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Apoptosis: In-Silico And In-Vitro Investigations Were Combined To Analyse The Acetylcholine: Bax Protein: Biomarker Derived From Clenome Gynandra Leaves

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Background: Virtual screening methods are promising and useful for designing new herbal remedies to satisfy numerous clinical needs. A major issue with the extensive use of herbs is the lack of information regarding the mechanisms of their action. **Materials and methods:** The aim of the present research was the molecular docking of compounds identified in the studied herbs to investigate the binding mechanism with suitable targets. The phytochemical screening, and free radical scavenging assay (FRAP, ABTS, DPPH, H_2O_2). **Results and discussion:** The presence of flavonoids, terpenoids, phenols, sterols, and glycosides were observed in the preliminary test. In the radical scavenging assay, ascorbic acid showed regression values FRAP (R^2=0.9834), ABTS (R^2=0.9865), DPPH (R^2 =0.9746), and H_2O_2 (R^2=0.988). In silico molecular docking, the binding energy of silico molecular docking was excellent, and the ADME profile was superior. Which have significant medicinal functions. **Conclusion:** The presence of these molecules helps the plant in identifying the diseases that are claimed by ethnomedicine. To establish the molecular mechanism of action of these metabolites, further work is required.

KEYWORDS: Clenome gynandra, Preliminary screening, In-vitro assay, In-silico molecular docking simulation

INTRODUCTION

The World Health Organization's (WHO) data suggests that the reliance on traditional medicine is alarmingly high in developing countries, particularly in India. [1] According to the WHO, approximately 80% of the Indian population depends on traditional medicine as their primary healthcare solution (WHO, 2005).[2] This figure is even more staggering in other rising

economies, where the rate of traditional medicine usage reaches up to 90% of the total population. [2] The reliance on plant-derived medicines is a testament to the remarkable versatility of the natural world. Nearly 40% of all marketed drugs have their origins in medicinal plants, showcasing the profound impact these botanical resources have had on the pharmaceutical industry. From alleviating ailments to providing crucial nutritional support, the role of medicinal plants extends far beyond their traditional uses. [3,4] Cleomaceae, a family of plants used for medicinal purposes, includes C. gynandra in its list of uses. This herb can be eaten and can reach a length of 0.5 to 1 m. It grows well in domestic drainage systems and is found in tropical and subtropical regions. [5] The phytochemical constitution includes ascorbic acid, violaxanthin, α and β-c] Thee, α, β, and γ-tocopherol, β-cryptoxanthin, and luteolin. The oil content contains polyunsaturated fatty acids which have health benefits, and this oil extracted by hand pressing. This is the cheapest and most rich assessable source of vitamins (A and C), protein (23.4%), and (8.3), essential minerals. [6] leaves possess higher concentrations of calcium, iron, phosphorus, potassium, and vitamin C. Gynandra uses nutraceuticals and can treat neuralgia, chronic malaria, diarrhea, hypotension, and hypertension. Gynandra leaf juice has been used in traditional systems of medicine to facilitate convulsion, childbirth, stomachache, threadworm inflection, and certain biliary disorders. [7] Meda N.T.R (2013), C. gynandra previous studies have shown that Gc-Ms analysis of ethyl acetate extract C. gynandra was performed- was observed that some very important molecules such as n-Hexadecenoic acid, 4-(2,4-Dimethylcyclohex-3-enyl)but-3-en-2-one, 3,7,11,15-Tetramethyl-2-hexadecen-1-ol, 9,12,15-Octadecatrienoic acid, methyl ester, (Z, Z, Z)-, 2-((Octan-2-yloxy)carbonyl)benzoic acid, Methyl stearidonate, dl-.alpha.-Tocopherol, Campesterol, Stigmasterol, .beta.-Sitosterol, i-Propyl 9,12,15-octadecatrienoate, which have important medicinal roles . –Sowunmi and Afolayan (2015), Phytochemical Constituents and Antioxidant Properties of Acetone Extract of C. Gynandra. Growing in the Eastern Cape, South Africa. it possesses high secondary metabolites which accounts for its strong antioxidant ability thus justifying its use as naturally occurring antioxidants in folkloric medicine. The study encourages regular consumption of this wild vegetable to avert oxidative stress-related diseases. [8] Meda (2013), Antioxidant activity of phenolic and flavonoid fractions of C. gynandra and Maerua angolensis of Burkina Faso. [9] Agustinus Widodo, Ritha Pratiwi (2018), Phytochemical screening of the ethanol extract was carried out qualitatively according to the standard methods. [10] Kommu S (2022), phytochemical screening and qualitative determination of phytochemicals in hydroalcoholic 70% ethanol in water and solvent to sample ratio 10:1 extract of C. gynandra L. Whole plant. [11] Consequently, we aim to investigate combining in-silico and in-vitro studies to evaluate the acetylcholine and Bax Protein of different accessions and the biomarker obtained from Clenome gynandra. The purpose of this study is to improve our understanding of C. gynandra and its potential for valuable and sustainable use, therefore contributing to food security and public health. As a result, there is still a need to research and confirm its nature in scientific and traditional medicine.

MATERIAL AND METHODS

MATERIAL

Chemical and Reagents

Dilute iodine solution, Wagner's reagent, Dragendorff's reagent, zinc powder, hydrochloric acid, sodium hydroxide solution, ferric chloride, Molisch's reagent, chloroform, gelatine, 10% lead acetate solution, methanol, ethanol, 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2'-azino-bis 3-Ethylbenzothiazoline-6- sulfonic acid (ABTS), Potassium persulfate, hydrogen peroxide solution, 50 mM phosphate buffer, Ascorbic acid (AA), 1% potassium ferricyanide, 10% Trichloro acetic acid (TCA), 0.1% ferric chloride purchased from Sigma-Aldrich Lab.

METHODS

Authentication af Specimen

C. gynandra was collected in an area of the Nagamangala market in the month of May. The specimen was authenticated by a Pharmacognosiste professor Dr. Nandeesh, Sree Siddaganga University, B.H. Road, Tumkur. specimen number SSCP/No-04/22.

Preparation of Plant Extract

The fresh 200g leaves of C. gynandra were washed and shade-dried to make a fine powder using a portable electric blender to reduce them into powder form. The collected powder was mixed with (70:20 v/v) Hydroethanolic solvent for 72 hours by cold maceration process. The homogenate was filtered via muslin cloth followed by Whatman No. 1 filter paper and lyophilized to obtain a dry powder of leaf extract. The lyophilized powder extract will be stored in air-tight amber-colour bottles, Yield percentage (w/w) from the dried extracts was calculated. [11]

Phytochemical Screening Test

The preliminary phytochemical analysis is done to verify the presence or absence of carbohydrates, protein, amino acids, alkaloids, glycosides, terpenoids, and Quantitation of total phenolic, flavonoid content, and tannic acid.[12]

Estimation of TPC

Briefly, 20 µL of C. gynandra (10 mg/mL) was ethanol to 5.0 mL with distilled water and 0.5 mL of 1: 1 FC reagent was added to this solution. After 5 minutes of incubation, 5.0 mL of 7% sodium carbonate was added and the mixture was incubated for 30 minutes at room temperature and then the absorbance was measured at 765 nm. Gallic acid was used as a standard. The results were expressed as µg of gallic acid equivalents per milligram of plant material (µg GAE/mg plant material). [12]

Estimation of TFC

Briefly, 20 µL of C. gynandra (10 mg/mL) was diluted to 5.0 mL with distilled water and then 0.1 mL of 10% $AlCl_3$ and 0.1 mL of 1 M potassium acetate were added. This mixture was incubated at 37°C for 40 minutes and the absorbance was measured at 415 nm. Quercetin was used as a standard, and the flavonoid concentration in C. gynandra

was calculated. The results were expressed as µg of quercetin equivalents per milligram of plant material (µg QE/mg plant material). [12]

