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Hypolipidemic and Antioxidant Effects of Phytochemicals Derived from *Centella Asiatica* Leaf Extract: An *In Vitro* and in Silico Approach

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KEYWORDS
Centella asiatica (L), Hypolipidemic, Antioxidant, Molecular Docking. Obesity,

ABSTRACT:

Introduction: Obesity is a part of metabolic syndrome instigated by a build-up of excess fat in body parts. In humans, obesity increases the expression of leptin, adipokines, and C-reactive protein plasminogen activators.

Objectives: The study investigated the antioxidant and hypolipidemic efficacy of bioactive components obtained from *Centella asiatica (L)* leaf extract using *in vitro* and in silico studies.

Methods: The phytochemicals in *C. asiatica* (L) extract were analysed using LC-MS analysis. The identified phytochemicals were used for in silico research to predict drug-likeness and molecular docking against anti-obesity targets.

Results: The results showed that IC_{50} values for DPPH, Nitric Oxide, and ABTS for plant extract are 46µg, 42µg, and 51µg, respectively, and the positive control butylated hydroxytoluene (BHT) showed 21µg, 24µg, and 18µg. The high amount of phenolic components in *Centella asiatica (L)* leaf extract contributes to the improved free radical scavenging action. The molecular docking results are based on interactions between proteins and ligands, as well as interactions between eight bioactive substances. The binding affinity of adiponectin protein is -9.86 kcal/mol, that of IGF1 protein is -8.95 kcal/mol, that of leptin protein is -8.3 kcal/mol, and that of GDF15 protein is -7.86 kcal/mol.

Conclusions: The bioactive phytochemicals from *Centella asiatica* have shown synergistic effects on multiple proteins involved in lipid metabolism based on the results of *in vitro* and *in silico* investigations. Hence, *Centella asiatica* can be used for obesity related disorders effectively.

1. Introduction

Obesity is a multifaceted syndrome marked by accumulation of adipose tissue. Obesity is a medical condition connected with a number of health issues [1]. It is a medical condition that raises the possibility of numerous other illnesses and health issues such as diabetes, high cholesterol, liver illness, heart disease sleep apnea, high blood pressure and certain cancers [2]. A cumulative effect of environmental, physiological and genetic factors, as well as decisions about diet, physical activity, and exercise typically causes obesity [3]. A prior study found that persons who were obese and had higher waist-hip ratios were 71% more likely to develop cancer than adults with lower ratios [4]. According to the World Obesity Federation's 2023 World Obesity Atlas, obesity is anticipated to have a noteworthy economic impact worldwide [5].

Recently found factors and signals related to appetite regulation and peripheral energy intake, preservation, and consumption. The primary melanocortin pathway, as well as external signals like leptin and gut hormones, have an impact on anti-obesity research [6]. Leptin is an adipocyte-derived hormone that is structurally related to the cytokine and plays a role in innate and acquired immunity [7]. Anti-obesity target proteins such as GDF15 (growth/differentiation factor 15) and its receptor (GDNF family receptor alpha-like) GFRAL/RET constitute a non-homeostatic mechanism that influences intake of food and body weight in preclinical species [8]. Adipose tissue produces the hormone adiponectin, which is decreased in conditions connected to obesity such as atherosclerosis, insulin resistance, and type 2 diabetes.

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FABP (fatty acid-binding protein) is an extracellular protein found solely in the gastrointestinal tract [9]. The FABP2 gene, according to the research, may be connected to obesity and insulin resistance. The FABP2 polymorphism has been reported to have an important role in the aetiology of at least some cases of obesity and insulin resistance, and it may be caused by an increase in inflammatory cytokines[10].IGF-1 is a dichotomous hormone and it is essential for cell cycle and mitogenesis development[11]. In this context, IGF1 may influence the progression of obesity, which contradicts its involvement in the capacity for oxidation. IGF2BP2 is an insulin-like growth hormone. Obesity development would be influenced by IGFBP-2 overexpression [12].

There are many medication approaches for the management of obesity. Usually, people advised to take sertraline, fluoxetine, sibutramine, topiramate, and orlistat for treatment, however, they cause side effects [13]. Many people turned to phytotherapy and pharmacology as an effective method to prevent obesity and weight loss. Plants have been used to treat many diseases; hence, ethno-pharmacological and ethano-botanical research examines the potential bioactive compounds that have the capacity to treat numerous disorders [14, 15]. Most of these medicinal plants have anti-hyperlipidemic properties, effectively treating obesity [16].

Centella asiatica (L) (*C. asiatica* (L) is an imperative traditional plant with extensive medicinal value [17]. *C. asiatica* (L), commonly known as Gotukola, Brahmi, Mandookaparni, is a traditional perennial herb found in Southeast Asian countries [18]. This rejuvenating medicinal herb has proven antibacterial, antifungal, antileprotic, antioxidative, antipyretic, anti-inflammatory, and wound-healing properties [19]. *C. asiatica (L)* was employed for diabetes treatment because it demonstrated anti-hyperglycemic and anti-hyperlipidemic properties in a rat model [20]. Some studies shows that the leaf extract has a potential effect on metabolic disease by reducing MAP (mean arterial pressure), inhibiting weight gain, regulating the heart rate, and decreasing the widened frontal QRS-T angle [21]. According to recent studies, treating mice with HFHSD-induced obesity with *C. asiatica (L)* dramatically decreased the amount of body fat they accumulated. The plant also has an anti-obesity action via controlling the genes expression linked to metabolism of lipid and cholesterol homeostasis in the liver [22]. Further, *in vivo* and *in vitro* experiments are is required to define the therapeutic effects of the plant's bioactive components in the treatment of obesity.

