. ويصل المحتوى الفينولى و الفلافونويد الكلي إلى أقصى حد في المستخلص الإيثانولي (EEGB)(٩٠,٦٩ ± ٢,٨٦ mg GAE/g) and ,(٢٦,٢١ ± ١,٥١ mg RE/g) على التوالى. وأظهر المستخلص الإيثانولي أشد نشاط كمضاد للاكسدة بقيم ٥٩,٢٤ ، ٥٩,٩٦ IC50 ميكروجرام/ مل. وقد ارتبطت نتائج المحتوى الفينولى و الفلافونويد الكلي مع اختبارات الكسح باستخدام DPPH و H2O2 ارتباطًا إيجابيًا.

كما ارتبطت نتائج النشاط المضاد للأكسدة مع طريقة استخدام الحاسوب باستخدام اهداف زانثين أوكسيديز (XOD) وسوبر اوكسيد ديسموتاز .(SOD) و تعتبر المعايير الميكروسكوبية والفيزيائية الكيميائية ذات قيمة للمصادقة على تحديد ونقاء الدواء. يعتبر نبات جرويا بيلاميلاتا مصدر منظور محتمل لمضادات الأكسدة.