Estimation of TTC

About 0.1 ml of the C. gynandra extract was added to a volumetric flask (10 ml) containing 7.5 ml of distilled water and 0.5 ml of Folin- Ciocalteu phenol reagent, 1 mL of 35% sodium carbonate solution, and dilute to 10 ml with distilled water. The mixture was shaken well and kept at room temperature for 30 min. A set of reference standard solutions of tannic acid (20, 40, 60, 80, 100 µg/ ml) were prepared in the same manner as described earlier. Absorbance for test and standard solutions was measured against the blank at 700 nm with a UV/ Visible spectrophotometer. The tannin content was expressed in terms of mg of tannic acid equivalents/ g of dried sample. [13,14]

IN-VITRO ANALYSIS

DPPH assay

The reaction mixture consisted of 0.5 mL of C. gynandra, 3 mL of methanol, and 0.3 mL of 0.5 mM 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical solution in ethanol. After incubation for 45 min, absorbance was determined in a spectrophotometer at 517 nm. [15]

ABTS assay

ABTS assay, the of equal amounts of 7.4 mM ABTS and 2.45 mM potassium persulfate was going to prepare a working solution that is put away in 12-13 h dark incubation to react and produce active ABTS radical cation which is the reaction solution with C. gynandra sample to estimate antioxidant activity. Ethanol was used for making the dilution of the ABTS solution. Then, 50 µL of samples and 1.9 ml of ABTS solution were transferred into a test tube and allowed to dark incubate for 6 mines. After incubation, absorbance was recorded at 734 nm. [16]

H_2O_2 assay

H_2O_2 assay, twenty millimolars of hydrogen peroxide solution were prepared in 50 mM phosphate buffer (pH 7.4). 0.4 mL of C. gynandra or ascorbic acid with varying concentrations (0-10 µg/mL) were mixed with 0.6 mL of hydrogen peroxide solution. After incubation for 10 minutes at room temperature, the absorbance was measured against the blank at 240 nm. The blank consisted of phosphate buffer and test C. gynandra without hydrogen peroxide. [16]

FRAP assay

FRAP assay, 1 mL of C. gynandra with different concentrations (0-10 ug/mL) was mixed with 2.0 mL of phosphate buffer (50 mM, pH 7.0) and 2.5 ml of 1% potassium ferricyanide. The C. gynandra was incubated at 50°C for 20 minutes followed by the addition of 2.5 ml of 10% TCA. The reaction mixture was centrifuged at 3000 × g for 10 minutes 1.25 ml supernatant from each sample was mixed with 1.25 ml of distilled

water and 0.5 ml of 0.1% ferric chloride. Absorbances were measured at 700 nm. Ascorbic acid (AA) was used as a standard.[17]

GC-MS ANALYSIS

The Clarus 680 GC was used in the analysis employed a fused silica column, packed with Elite-5MS (5% biphenyl 95% dimethylpolysiloxane, 30 m × 0.25 mm ID × 250μm), and the components were separated using Helium as carrier gas at a constant flow of 2 mL/min. The injector temperature was set at 280°C during the chromatographic run. The 1 μL of extract sample was injected into the instrument the oven temperature was as follows: 100°C (2 min); followed by 200°C at the rate of 10 per min and 200°C, where it was held for 3min and then followed by 300°C at the rate of 25 per min; it was held for 10 min. The mass detector conditions were an Inlet line temperature of 250°C; an ion source temperature of 230°C; an ionization mode electron impact at 70 eV, a scan time of 0.2 sec, and a scan interval of 0.1 sec. The fragments from 40 to 440 Da. The spectrums of the components were compared with the database of a spectrum of known components stored in the GC-MS NIST library (2014).

COMPUTERATED MOLECULAR DOCKING SIMULATION

Preparation of Ligands: From the literature, we selected the set of thirty phytoconstituents structures, known for their brain stimulant action. The phytoconstituents were extracted in their 2D conformation from PubChem and were in SDF format. Donepezil was used as the reference standard, as the first line of treatment for various neurological problems.

Retrieval of Protein Structure and Preparation

The X-ray-co-crystallized structures of the protein molecules were chosen from the Protein data bank, and the receptor file was saved in "Mol2" format. Molecular Docking Studies: The Schrodinger software was used to load the potential binding sites between the various ligands and the target protein. Prediction of protein–ligand interactions are performed. software. The docking results were then compared with the docked result of the reference ligand obtained from the corresponding PDB ID. The docking scores, 2D pose views, as well as the binding affinities of the selected natural compounds, and verifying ADME / Lipinski rule 5, were analysed for further investigation. The best-docked phytoconstituent for neuroprotective action was identified based on binding energy and interaction with amino acid residues.

STATISTICAL ANALYSIS

All the values are expressed as mean±SEM (n=6) statistical analysis by one-way ANOVA followed by Tukey's test.

RESULTS

Table: 1 Phytochemical screening of C. gynandra

Sl. No	Phytoconstituents	Observation plant extract
	Chemical constituent	Hydroethanolic extract (70%)
1.	Alkaloid	Negative

2.	Carbohydrates	Positive
3.	Proteins & Amino Acids	Negative
4.	Steroids	Positive
5.	Glycosides	Positive
6.	Saponins	Positive
7.	Flavonoids	Positive
8.	Tannins	Positive
9.	Triterpenoids	Positive
10.	Phenolics	Positive

Table 02: Quantitative Estimation of Total phenolic and flavonoids, Tannic acid content of extracts Clenome gynadra

Clenome gynadra	**Total phenolic (mg/g)**	**Total flavonoids (mg/g)**	**Total** Tannic acid **(mg/g)**
Hydroethanolic	49.67 ± 0.22	17.3 ± 1.21	38.73 ± 0.15

Quantitative estimation of TPC and TFC and TTC of extracts Clenome gynadra.