C. asiatica (L) leaf extract phytochemical screening and in vitro antioxidant activities (DPPH, ABTS, and nitric oxide) were done in this study. GDF15, leptin, adiponectin, FABP2, IGF1, IGF2BP2, lipase, and ghrelin proteins were also chosen as anti-obesity targets [23]. The phytochemical components discovered from the *C. asiatica* (L) extract were compared to the reference medicine Orlistat, which is being researched to understand its inhibitory action on anti-obesity target proteins as potential therapeutic qualities. For the initial evaluation of appropriate medicines for obesity, we studied computational drug discovery approaches for selecting molecules and receptor targets based on methodology, in addition, by employing computational drug discovery software to study protein-ligand interaction.

2. Methods

2.1. The leaf extract Preparation

C. asiatica (L) leaves were thoroughly cleansed in tap water, shade dried and then crushed in an electric mixer grinder. The Soxhlet extraction procedure generated 4% of the yield from 20 g of shade-dried powder in 250 mL of methanol. After that, evaporation of the solvent done by using rotary evaporator, and the extracts were concentrated to create a thick mixture. The extract was kept at 4°C until further testing [24]

2.2. Phytochemical screening of *C. asiatica (L)*

Investigation for alkaloids.
Mayers reagent 1ml was added to the1ml of extract solution, but the development of an orange or reddish-brown colour did not occur, indicating the absence of alkaloids [25].
Investigation for carbohydrates.
To the 2-3 ml of extract solution, α-naphthol solution about two drops were added, and then 0.5ml of concentration H_2So_4 was added to the test tube. There was no development of the violet ring, which indicates the absence of carbohydrates [26].
Investigation for terpenoids.
Chloroform 2 ml and concentrated sulphuric acid 3 ml were added to 5 ml of extract sample, the development of a reddish-brown ring was observed and confirmed the terpenes presence [27,28].
Investigation for steroids.
The extract sample about 1 ml was dissolved in 10 ml of chloroform, and a comparable amount of sulphuric acid was then added *via* the test tube's sides. There was no formation of a superior layer that turns red, and the layer of sulphuric acid showed green fluorescence with

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yellow. This results shows that steroids were not present [27,28].

Investigation for saponins.

One ml of the extracted solution was shaken vigorously with water about10 ml, and the formation of persistent foam indicated the saponins [28].

Investigation for flavonoids.

Few drops of concentrated hydrochloric acid was added to a small volume of extract sample. Development of red colour showed the presence of flavonoids [28].

Test for tannins and phenolic compounds.

1 ml sample of extract was combined with 1 ml of acetic acid. The development of a pink color shows the presence of phenolic and tannin compounds [28].

Test for cardiac glycosides.

One milliliter of the extract solution was taken and added one drop of ferric chloride solution, two milliliters of glacial acetic acid and one milliliter of concentrated H_2SO_4 to it, and looked for the brown ring. The appearance of a brown ring specifies the presence of cardiac glycosides [28].

2.3. Liquid chromatography–mass spectrometry (LC–MS) analysis

An XDB column was used for separation by chromatography. The mobile phase was composed of (A) methanol and (B) bi-distilled water containing formic acid about 0.1%. Separation was performed as follows: (10:90; v/v) methanol/water with the methanol/water ratio reaching about 100:0 (v/v) in 20 min with 25 min. It takes 10 minutes for the methanol/water ratio to rise to 10:90 (v/v). The mobile phase, which had been previously degassed with extremely pure helium., was pumped at an average speed of 0.3 mL/min with a volume addition of 10 µL. In the case of the positive ion mode, the electromagnetic radiation ionization-mass spectrometer was used. The electrospray capillaries perspective was 50 V, whereas the shield potential was 225 V. Nitrogen was utilized in the evaporation of solvents as a drying gas at 49 mTorr. The drying gas and ionization at atmospheric pressure vessel were kept at a constant internal temperature of 380 degrees Celsius and 50 degrees Celsius, respectively. One second time was used for scanning, and the detector amplification 1500 V voltage was set to for quadrupole 1 and quadrupole 3 isolation widths of m/z 1.2 and m/z 2.0, respectively. Scanning the mass range 20-480 yielded the ESI mass spectra [29].

2.4. Anti-oxidant activity of *C. asiatica* extract

2.4.1. DPPH + radical scavenging activity

The hydrogen-donating capability of the *C. asiatica* (L) extract was examined by following the method with minor modifications [30]. BHT (butylated hydroxyl toluene) has been dissolved with methanol to provide as a positive control, sample solutions of 5, 10, 20, 50, and 100µg of sample/ml solution were used. Adding one milliliter of 0.25% DPPH into a clean tube after the sample solution was transferred at different concentrations. For 30 minutes, the resulting mixture was incubated at 37ºC. The absorbing capacity of the reaction solution was decisive at 515 nm in a UV-Vis spectrophotometer. The experiment was done in triplicates. The following formula was used to compute the activity of scavenging free radicals:

$$\% \text{ Scavenging} = [(A_0 - A_1) / A_0] \times 100$$

where A_0 represents the control's absorbance and A_1 represents the extract's or standard's absorbance, which is BHT (butylated hydroxyl toluene).