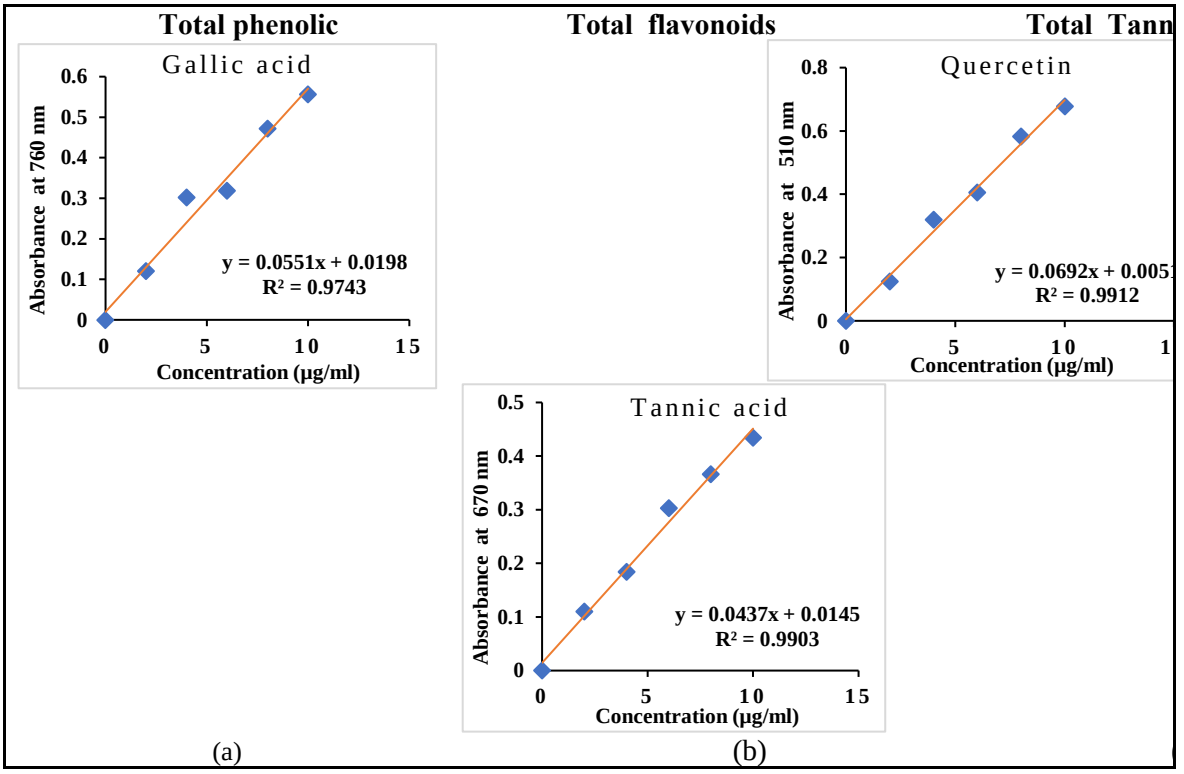

Figure 1: Standard Curve of Gallic acid, Quercetin, and Tannic acid

Table 03: In-vitro radical scavenging activity Assay

Clenome gynadr	FRAP	ABTS	DPPH	H_2O_2
Hydroethanolic	16.88 ± 0.08	25.70 ± 0.21	0.95 ± 0.13	43.09 ± 0.21

In-vitro Radical Scavenging activity

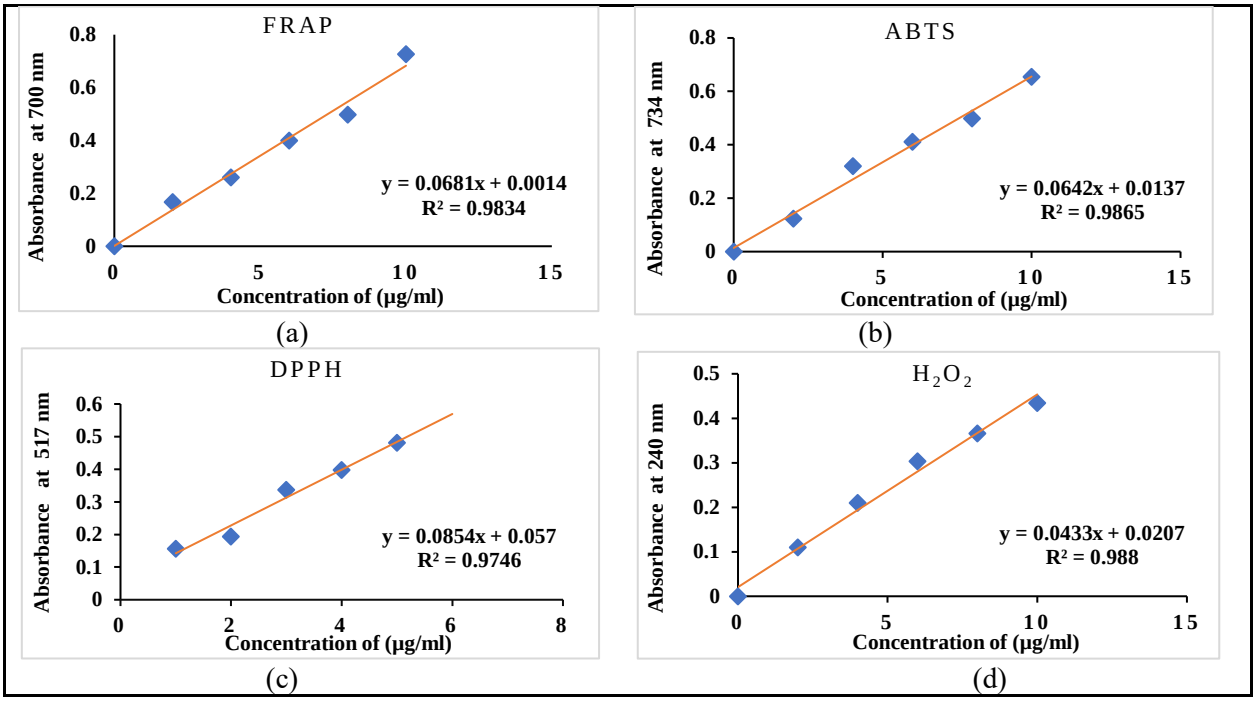

Figure 2: Radical scavenging activity Standard Curve of Ascorbic acids a, b c, and d.

Table 04: Bioactive derivative of C. gynandra in GC-MS analysis

Sl.No	Compound	MF	MW	Peak Area (%)
1.	l-Leucine	$C_{27}H_{51}NO_3$	437	89.3
2.	Isonipecotic acid	$C_{27}H_{51}NO_3$	437.3	3.95
3.	Leucine, N-(thiophen-2-carbonyl)-, tetradecyl ester	$C_{25}H_{43}NO_3S$	437.2	3.64
4.	S-Triazine	$C_{17}H_{17}F_6N_5O_2$	437.1	1.44
5.	N-Trifluoro acetyl-O	$C_{18}H_{30}F_3NO_4Si_2$	437.1	0.98
6.	Benzenepropenoic acid	$C_{28}H_{23}NO_4$	437.1	0.24
7.	4-(4-Diethylamino-2-methylphenylimino)-1-oxo-N-phenyl-1,4-dihydro-2-naphthalenecarboxamide	$C_{28}H_{27}N_3O_2$	437.2	0.15
8.	Isonipecotic acid	$C_{25}H_{37}F_2NO_3$	437.2	0.15

9.	N-(5-Hydroxy-1,2-dimethoxy-6,7,8,9-tetrahydro-5H-benzo[a]cyclohepten-6-yl propenamide ditms	$C_{22}H_{39}NO_4Si_2$	437.2	0.04
10.	Benzamide	$C_{24}H_{27}F_4NO_2$	437.1	0.02
11.	Dihydrocapsaicin	$C_{21}H_{37}NO_3Si$	379.2	7.99
12.	1,2-Dioxin-3-acetic acid, 6-hexadecyl-3,6-dihydro-6-methoxy-methyl ester	$C_{24}H_{44}O_5$	412.3	7.99
13.	6-quinolinamine	$C_{20}H_{20}N_4O_4$	380.1	7.99
14.	3-(1H-Indol-3-ylmethyl)-7-methyl-5-phenyl-1,3-dihydro-benzo[e][1,4]diazepin-2-one	$C_{25}H_{21}N_3O$	379.1	7.99
15.	16,19-Secostrychnidine-10,16-dione, 14-hydroxy-19-methyl	$C_{22}H_{24}N_2O_4$	380.1	7.99
16.	Voachalotine oxindole	$C_{22}H_{24}N_2O_4$	380.1	7.99
17.	N,N'-Bisethoxymethylene-2,6-diamino-4,8-dihydro-4,8-dioxo-benzo(1,2-b:4,5-b')difuran-3,7-dicarbonitrile	$C_{18}H_{12}N_4O_6$	380	7.99
18.	Naphtho[2,3-c] furan-1(3H)-one, 9-(1,3-benzodioxol-5-yl)-7-hydroxy-4,8-dimethoxy	$C_{21}H_{16}O_7$	380	7.99
19.	Vomicine	$C_{22}H_{24}N_2O_4$	380.1	7.99
20.	2-Naphthalenol, 1-[[2-methyl-4-[(2-methyl phenyl) azo] phenyl] azo]	$C_{24}H_{20}N_4O$	380.1	4.84
21.	Pyridine-2,6-dimethanol, O, O'-bis[4-[(butylamine)methyl] phenyl]	$C_{29}H_{35}N_3O_2$	457.2	63.6
22.	Phosphinous bromide, bis[2-(trifluoromethyl)phenyl]	$C_{14}H_8BrF_6P$	399.9	4.12
23.	N-(5-Bromo-quinoline-8-yl)-3,3,3-trifluoro-2-trifluoromethyl-propionamide	$C_{13}H_7BrF_6N_2O$	399.9	3.07
24.	Spirostan-3-one, (5β,25S)	$C_{27}H_{42}O_3$	414.3	3.07
25.	Naphthalene, 1,1'-(1,10-decanediyl) bis[decahydro	$C_{30}H_{54}$	414.4	2.35
26.	N-(3-Bromo-4-hydroxy-phenyl)-4-nitro-benzenesulfonamide, N,O-dimethyl	$C_{14}H_{13}BrN_2O_5S$	399.9	1.52
27.	3-Methoxy-2,4,5-trifluoro benzoic acid, 2,4-dichloro naphthyl-1 ester	$C_{18}H_9Cl_2F_3O_3$	399.9	1.01
28.	2-Thiophenecarboxylic acid, 4-(chloromethyl)-5-pentadactyl-, methyl ester	$C_{22}H_{37}ClO_2S$	400	0.93
29.	3-Carbomethoxy-5,5-dimethyl-4-	$C_{21}H_{21}ClN_2O_4$	400.1	0.78

	phenyl-2-[N'-[p-chlorophenyl] ureido]-4,5-dihydro furane			
30.	Fumaric acid	$C_{21}H_{14}Cl_2O_4$	400	0.75