2.4.2. Nitric oxide scavenging activity

Nitric oxide (NO) was generated when sodium nitroprusside in an H2O at the functional pH to form nitrite ions that react with oxygen, which were quantified using the Griess reaction [31]. Three millilitres of 10 mM sodium nitroprusside in phosphate buffer were mixed with two millilitres of *C. asiatica* extract, the reference ingredient, in various concentrations (5, 10, 15, 20 and 25µg/ml). The resultant solution was subsequently left to incubate for 60 minutes at 25°C. A similar method was carried out with methanol as control. 5 ml of Griess reagent (diamine hydrochloride, 0.1% naphthylethylene, 1% sulphanilamide in 2% H_3PO_4) was added to 5 ml of the incubated sample. Using BHT (butylated hydroxyl toluene) as a reference, at 546 nm, nitrite and sulphanilamide undergo diazotization due to the chromophore's absorption and combining it with naphthylethylene diamine was measured.

2.4.3. ABTS assay

The ABTS assay based on light scavenging by ABTS radicals, and it was carried out in accordance with the previously published protocols [32,33]. A stable free radical will be quenched by an antioxidant that can provide a hydrogen atom, changing absorption in a manner that may be observed spectroscopically. The

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reasonably stable green in color, the ABTS radical can be measured spectrophotometrically at 734 nm. By reacting an equivalent amount of ABTS with APS and incubation the combination at ambient temperature in darkness for 14-16 hrs, ABTS radical cations were generated. Using a spectrophotometer, the solution was further diluted with methanol (1:60) to provide an absorbance of 1.00 at 734 nm. 950ml of ABTS working solution was mixed with differential test sample and standard of reference readings (maximum amount collected was 50 ml) to yield a final volume of 1ml. The absorbance was recorded immediately at 734nm. GraphPad Prism software was used for determining the percent inhibition and IC_{50} values at various doses. The complete formulation was used to compute the measurement inhibition of growth:

$$\% \text{ Inhibition} = \frac{(\text{Abs of Control} - \text{Abs of Sample})}{(\text{Abs of Control})} \times 100$$

2.5. Computational studies

2.5.1. Ligand preparation

The structure data files (SDF) structure of the ligands (identified compounds in *C.asiatica* extract by LC-MS Analysis) and reference drug like Orlistat was attained from the PubChem database (www.pubchem. ncbi. nlm.nih.gov) [34]. The molecular graphics system PYMOL (1.7.4.5 Edu) was utilised for changing the molecules to.pdb chemical format [35]. The dockable ligand molecules were then transformed. pdbqt format by ligand preparing the number of rotation bonds and computations using the Autodock vina program Charged by Gasteiger, non-polar hydrogens are merged.

2.5.2. Macromolecule preparation

The target proteins such as GDF15, Leptin, Adiponectin, FABP2, IGF1, IGF2BP2, Lipase and Ghrelin proteins were taken for the study. Protein BLAST (an NCBI tool) was used to obtain sequences that had been submitted to NCBI [36]. Additionally, understanding the complexity of the protein structure is aided through using the Ramachandran Plot and SAVES to analyze the stereochemical association of residue-by-residue molecules to anticipate the type of bond interaction [37]. The Swiss Model & Modeller-v9.23 was used to generate the 3D structure depends on the target-template selection for template identification. Docking analysis of the reference pharmaceutical molecule Orlistat and bioactive substances found in LC-MS analysis were taken against targets.

3. Results and Discussion

Qualitative examination discovered the presence of tannin, terpenoids, saponin, flavonoids and glycosides compounds in methanolic leaf extracts of *C. asiatica* [L] and the data was presented in [**Table.1**]. Total phenolics, flavonoids and tannins were found in significant amounts in *C. asiatica* leaf extracts [**Fig.1**].

Figure 1. Qualitative analysis of *Centella asiatica* leaf crude extract

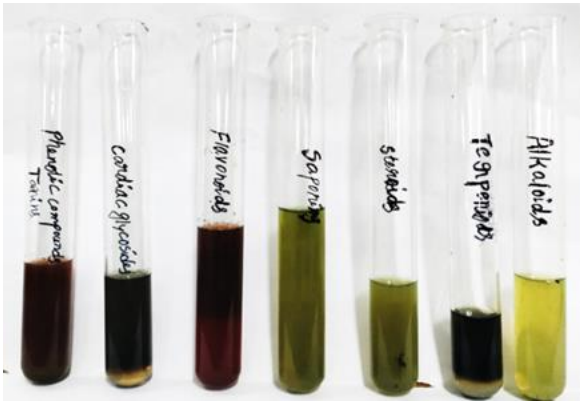

Table: 1. Phytochemical screening of C. asiatica leaf extract

Constituents	Tests	Result
Alkaloids	Dragendoff's Test	-
Tannins	Acetic acid test	+
Terpenoids	Terpenoids Test	+
Steroid	Steroid Test	-
Saponin	Foam Test	+
Flavonoid	Alkaline reagent Test	+
Phenolic compounds	Acetic acid Test	+
Cardiac Glycosides	Keller-Killani test	+

3.1. LC-MS analysis

The presence of phytochemicals in the *C. asiatica* [L] leaf extracts is additionally confirmed by LC-MS analysis [**Fig.2**] **[Table.2]** [38].

3.2. The *C. asiatica* (L) extract anti-oxidant activity

The antioxidant activity was assessed by assessing its ability to scavenge various free radicals. The ability to obtain stable free radicals such as DPPH was calculated by estimating the percentage of inhibition observed through gradual reduction at the absorbance 517 nm. The Griess reagent was used to determine how much nitric oxide (NOx) was generated by sodium nitroprusside [39]. The percentage of inhibition was estimated by reading the chromophore absorbance at 546 nm, which is produced when nitrite is diazotized with sulphanilamide and then coupled with naphthalene diamine. *C. asiatica* (L) extract showed significantly higher DPPH, NOx and ABTS radical scavenging activity (**Fig 3a, 3b, 3c)** with increasing concentrations (5-100 µg/ml). Butylated hydroxy toluene (BHT) was used as positive control and it is showed with maximum scavenging activity at 100 µg/ml.

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S. No	CAS No	Chemical Name	Molecular weight	Molecular Formula
1	72654-88-7	Androst-11-en-17-one, 3-formyloxy-, (3α, 5α)-	316.4	$C_{20}H_{28}O_3$
2	3593-85-9	Methandriol dipropionate	416.6	$C_{26}H_{40}O_4$
3	33512-86-6	3β-Hydroxy-21-oxoolean-12-en-28-oic acid methyl ester	484.7	$C_{31}H_{48}O_4$
4	2665-03-4	Lanosterol, trimethylsilyl ether	458.8	$C_{30}H_{54}OSi$
5	17846-09-2	Silane,[[(3α,5α,20S)-pregnane-3,17,20-triyl]tris(oxy)]tris [trimethyl-	553.0	$C_{30}H_{60}O_3Si_3$
6	69688-39-7	Estra-1,3,5(10)-trien-17-one, 3,12-bis[(trimethylsilyl)oxy]-, (12β)-	430.7	$C_{24}H_{38}O_3Si_2$
7	54845-27-1	2-Hexenoic acid, 6-cyclohexyl-	196.29	$C_{12}H_{20}O_2$
8	56312-71-1	Cholesta-5,17(20)-dien-3-ol, acetate, (3β, 17E)-	426.7	$C_{29}H_{46}O_2$