In-SILICO DOCKING SIMULATION

Pro-apoptosis crystal

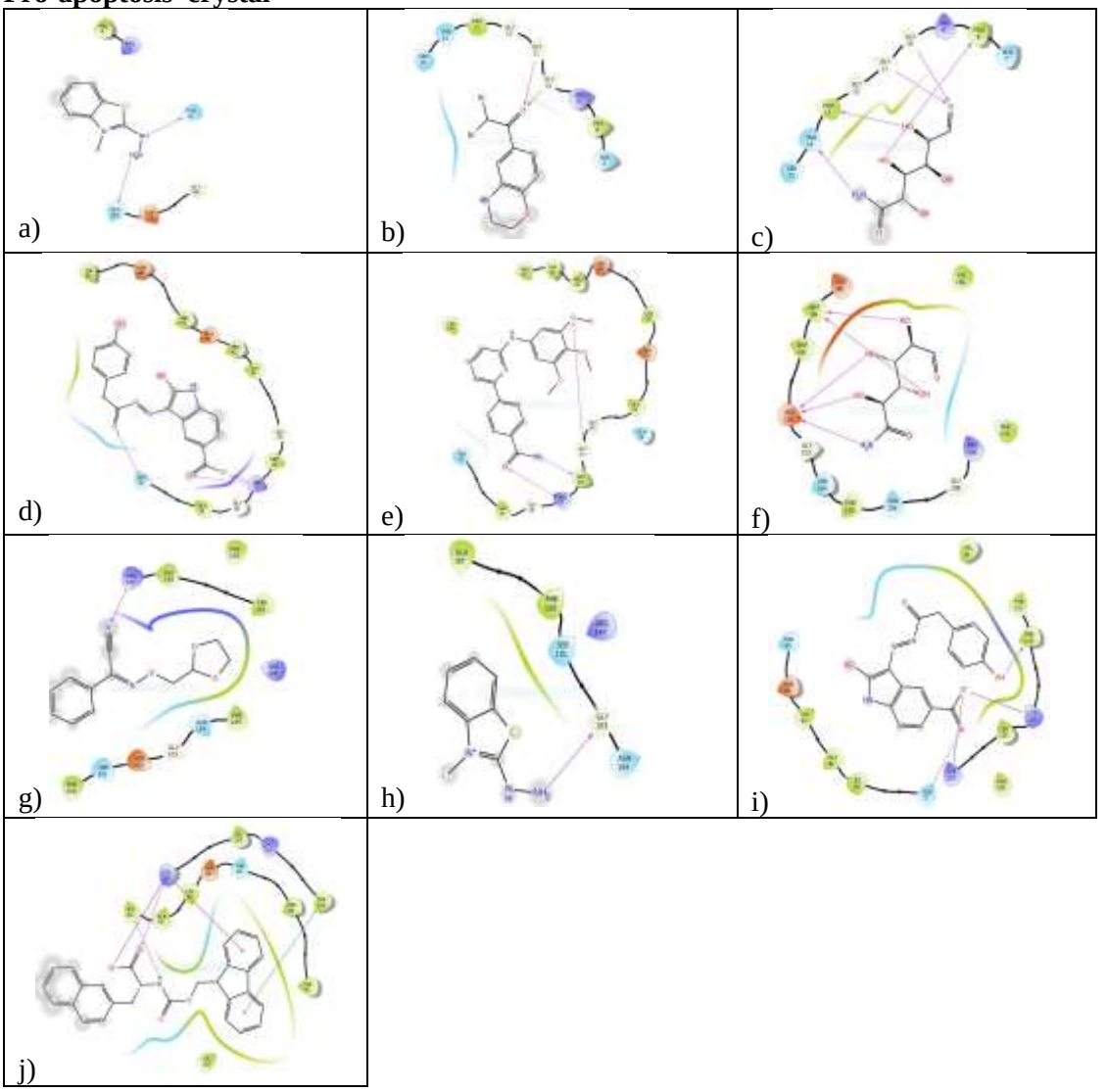

Figure 3: Protein-ligand interaction of pro-apoptotic crystal

ACETYLCHOLINE CRYSTAL

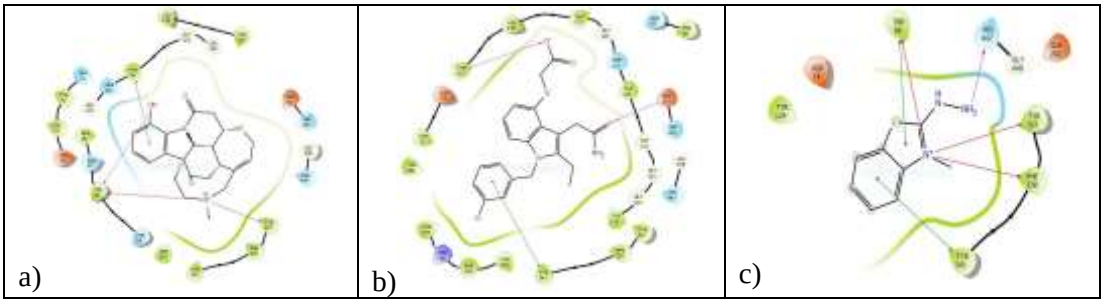

Figure 4: Protein-ligand interaction of acetylcholine

Table 05: List of interaction of Pro-apoptosis BAX protein on C. gynandra ligands

Sl. N	Compound Name	MF	MW	RB	DS	LogP	RMSD	Iupac_Inchi key
1.	3-methyl-1,3-benzothiazole-2-one hydrazone	$C_8H_9N_3S$	179.24	1	-5.589	-0.224	19.146	PHOLIFLKGONSGY-NTMALXAHSA-N
2.	(2S,3S,4S,5R)-2,3,4,5-tetrahydroxy-6-oxohexanamide	$C_6H_{11}NO_6$	193.15	9	-5.314	-0.266	25.213	JRIOKBXQMHEJOZ-QTBDOELSSA-N
3.	2,2-dibromo-1-(3,4-dihydro-2H-1,4-benzoxazin-6-yl)ethanone	$C_{10}H_9Br_2NO_2$	334.99	2	-5.107	-1.028	27.008	FAFPNGFYWAOZHF-UHFFFAOYSA-N
4.	4-[6-(3,4,5-trimethoxyanilino)-2-pyrazinyl]benzamide	$C_{20}H_{20}N_4O_4$	380.4	7	-5.058	-1.544	20.839	LZLKDDDQYGMZTB-UHFFFAOYSA-N
5.	(2Z)-2-(1,3-dioxolan-2-ylmethoxyimino)-2-phenyl acetonitrile	$C_{12}H_{12}N_2O_3$	232.23	4	-4.415	-0.607	23.081	WFVUIONFJOAYPK-