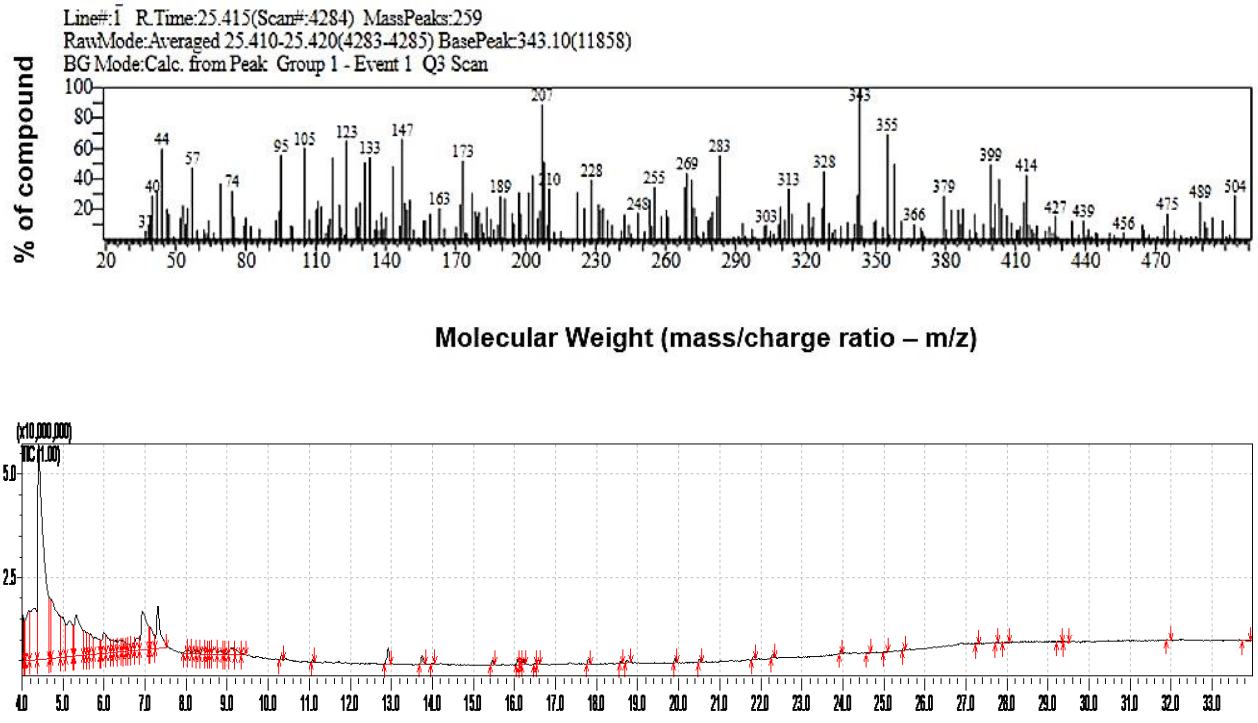

Figure 2. LC-MS examination of *Centella asiatica* leaf extract

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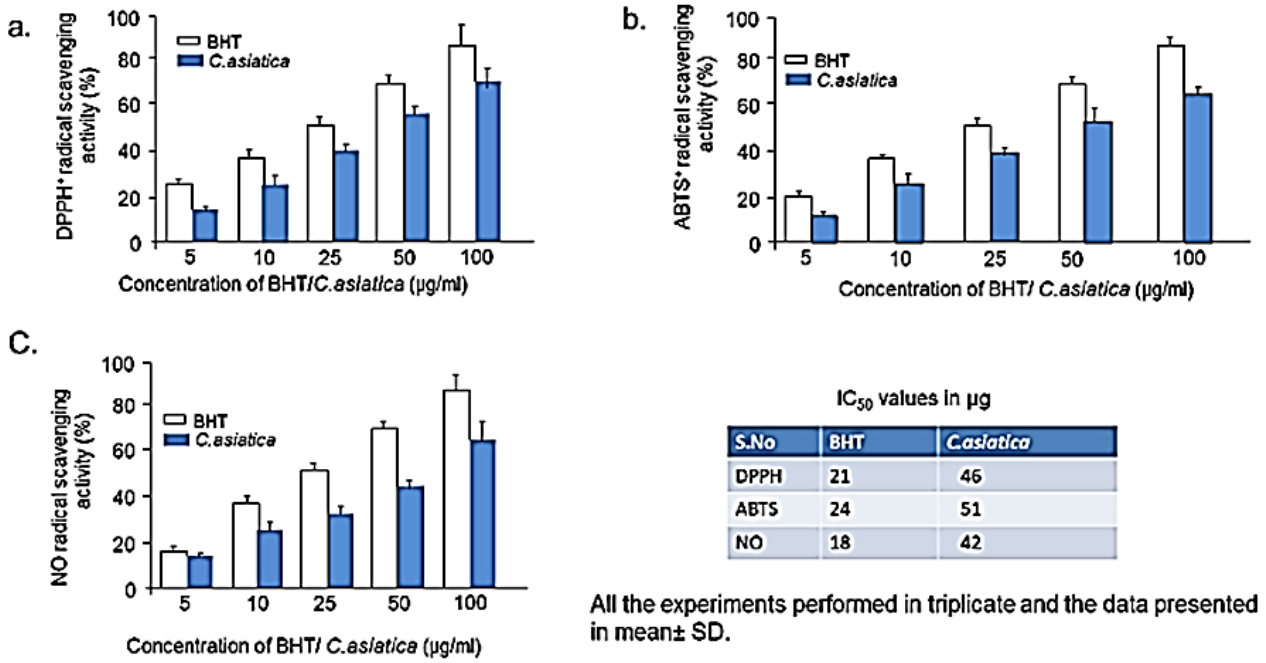

Figure 3. Anti-oxidant activity of *C. asiatica* (L) extract. *C. asiatica* (L) extract showed significantly higher DPPH, NOx and ABTS radical scavenging activity (**Fig 3a, 3b, 3c)** with increasing concentrations (5-100µg/ml). Butylated hydroxy toluene (BHT) was used as a positive control and it is showed with maximum scavenging activity at 100 µg/ml.

3.3. *In–silico* studies

3.3.1. Phytochemical selection by AMDET properties

Lipinski rule of 5 for various descriptors. The independent descriptors were determined for each of the 8 phytochemicals using molinspiration and represented in (**Table.3).**

3.3.2. *In silico* bioactivity study

The tool Molinspiration Cheminformatics server was also used to assess the biological activity score of phytochemicals of phytochemicals (http://www.molinspiration.com) [40]. Large chemical databases are examined in this *in-silico* chemistry approach in order to identify potential novel medication compounds. Utilizing sophisticated Bayesian statistics, the Molinspiration program examines a preparation for set of active molecules and contrasts it with inactive molecules. For instructional purposes, only the SMILES or SDfile structures of active compounds are required; no prior understanding of the active site or binding mechanism is required to understand how phytochemicals function biologically showed in (**Table.4)**.

3.3.3. Receptors and template structures predicted using Swiss Model

Using the NCBI tool the submitted protein in protein BLAST algorithm, and finding the target proteins, including GDF15, Leptin, Adiponectin, FABP2, IGF1, IGF2BP2, Lipase, and Ghrelin sequences, were located (**Table.5**). A query sequence of the targeted proteins has been uploaded to the SWISS-MODEL server (https://swissmodel.expasy.org/templates/), which uses the BLAST and HHblits approach to look for evolutionary-related protein structures (**Fig.4**) (41).

3.3.4. Molecular docking analysis by Autodock vina 4.2

Protein-ligand identification and biochemical interactions are studied using the molecular docking approach. Explicit water hydration, specific metal coordination models, and macrocyclic flexibility, and simultaneous docking of multiple ligands AutoDock Vina computes the grid maps automatically and groups the results directly for the user[42]. Hydrogen atoms are added to the polarity group of the amino acids in the receptor using AutoDock v.4.2 in order to construct the PDBQT files for the ligand and protein structures.

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Table. 3. Drug likeliness prediction of phytochemicals using molinspiration

Chemicals	miLogP	TPSA	nAtoms	MW	nON	nOHNH	Nrotb	Volume	N Violations
Ligand_1	3.35	43.38	23	316.44	3	0	2	311.53	0
Ligand_2	6.00	52.61	30	416.60	4	0	6	420.27	1
Ligand_3	6.24	63.60	35	484.72	4	1	2	490.85	1
Ligand_4	9.51	9.23	35	498.91	4	0	6	546.15	1
Ligand_5	9.63	27.70	36	553.07	3	0	7	585.29	2
Ligand_6	8.06	35.54	29	430.74	3	0	4	431.32	1
Ligand_7	3.83	37.30	14	196.29	2	1	5	207.45	0
Ligand_8	8.19	26.30	31	426.69	2	0	6	453.40	1
Orlistat	9.01	81.71	35	495.75	6	1	23	525.13	1

Table: 4. Bioactive properties of phytochemicals from *Centella asiatica* leaf extract

Chemicals	GPCR ligand	Ion channel modulator	Kinase inhibitor	Nuclear receptor ligand	Protease inhibitor	Enzyme inhibitor
Ligand_1	0.25	0.36	-0.41	0.68	0.24	0.85
Ligand_2	0.08	-0.11	-0.53	0.68	-0.01	0.49
Ligand_3	0.19	-0.02	-0.50	0.69	0.06	0.65
Ligand_4	0.31	0.04	-0.29	0.61	0.25	0.95
Ligand_5	0.31	0.29	-0.10	0.45	0.33	0.84
Ligand_6	0.46	0.31	-0.19	0.39	0.64	0.99
Ligand_7	-0.21	-0.05	-0.79	-0.14	-0.27	0.11
Ligand_8	-0.07	0.06	-0.56	0.69	0.01	0.54
Orlistat	0.39	0.14	-0.18	0.12	0.51	0.57

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All active bonds that are non-rotatable and dynamic amide bonds have their torsion numbers increased [42]. In the Grid box the Using grid spacing of 0.558 and grid box sizes of x = 40, y = 40, and z = 40, Auto Grid space was evidently taxing and provided search space. The chosen grid center's measurements were x = 30.796, y = -1.705, and z = -12.920. With the aid of these elements and a 10 exhaustiveness score, grid parameters were created to assist in understanding the grid's distribution of energy. Proteins were docked with bioactive substances using Auto Dock Vina, comparable to the default-valued Lamarckian genetic algorithm (LGA).

After determining the optimal protein-ligand docking structure utilizing an energy values expressed in kcal mol1, the hydrophobic interactions and hydrogen bond affinity are examined[43,46]. A tool for improving protein structure, AutoDock Vina 4.2 adds hydrogens with polarity and gasteiger charges to atomically charged groups. The ligand can be changed to solve the ligand-protein relationship by discovering a rotatable bond and assessing energy investments. Grid maps were created to assist with docking by producing different docking scores with structural (ligand) molecules as they engage with proteins [44,46].

Table: 5. Target protein selection based on sequence similarity

Target Protein	Template ID	% of Similarity	E-Value	Sequence Coverage	Total Score
GDF15	5VT2_A	100%	3e-80	197-308	239
	6Q2J_A	100%	3e-80	197-308	240
Leptin Receptor	3V6O_A	100%	2e-142	428-633	429
MC4R	7F53_R	100%	0.0	1-332	675
	7PIU_R	100%	0.0	1-332	674
Ghrelin precursor	7F9Y_C	100%	2e-12	24-51	58.2
	2JSH_A	91%	9e-08	76-98	45.8
Adiponectin precursor	6U66_A	99.29%	3e-102	104-244	294
	4DOU_A	98.60%	1e-98	102-244	296
FABP2	1KZX_A	100%	4e-92	2-132	263
	2MO5_A	99%	5e-92	2-142	263
PPARG	3DZU_D	100%	0.0	72-475	835
	2YFE_A	99%	0.0	192-475	574
SREBP1	1AM9_A	100%	2e-48	349-430	167
IGF1	8EYR_C	100%	4e-146	1-195	404
	1BQT_A	100%	7e-47	49-118	149
IGF2BP2	6ROL_A	100%	1e-108	426-588	324
Lipase	6OAU_A	100%	0.0	28-475	941
	6E7K_A	99.7%	0.0	28-475	939

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Table: 6. Docking of phytochemicals from *Centella asiatica leaf* extract against selected target proteins.

Target protein	Set of 8 ligands	Binding (kcal/mol)	Inhibitory constant (Ki)	Amino Acids	No. of H-bonds
GDF15 (5VT2_A)	1	-6.67,	12.93 µM	CYS44, LEU104	2
	2	-7.36,	4.03 µM	-	-
	3	-7.86,	1.73 µM	CYS77, ASP103, ASP103,	3
	4	-4.96	232.38 µM	LEU104	2
	5	-7.32	4.28 µM	-	-
	6	-6.17	30.06 µM	-	-
	7	-6.15	31.13 µM	-	-
	8	-0.59	369.61 mM	GLY46, CYS78	2
	Orlistat	-7.5	60.48 mM	CYS44, LEU104	2
Leptin (3V6O_A)	1	-6.07	35.53 µM	TRP625	1
	2	-5.48	96.71 µM	LYS614, TRP625	2
	3	-4.99	219.12 µM	TRP625	1
	4	-1.74	53.31 mM	-	-
	5	-5.14	169.96 µM	-	2
	6	-4.78	311.17 µM	-	-
	7	-5.74	61.78 µM	GLU437, THR446	2
	8	-7.85	16.11 µM	LYS614, TRP625	2
	Orlistat	-7.4	69.48 mM	LYS536, ASN624	2
Adiponectin (6U66_A)	1	-8.42	726.10nM	GLN196, ASP165	2
	2	-8.15	326.18nM	GLN196	1
	3	-7.66	54.54nM	GLN196, ASP165	2
	4	-9.18	126.75nM	GLN196, ASP165, ASP96	3
	5	-6.13	185.11nM	- GLN196, ASP165, ASP96	-
	6	-7.76	290.12nM	GLN196	3
	7	-9.96	187.16µM	GLN196, ASP165, ASP96 TRP625	1
	8	-9.86	90.17nM		3
	Orlistat	-6.5	61.78 µM		1
FABP2 (2MO5A)	1	-8.23	933.17 nM	-	-
	2	-9.29	155.28 nM	-	-
	3	-9.74	72.27 nM	-	-
	4	-8.74	392.4 nM	-	-
	5	-8.96	269.14 nM	TRP82	1
	6	-8.96	272.56 nM	-	-
	7	-6.74	11.47 µM	GLN115	1
	8	-3.95	9.85 µM	-	-
	Orlistat	-6.7	90.17 nM	GLN115	1