								SDNWHVSQSA-N
6.	2-hydroxy-3-[2-(4-hydroxyphenyl)-1-oxoethyl]azo-1H-indole-5-carboxylic acid	$C_{17}H_{13}N_3O_5$	339.3	7	-3.971	-0.763	24.705	DXMNZMFMHRNRIL-UHFFFAOYSA-N
7.	2-(2,3-dihydro benzofuran-5-yl)-5-[(4-methoxyphenyl) methylthio]-1,3,4-oxadiazole	$C_{18}H_{16}N_2O_3S$	340.4	5	-3.88	-1.506	24.56	KDBVFKKUBSCXLU-UHFFFAOYSA-N
8.	(1S,10S,22R,23R,24S)-15-hydroxy-4-methyl-9-oxa-4,13-diazahexacyclo[11.6.5.01,24.06,22.010,23.014,19]tetracosa-6,14(19),15,17-tetraene-12,20-dione	$C_{22}H_{24}N_2O$	380.4	1	-10.65	-4.057	53.452	ZMTYENXGROJCEA-LNKPQSDASA-N
9.	2-[[3-(2-amino-2-oxoethyl)-1-[(3-chlorophenyl)methyl]-2-ethyl-4-indolyl]oxy]acetic acid	$C_{21}H_{21}ClN_2O_4$	400.9	8	-9.536	-3.504	53.091	LYSUZOPMYPBNJF-UHFFFAOYSA-N
10.	3-methyl-1,3-benzothiazol-2-one hydrazone	$C_8H_9N_3S$	179.24	1	-9.277	-1.834	55.876	PHOLIFLKGONSGY-NTMALXAHSA-N
11.	(2R)-2-[[9H-fluoren-9-ylmethoxy(oxo)methyl]amino]-3-(2-naphthalenyl)propanoic acid	$C_{28}H_{23}NO_4$	437.5	8	-8.831	-5.084	54.677	JYUTZJVERLGMQZ-AREMUKBSSA-N
12.	(2R)-2-[[9H-fluoren-9-ylmethoxy(oxo)methyl]amino]-3-(2-naphthalenyl)propanoic acid	$C_{28}H_{27}N_3O_2$.	437.5	8	-8.831	-5.084	54.677	JYUTZJVERLGMQZ-AREMUKBSSA-N
13.	2,4,5-trifluoro-3-methoxy benzoic acid (2,4-dichloro-1-naphthalenyl) ester	$C_{18}H_9Cl_2F_3O_3$	401.2	4	-8.805	-3.77	56.94	ROSUSCOOUSFPSY-UHFFFAOYSA-N
14.	2-hydroxy-3-[2-(4-hydroxyphenyl)-1-	$C_{17}H_{13}N_3O_5$	339.3	7	-8.682	-3.081	54.825	DXMNZMFMHRNRIL-

	oxoethyl]azo-1H-indole-5-carboxylic acid							UHFFFAOYSA-N
15.	4-[6-(3,4,5-trimethoxyanilino)-2-pyrazinyl]benzamide	$C_{20}H_{20}N_4O_4$	380.4	7	-8.426	-4.013	56.543	LZLKDDDQYGMZTB-UHFFFAOYSA-N
16.	4-(1-imidazolylmethyl)-2-[2-(4-phenyl phenyl)ethoxy]benzonitrile	$C_{25}H_{21}N_3O_1$	379.5	7	-8.384	-4.091	52.651	ZMHFYICSWNADEI-UHFFFAOYSA-N
17.	(1S,10S,22R,23R,24S)-15-hydroxy-4-methyl-9-oxa-4,13-diazahexacyclo[11.6.5.01,24.06,22.010,23.014,19]tetracosa-6,14(19),15,17-tetraene-12,20-dione	$C_{22}H_{24}N_2O4_1$	380.4	1	-8.295	-2.189	50.46	ZMTYENXGROJCEA-LNKPQSDASA-N
18.	2-(3,4-dichlorophenyl)-5-hydroxy-3-benzo[g]benzofurancarboxylic acid ethyl ester	$C_{21}H_{14}Cl_2O_4$	401.2	5	-8.169	-4.145	53.955	IECRKIGLIYLQST-UHFFFAOYSA-N
19.	9-(1,3-benzodioxol-5-yl)-4-hydroxy-6,7-dimethoxy-3H-benzo[f]isobenzofuran-1-one	$C_{21}H_{16}O_7$	380.3	4	-8.11	-2.782	55.513	VMEJANRODATDOF-UHFFFAOYSA-N
20.	2-(2,3-dihydrobenzofuran-5-yl)-5-[(4-methoxyphenyl)methylthio]-1,3,4-oxadiazole	$C_{18}H_{16}N_2O_3S$	340.4	5	-7.918	-3.658	54.555	KDBVFKKUBSCXLU-UHFFFAOYSA-N
21.	1-[(2,6-difluoro-3-methylphenyl)-oxomethyl]-4-piperidinecarboxylic acid undecyl ester	$C_{25}H_{37}F_2NO_3$	437.6	14	-7.465	-3.941	57.641	QDAVBCMMOWIPJV-UHFFFAOYSA-N
22.	(2Z)-2-(1,3-dioxolan-2-ylmethoxyimino)-2-phenylacetonitrile	$C_{12}H_{12}N_2O_3$	232.23	4	-7.456	-2.094	54.253	WFVUIONFJOAYPK-SDNWHVSQSA-N
23.	1-(4-methoxyphenyl)-4-phenyl-1-butanone	$C_{17}H_{18}O_2$	254.32	6	-7.297	-3.028	54.954	XBNJLAPERJEQAO-UHFFFAOYSA-N

24.	N-[2-[(4-bromophenyl)methoxy]-4-nitrophenyl]methanesulfonamide	$C_{14}H_{13}BrN_2O_5S$	401.23	6	-7.272	-2.672	54.722	ZJHDHIFNJKSKJN-UHFFFAOYSA-N
25.	1-chloro-4-hexadecylsulfonylbenzene	$C_{22}H_{37}ClO_2S$	401	16	-7.096	-4.86	52.14	DZQLGPCNQDVJIK-UHFFFAOYSA-N
26.	2,2-dibromo-1-(3,4-dihydro-2H-1,4-benzoxazin-6-yl)ethanone	$C_{10}H_9Br_2NO_2$	334.99	2	-7.055	-2.212	53.181	FAFPNGFYWAOZHF-UHFFFAOYSA-N
27.	2-[[oxo-(7-oxo-5-phenyl-1H-pyrazolo[1,5-a]pyrimidin-3-yl)methyl]amino]acetic acid	$C_{15}H_{12}N_4O_4$	312.28	5	-7.007	-2.474	54.334	YWRBFQXZTWMBLH-UHFFFAOYSA-N
28.	(1S,2S,4S,5'R,6R,7S,8R,9S,12S,13R,16S)-5',7,9,13-tetramethyl-16-spiro[5-oxapentacyclo[10.8.0.02,9.04,8.013,18]eicos-18-ene-6,2'-oxane]ol	$C_{27}H_{42}O_3$	414.6	1	-6.911	-3.558	54.187	WQLVFSAGQJTQCK-VKROHFNGSA-N
29.	1-(3-methyl-1-oxo butyl)-4-piperidine carboxylic acid hexadecyl ester	$C_{27}H_{51}NO_3$	437.7	20	-6.804	-3.397	55.076	OBOFWDUNCIRFIH-UHFFFAOYSA-N
30.	4-(1,1,1,3,3,3-hexafluoropropan-2-yloxy)-N-(4-methyl phenyl)-6-(4-morpholinyl)-1,3,5-triazine-2-amine	$C_{17}H_{17}F_6N_5O_2$	437.34	7	-6.553	-2.215	53.972	FVFOZCBFMTVJII-UHFFFAOYSA-N

Table 06: List of the interaction of Acetylcholine protein on C. gynandra ligands

Sl. N	Compound Name	MF	MW	RB	DS	Log P	RMSD	Iupac_Inchikey
1.	(1S,10S,22R,23R,24S)-15-hy 4-methyl-9-oxa-4,13-diazahexacyclo-19-tetracosa-6,14(19),15,17-tetraene-12,2(	$C_{22}H_{24}N_2O_4$	380.4	1	-10.863	-4.057	55.034	ZMTYENXGROJCEA-LNKPQSDASA-N
2.	2-[3-(2-amino-2-oxo-ethyl)-1-[(3-chlorophenyl)methyl]-2-	$C_{21}H_{21}ClN_2O_4$	400.9	8	-9.536	-3.504	55.537	LYSUZOPMYPBNJF-

	ethyl-indol-4-yl]oxyacetic acid							UHFFFAOYSA-N
3.	3-methyl-1,3-benzothiazole-2-one hydrazone	$C_8H_9N_3S$	179.24	1	-9.277	-1.834	51.934	PHOLIFLKGONSGY-NTMALXAHSA-N
4.	(2R)-2-(9H-fluorene-9-ylmethoxycarbonylamino)-3-(2-naphthyl)propanoic acid	$C_{28}H_{23}NO_4$	437.58	1	-8.831	-5.084	54.112	JYUTZJVERLGMQZ-AREMUKBSSA-N
5.	(2R)-2-(9H-fluoren-9-ylmethoxycarbonylamino)-3-(2-naphthyl)propanoic acid	$C_{28}H_{23}NO_4$	437.58	1	-8.831	-5.084	53.791	JYUTZJVERLGMQZ-AREMUKBSSA-N
6.	(2,4-dichloro-1-naphthyl) 2,4,5-trifluoro-3-methoxy-benzoate	$C_{18}H_9Cl_2F_3O_3$	401.24	4	-8.805	-3.77	53.145	ROSUSCOOUSFPSY-UHFFFAOYSA-N
7.	2-hydroxy-3-[2-(4-hydroxyphenyl)acetyl]azo-1H-indole-5-carboxylic acid	$C_{17}H_{13}N_3O_5$	339.3	7	-8.715	-3.081	51.737	DXMNZMFMHRNRIL-UHFFFAOYSA-N
8.	4-[6-(3,4,5-trimethoxyanilino)pyrazin-2-yl]benzamide	$C_{20}H_{20}N_4O_4$	380.4	7	-8.426	-4.013	54.034	LZLKDDDQYGMZTB-UHFFFAOYSA-N
9.	4-(imidazol-1-ylmethyl)-2-[2-(4-phenyl phenyl)ethoxy]benzonitrile	$C_{25}H_{21}N_3O$	379.5	7	-8.55	-4.091	54.021	ZMHFYICSWNADEI-UHFFFAOYSA-N
10.	(1S,10S,22R,23R,24S)-15-hydroxy-4-methyl-9-oxa-4,13-iazahexacyclo[11.6.5.01,24.06,22.010,23.014,19]tetracosa-6,14(19),15,17-tetraene-12,20-dione	$C_{22}H_{24}N_2O_4$	380.4	1	-8.508	-2.189	55.868	ZMTYENXGROJCEA-LNKPQSDASA-N
11.	ethyl 2-(3,4-dichlorophenyl)-5-hydroxy-	$C_{21}H_{14}Cl_2O_4$	401.25	5	-8.417	-4.145	55.788	IECRKIGLIYLQST-

	benzo[g]benzofuran-3-carboxylate							UHFFFAOYSA-N
12.	9-(1,3-benzodioxol-5-yl)-4-hydroxy-6,7-dimethoxy-3H-benzo-isobenzofuran-1-one	$C_{21}H_{16}O_7$	380.3	4	-8.11	-2.782	53.002	VMEJANRODATDOF-UHFFFAOYSA-N
13.	2-(2,3-dihydro benzofuran-5-yl)-5-[(4-methoxyphenyl)methylsulfanyl]-1,3,4-oxadiazole	$C_{18}H_{16}N_2O_3S$	340.4	5	-7.918	-3.658	54.834	KDBVFKKUBSCXLU-UHFFFAOYSA-N
14.	undecyl 1-(2,6-difluoro-3-methyl-benzoyl)piperidine-4-carboxylate	$C_{25}H_{37}F_2NO_3$	437.6	14	-7.465	-3.941	53.068	QDAVBCMMOWIPJV-UHFFFAOYSA-N
15.	(Z)-N-(1,3-dioxolan-2-ylmethoxy)benzimidoyl cyanide	$C_{12}H_{12}N_2O_3$	232.23	4	-7.456	-2.094	52.589	WFVUIONFJOAYPK-SDNWHVSQSA-N
16.	1-(4-methoxyphenyl)-4-phenyl-butane-1-one	$C_{17}H_{18}O_2$	254.32	6	-7.297	-3.028	53.984	XBNJLAPERJEQAO-UHFFFAOYSA-N
17.	N-[2-[(4-bromophenyl)methoxy]-4-nitro-phenyl]methanesulfonamide	$C_{14}H_{13}BrN_2O_5S$	401.23	6	-7.542	-2.672	54.701	ZJHDHIFNJKSKJN-UHFFFAOYSA-N
18.	ethyl 4-(1-methyl-5-nitro-benzimidazol-2-yl)butanoate	$C_{14}H_{17}N_3O_4$	291.3	7	-7.161	-1.748	56.355	VJVBGSJZBDBEIF-UHFFFAOYSA-N
19.	1-[4-(hexadecyl amino)phenyl]-2-methylsulfonyl-ethanone	$C_{25}H_{43}NO_3S$	437.7	19	-7.133	-3.904	53.737	HHUIKUMVRXDQPB-UHFFFAOYSA-N
20.	1-chloro-4-hexadecylsulfonyl-benzene	$C_{22}H_{37}ClO_2S$	401	16	-7.096	-4.86	54.033	DZQLGPCNQDVJIK-UHFFFAOYSA-N
21.	2,2-dibromo-1-(3,4-dihydro-2H-1,4-benzoxazin-6-yl)ethanone	$C_{10}H_9Br_2NO_2$	334.99	2	-7.055	-2.212	55.6	FAFPNGFYWAOZHF-

								UHFFFAOYSA-N
22.	2-[(7-oxo-5-phenyl-1H-pyrazolo[1,5-a]pyrimidine-3-carbonyl)amino]acetic acid	$C_{15}H_{12}N_4O_4$	312.28	5	-7.059	-2.474	56.05	YWRBFQXZTWMBLH-UHFFFAOYSA-N
23.	(1S,2S,4S,5'R,6R,7S,8R,9S,12S,13R,16S)-5',7,9,13-tetramethylspiro[5-oxapentacyclo[10.8.0.02,9.04,8.013,18]icos-18-ene-6,2'-tetrahydropyran]-16-ol	$C_{27}H_{42}O_3$	414.6	1	-6.911	-3.558	53.862	WQLVFSAGQJTQCK-VKROHFNGSA-N
24.	hexadecyl 1-(3-methylbutanoyl)piperidine-4-carboxylate	$C_{27}H_{51}NO_3$	437.7	20	-6.804	-3.397	57.014	OBOFWDUNCIRFIH-UHFFFAOYSA-N
25.	4-morpholino-N-(p-tolyl)-6-[2,2,2-trifluoro-1-(trifluoromethyl)ethoxy]-1,3,5-triazin-2-amine	$C_{17}H_{17}F_6N_5O_2$	437.34	7	-6.553	-2.215	53.355	FVFOZCBFMTVJII-UHFFFAOYSA-N
26.	4-benzyl-3-(4-ethoxy phenyl)-1H-1,2,4-triazole-5-thione	$C_{17}H_{17}N_3OS$	311.4	5	-6.513	-2.632	55.874	GBHZFUVNXPAEKT-UHFFFAOYSA-N
27.	N-(2,6-difluoro-3-methyl-benzoyl)-2,6-difluoro-3-methyl-N-octyl-benzamide	$C_{24}H_{27}F_4NO_2$	437.5	11	-6.434	-3.765	55.834	FSQGTRSCOAJXSS-UHFFFAOYSA-N
28.	1,6,6-tricyclohexylhexylcyclohexane	$C_{30}H_{54}$	414.7	9	-6.221	-4.389	54.289	VSAQRYVOTNSXBL-UHFFFAOYSA-N
29.	(3Z)-3-[[2-(1,1-dimethylallyl)-4,5-bis(3-methylbut-2-enyl)-1H-indol-3-yl]methylene]-6-methylene-piperazine-2,5-dione	$C_{29}H_{35}N_3O_2$	457.6	7	-6.07	-3.732	56.091	HPZFXKBNCMYWKJ-JLPGSUDCSA-N
30.	methyl 2-[(3S,6S)-6-hexadecyl-6-methoxy-3H-1,2-dioxin-3-yl]acetate	$C_{24}H_{44}O_5$	412.6	19	-5.899	-3.495	55.537	ZKFUKHCEACWDKL-VWNXMTODSA-N