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IGF1(8EYR C)	1	-8.68	433.22 nM	-	-
	2	-7.36	4.01 µM	-	-
	3	-8.95	272.94 nM	ARG36	1
	4	-6.15	30.97 µM	-	-
	5	-8.43	661.37 nM	ARG36	1
	6	-6.86	9.40 µM	-	-
	7	-6.45	18.70 µM	ASN26	1
	8	-2.38	18.07 mM	-	-
	Orlistat	-7.9	25 µM	ARG36	1
IGF2BP2 (6ROL_A)	1	+255.50	-	VAL559	1
	2	+432.65	-	GLN569	1
	3	+2.25e+003	-	-	-
	4	-4.83	289.51 µM	-	-
	5	+435.45	-	-	-
	6	+897.58	-	-	-
	7	+33.74	-	-	-
	8	+7.07e+003	-	-	-
	Orlistat	-7.8	72.05 µM	-	-
Lipase (6OAU_A)	1	-5.67		LYS455, TRP421	2
	2	+722.52	70.04 µM	ASN235, TYR315	2
	3	+2.53e+003	-	-	-
	4	+1.21e+003	-	ASN235	1
	5	+508.78	-	VAL340	1
	6	+484.93	-	-	-
	7	+24.76	-	-	-
	8	+3.77e+003	-	-	-
	Orlistat	-8.6	-	ASN235, VAL40	2
Ghrelin (7F9Y_C)	1	-4.55	459.77 µM	SER5	1
	2	-5.01	214.37 µM	-	-
	3	-5.21	151.05 µM	-	-
	4	-3.59	2.35 mM	-	-
	5	-4.59	433.25 µM	PHE3, SER5	2
	6	-3.82	1.59 mM	-	-
	7	-4.27	745.82 µM	HIS8	1
	8	-3.71	1.90 mM	-	-
	Orlistat	-7.5	151.05 µM	SER5	1

Eight compounds were selected from the crude extract based on LC-MS analysis results. These compounds such as Androst-11-en-17-one, 3-formyloxy-, [3α, 5α], Methandriol dipropionate, 3β-Hydroxy-21-oxoolean-12-en-28-oic acid methyl ester, Lanosterol, trimethylsilyl ether, Silane, [[[3α,5β,20S]-pregnane-3,17,20-triyl]tris[oxy]]tris[trimethyl,Estra-1,3,5(10)-trien-17-one,3,12-bis[[trimethylsilyl]oxy]-, [12β], 2-Hexenoic acid, 6-cyclohexyl, Cholesta-5,17(20)-dien-3-ol, acetate, [3β,17E]. Further, these chemical structures were used to forecast pharmacological effects against anti-obesity target proteins, together with the reference drug molecule orlistat, such as GDF15, Leptin, Adiponectin, FABP2, IGF1, IGF2BP2, Lipase, and Ghrelin proteins. The five-rule of Lipinski was applied to assess the "drug-like" character of compounds by polar surface area (PSA), molecular weight (MW<500 Da), estimated lipophilicity (ALogP), number of hydrogen bond donors (HBD), and number of hydrogen bond acceptors (HBA)[45].

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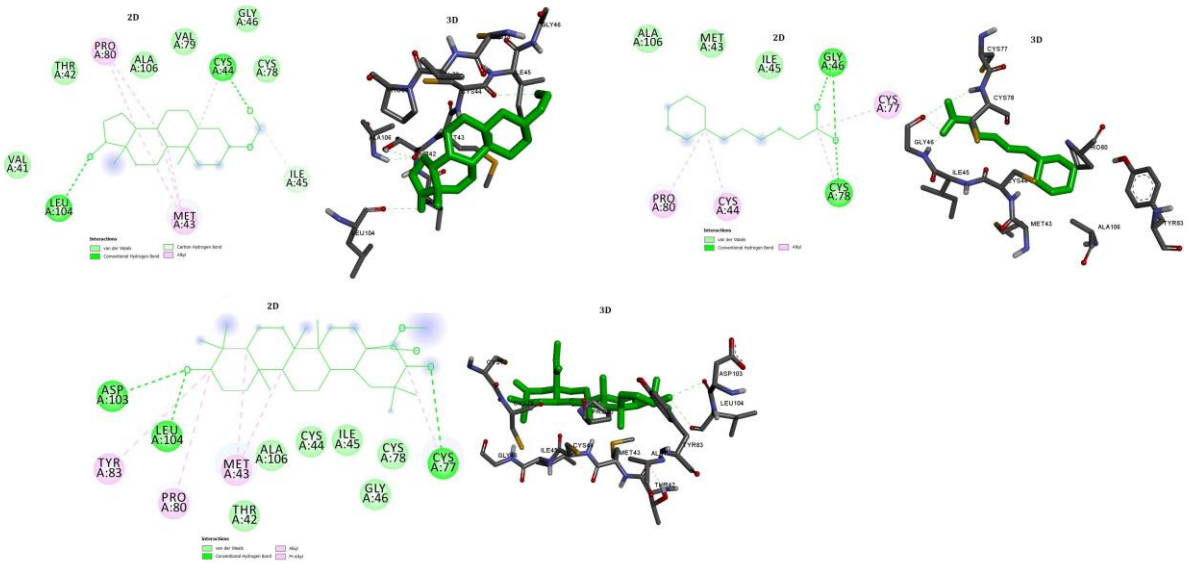
The plant extract has an IC50 value of 46 µg for DPPH and a positive control of 21 µg for BHT, indicating free radical scavenging action. The IC50 value for ABTs in plant extract is 51µg, while the positive control [BHT] is 24µg. The IC50 value for nitric oxide in plant extract is 42µg, while the positive control [BHT] is 18µg. The increased presence of phenolic compounds is the explanation for the higher capacity to scavenge free radicals present in C. asiatica [L] leaf extract, and these results were validated with previous published work [46, 47]. The molecular docking study analysis reveals that anti-obesity target proteins such as GDF15, Leptin, Adiponectin, FABP2, IGF1, IGF2BP2, Lipase, and Ghrelin are docked with phytochemicals from Centella asiatica leaf extract. The target GDF15 protein interacts with phytochemicals by forming 2-4 hydrogen bonds with an affinity range of -6.67 to -7.86 kcal/mol [Fig. 5]. CYS77, ASP103, LEU104, CYS44, GLY46, and CYS78 exhibit conformational binds with amino acids, with inhibitory constant values of 12.93µM, 1.73µM, and 369.61 mM, respectively. The reference drug Orlistat has two hydrogen bonds with amino acids CYS44 and LEU104 with binding energies of -7.5 kcal/mol and 60.48 mM, respectively [Table 6]. Associated with the target Leptin protein *via* two bonds of hydrogen by a binding affinity of 8.3 kcal/mol [**Fig. 6**]. The conformational bindings with amino acids are LYS614, TRP625 with inhibitory constant values of 16.11 µM, and the reference drug Orlistat has two hydrogen bonds with amino acids LYS536, ASN624 with binding energies of -7.4 kcal/mol and 69.48 mM, respectively [**Table 6**].