DISCUSSION

C. gynandra was identified and standardized through pharmacological methods. A hydroethanolic extract of C. gynandra underwent qualitative phytochemical analysis, which included TPC, TFC, TTC, and antioxidant activity tests (FRAP, ABTS, DPPH, H_2O_2). Analysis of the extract indicated the presence of tannic acid, phenols, flavonoids, glycosides, terpenoids, and free anthraquinone, with phytochemical concentration visually assessed based on color intensity. Phenolic, flavonoid, and tannic compounds, are well known for their antioxidant properties, these secondary metabolites neutralize free radicals by donating hydrogen and terminating the chain for the presence of polyphenolic in the leaves of C. gynandra leaf the TPC of HEE was found to be 49.67±0.22 µg GAE/g. The TPC of the extract was calculated from the regression question of the calibration curve (R^2=0.9743). The TFC of the extract was calculated from the regression question of the calibration curve (R^2=0.9912), and flavonoid content was determined to be 17.3±1.21 mg QE/g. and TTC of the extract was calculated from the regression question of the calibration curve (R^2=0.9903), tannic content was determined found to be 38.73±0.15 mg TAE/g. Determination of antioxidant potential: FRAP Assay- antioxidants neutralize free radicals by donating an electron. The higher the reducing powder, the greater the absorbance of the reaction mixture. which was comparable to that of standard AA. (fig: 02 & Table 03). A concentration-dependent increase in absorbance was observed with both HEE (16.88 ± 0.08) and AA (R^2=0.9834). This observation reflects the HEE's ability to donate electrons, which may be a major contributor to neutralizing free radicals. Scavenging activity of ABTS Radical: The absorbance was observed with both HEE (25.70±0.21) and AA (R^2=0.9865). which was comparable to that of standard AA. (fig: 02 & Table 03). the results were expressed by mmol AA E/g. DPPH Radical: Antioxidant activity of the C. gynandra is measured by the standard curve of AA (fig: 02 & Table 03). The absorbance was observed with both HEE (0.95±0.13) and AA (R^2 = 0.9746). the results were expressed by mmol AA E/g. H_2O_2 Assay: The effect of HEE on H_2O_2 was investigated, and AA served as standard. The absorbance was observed with both HEE (43.09±0.21) and AA (R^2=0.988). which was comparable to that of standard AA. (fig: 02 & Table 03). the results were expressed by mmol AA E/g. Bioactive components identified in the Hydro-ethanol extract of C. gynandra by GC-MS: l-Leucine, Isonipecotic acid, Leucine, N-(thiophen-2-carbonyl)-, tetradecyl ester,S-Triazine, N-Trifluoro acetyl-O, Benzenepropenoic acid,4-(4-Diethylamino-2-methylphenylimino)-1-oxo-N-phenyl-1, 4-dihydro-2-naphthalenecarboxamide, Isonipecotic acid, N-(5-Hydroxy-1,2-dimethoxy-6,7,8,9-tetrahydro-5H-benzo[a]cyclohepten-6-yl) propenamide ditms, Benzamide, Dihydrocapsaicin, 1,2-Dioxin-3-acetic acid, 6-hexadecyl-3,6-dihydro-6-methoxy-methyl ester, Dihydrocapsaicin, 1,2-Dioxin-3-acetic acid, 6-hexadecyl-3,6-dihydro-6-methoxy-methyl ester, 6-quinolinamine, 3-(1H-Indol-3-ylmethyl)-7-methyl-5-phenyl-1,3-dihydro-benzo[e][1,4]diazepin-2-one,16,19-Secostrychnidine-10,16-dione, 14-hydroxy-19-methyl, Voachalotine oxindole,N,N'-Bisethoxymethylene-2,6-diamino-4,8-dihydro-4,8-dioxo-benzo(1,2-b:4,5-b')difuran-3,7-dicarbonitrile, Naphtho[2,3-c] furan-1(3H)-one, 9-(1,3-benzodioxol-5-yl)-7-hydroxy-4,8-dimethoxy, Vomicine, 2-Naphthalenol, 1-[[2-methyl-4-[(2-methylphenyl) azo] phenyl] azo], Pyridine-2,6-dimethanol, O'-bis[4-

[(butylimino)methyl] phenyl], Phosphinous bromide, bis[2-(trifluoromethyl)phenyl],N-(5-Bromo-quinolin-8-yl)-3,3,3-trifluoro-2-trifluoromethyl-propionamide, pirostan-3-one, (5β,25S), Naphthalene, 1,1'-(1,10-decanediyl) bis[decahydro, N-(3-Bromo-4-hydroxy-phenyl)-4-nitro-benzenesulfonamide, N,O-dimethyl, 3-Methoxy-2,4,5-trifluorobenzoic acid, 2,4-dichloronaphthyl-1 ester, 2-Thiophenecarboxylic acid, 4-(chloromethyl)-5-pentadecyl-, methyl ester, 3-Carbomethoxy-5,5-dimethyl-4-phenyl-2-[N'-[p-chlorophenyl] ureido]-4,5-dihydrofurane, Fumaric acid. Interaction between pro-apoptotic and acetylcholine protein: (a) 2(3H)-Benzothiazolone, 3-methyl-, hydrazone interact with THR14 and GUN153; (b) (2S,3S,4S,5R)-2,3,4,5-tetrahydroxy-6-oxohexanamide interact with THR14, PRO13, GLY11, GLY10, PRO8; (c) 2,2-dibromo-1-(3,4-dihydro-2H-1,4-benzoxazin-6-yl)ethenone interact with GLY11, GLY10; (d) 2-hydroxy-3-[[2-(4-hydroxyphenyl)acetyl]diazenyl]-1H-indole-5-carboxylic acid interact with GLN32, ARG37; (e) 2-[(7-oxo-5-phenyl-1H-pyrazolo[1,5-a]pyrimidine-3-carbonyl)amino]acetic acid interact with NET38,ARG37; (f) 4-[6-(3,4,5-Trimethoxyanilino)pyrazin-2-yl]benzamide interact with ASP102,MET99; (g) (2S,3S,4S,5R)-2,3,4,5-tetrahydroxy-6-oxohexanamide interact with GLY103; (h) 2(3H)-Benzothiazolone,3-methyl-,hydrazone interact with ARG147;(i) Oxabetrinil interact with GLN77, LYS123, LYS119,PHE116; (j) 2-hydroxy-3-[[2-(4-hydroxyphenyl)acetyl]diazenyl]-1H-indole-5-carboxylic acid interact with PHE116, LYE123. Acetylcholine protein interaction with (a) Vomicine interact with TRP86, TYR124, TYR337; (b) [3-Carbamoylmethyl-1-(3-chloro-benzyl)-2-ethyl-1H-indol-4-yloxy]-acetic acid interact with TYR133, GLH202, TYR341; (c) 2(3H)-Benzothiazolone, 3-methyl-, hydrazone interact with TRP86, TYR337, TYR341, PHE338, HIS447. Further research is needed to establish how these molecules may cure the diseases mentioned in ethno-medicine. Collaboration between traditional healers and scientific researchers is crucial to unlocking the full potential of these medicinal compounds. By combining age-old knowledge with modern analytical techniques, we can pave the way for innovative treatments that benefit both human health and traditional healing practices.

CONCLUSION

Bioactive compounds, especially phenolic compounds, play a key role in inhibiting free radical formation and possess antioxidant properties. Other phytocompounds also impact antioxidant activity, with glycosides showing therapeutic value. Polyphenolic compounds and other phytocompounds collectively influence antioxidants. Phytochemical screening of C. gynandra extract indicates high total phenolic content (TPC) and total tannin content (TTC). Among antioxidant assays, H_2O_2 exhibits the highest activity, while ABTS and FRAP show moderate antioxidative activity, and DPPH shows the least. Antioxidants are crucial for human health, addressing issues like aging, neurological disorders, obesity, gastrointestinal problems, and diabetes. They are enzymatic or non-enzymatic, with non-enzymatic antioxidants categorized into hydrophobic and hydrophilic types. Hydrophobic antioxidants protect cell membranes from lipid peroxidation, whereas hydrophilic one's dissolve blood and cytoplasm to counter free radicals. GC-MS analysis identified thirty different beneficial compounds in the hydro-ethanolic extract of C. gynandra, suggesting its therapeutic potential and the need for further exploration.

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There is no Ethical Approval needed for this study.

UNIQUENESS OF THE RESEARCH
Previous research suggests this plant possesses antioxidant and anti-inflammatory properties, although evidence is limited. Despite the ongoing study project, we have decided to focus on this species. Our team will conduct a thorough analysis of its benefits and applications, in line with our dedication to precise research. Stay tuned for updates on our progress as we explore the properties of this plant further.

CONFLICT OF INTEREST
The authors declare that we have no competing financial investments or personal relationships. Which can influence the results of the study and do not have any conflict with any other research work.

ABBREVIATIONS
C. gynadra: Clenome gynadra; **HEE:** Hydro ethanolic extract; **TPC:** Total phenolic content; **TFC:** Total flavonoid content; **TTC:** Total tannic acid content; **FRS:** Free radical scavenging activity; **DPPH:** 2,2-diphenyl-1-picrylhydrazyl; **ABTS:** (2,2'-azino-bis 3-Ethylbenzothiazoline-6- sulfonic acid); **H_2O_2:** Hydrogen peroxide scavenging; **TCA:** Trichloro acetic acid, **NaOH:** Sodium hydroxide; **FC:** Folin–Ciocalteu; **GC-MS:** Gas chromatography-mass spectroscopy; PDB: Protein data bank; **NIST:** National institute of standard and technology; **MF:** Molecular formula; **MW:** Molecular weight; **RB:** Rotatable bond; **DS:** Docking Score; **Logp:** Lipophilicity; **RMSD:** Rotatable Mean Square Deviation.

REFERENCE
1. Nyeko R, Tumwesigye N.M, Halage A.A. Prevalence and factors associated with use of herbal medicines during pregnancy among women attending postnatal clinics in Gulu district, Northern Uganda. BMC pregnancy and childbirth. 2016;16:1-2. doi: 10.1186/s12884-016-1095-5
2. Nasim N, Sandeep I. S, Mohanty S. Plant-derived natural products for drug discovery: current approaches and prospects. The Nucleus. 2022;65(3):399-411. doi: 10.1007/s13237-022-00405-3
3. Tamanna N, Mahmood N. Food processing and maillard reaction products: effect on human health and nutrition. International journal of food science. 2015;2015(1):526762. doi: 10.1155/2015/526762

4. Avgoustaki D. D, Xydis G. How energy innovation in indoor vertical farming can improve food security, sustainability, and food safety?. In Advances in food security and sustainability 2020;1(l)5:1-51. Elsevier. doi: 10.1016/bs.af2s.2020.08.002
5. Mashamaite C.V, Manyevere A, Chakauya E. Cleome gynandra: A wonder climate-smart plant for nutritional security for millions in semi-arid areas. Frontiers in Plant Science. 2022;23:13:1003080. doi: 10.3389/fpls.2022.1003080
6. Moyo M, Amoo S. O, Aremu A. O, Gruz J, Šubrtová M, Jarošová M, Tarkowski P, Doležal K. Determination of mineral constituents, phytochemicals and antioxidant qualities of Cleome gynandra, compared to Brassica oleracea and Beta vulgaris. Frontiers in Chemistry. 2018;4:5:128. doi: 10.3389/fchem.2017.00128
7. Mashamaite C. V, Manyevere A, Chakauya E. Cleome gynandra: A wonder climate-smart plant for nutritional security for millions in semi-arid areas. Frontiers in Plant Science. 2022;23:13:1003080. doi: 10.3389/fpls.2022.1003080
8. Mangoale R. M, Afolayan A. J. Comparative phytochemical constituents and antioxidant activity of wild and cultivated Alepidea amatymbica Eckl & Zeyh. BioMed research international. 2020;2020(1):5808624. doi: 10.1155/2020/5808624
9. Karar M. G, Kuhnert N. Herbal drugs from Sudan: traditional uses and phytoconstituents. Pharmacognosy reviews. 2017;11(22):83. doi: 10.4103/phrev.phrev-15-15
10. Traoré K, Parkouda C, Savadogo A, Kamga R, Traoré Y. Effect of processing methods on the nutritional content of three traditional vegetables leaves: Amaranth, black nightshade and jute mallow. Food Science & Nutrition. 2017;5(6):1139-44. doi:10.1002/fsn3.504.
11. Imanirampa L, Alele P. E. 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. doi: 10.1186/s12906-016-1187-9
12. Moyo M, Amoo S. O, Aremu A. O, Gruz J, Subrtova M, Jarosova M, Tarkowski P. Determination of mineral constituents, phytochemicals and antioxidant qualities of Cleome gynandra, compared to Brassica oleracea and Beta vulgaris. Frontiers in Chemistry. 2018;4:5:128. doi: 10.3389/fchem.2017.00128
13. Sankhalkar S, Vernekar V. Quantitative and Qualitative analysis of Phenolic and Flavonoid content in Moringa oleifera Lam and Ocimum tenuiflorum L. Pharmacognosy research. 2016;8(1):16. doi: 10.4103/0974-8490.171095
14. Sharma T, Gamit R, Acharya R, Shukla V.J. Quantitative estimation of total tannin, alkaloid, phenolic, and flavonoid content of the root, leaf, and whole plant of Byttneria herbacea Roxb. An International Quarterly Journal of Research in Ayurveda. 2021;1:42(3):143-7. doi: 10.4103/ayu.AYU-25-19
15. Tashim N. A, Lim S. A, Basri A. M. Synergistic antioxidant activity of selected medicinal plants in Brunei Darussalam and its application in developing fortified pasta. Journal of the Science of Food and Agriculture. 2022;102(15):7331-42. doi: 10.1002/jsfa.12099
16. Ersoy E, Suvari G, Ercan S, Ozkan E. E, Karahan S, Tuncay E. A, Canturk Y. Y, Kara E. M, Zengin G, Boga M. Towards a better understanding of commonly used medicinal plants from Turkiye: detailed phytochemical screening and biological activity studies of two Teucrium L. species with in vitro and in silico approach. Journal of Ethnopharmacology. 2023;10:312:116482. doi: 10.1016/j.jep.2023.116482
17. Benzie IF, Strain J. J. Ferric reducing/antioxidant power assay: direct measure of total antioxidant activity of biological fluids and modified version for simultaneous measurement of total antioxidant power and ascorbic acid concentration. In Methods in enzymology 1999;299:15-27. doi: 10.1016/s0076-6879(99)99005-5