Adiponectin protein generates 2-3 hydrogen bonds with a binding affinity of 9.86 kcal/mol. The conformational bindings with amino acids are GLN196, ASP165, and ASP96, with inhibitory constant values of 90.17 nM, and the reference drug Orlistat has two hydrogen bonds with amino acids CYS44 and EU45, with binding energies of -7.4 kcal/mol and 69.48 mM, respectively. Bioactive compounds interact with selected target FABP2 proteins by forming one hydrogen bond with a binding energy of -8.96 kcal/mol. The conformational binding with amino acids is TRP82, with inhibitory constant values of 269.14nM, and the reference drug Orlistat has one hydrogen bond of amino acids GLN115, with binding affinity -6.7kcal/mol and 69.48 mM, respectively. They form two hydrogen bonds to link with the target IGF1 protein, which has a binding affinity of -8.95 kcal/mol. ARG36 binds to amino acids with an inhibition constant of 272.94 nM, while Orlistat has a binding affinity of -7.9 kcal/mol and 25 µM. Lipase protein forms two hydrogen bonds with a binding affinity of +722.52 kcal/mol [Fig. 7]. ASN235 and TYR315 exhibit conformational binding with amino acids and have an inhibition constant of 70.04 µM. Ghrelin protein is composed of two hydrogen bonds with a binding energy of -4.59 kcal/mol. The conformational binding with amino acids is PHE3, SER5, with an inhibitory constant value of 433.25 µM. The reference medicine Orlistat contains one hydrogen bond of amino acids SER5, with binding energy of -7.5 kcal/mol and 151.05 µM, respectively [**Table 6**] [48].

Figure .5. The interaction of phytochemicals with GDF15 protein



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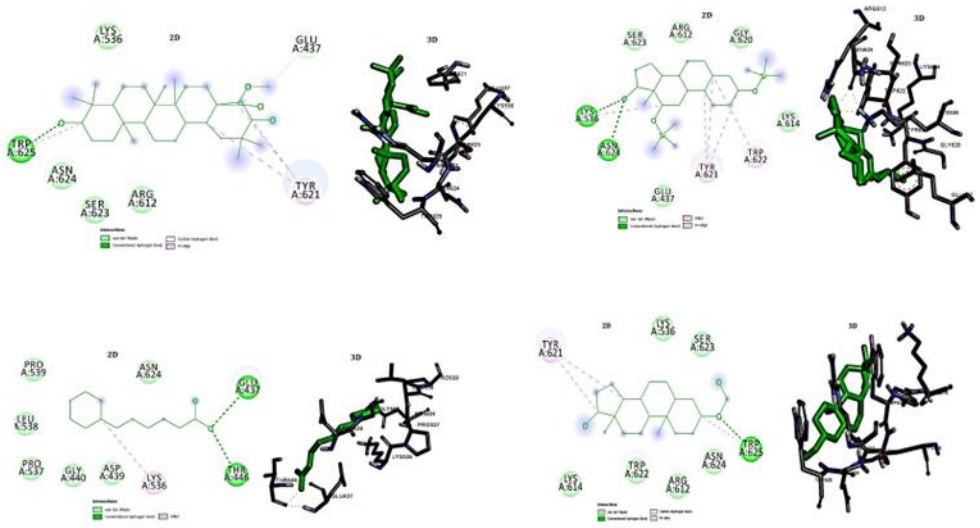

Figure.6. The interaction of phytochemicals with Leptin protein

4. Conclusion

In conclusion, after identifying bioactive components using LC-MS analysis and doing qualitative analysis, the phytochemicals discovered in C. asiatica (L) extract exhibit high antioxidant activity. In vitro experiments revealed that C. asiatica (L) leaf extract has a greater potential to scavenge free radicals due to its higher concentration of phenolic compounds. The studies investigated computational strategies for bioactive chemicals found via LC-MS analysis using molecular docking studies. The pharmacokinetic and pharmacophore studies demonstrated that drugs bind strongly to crystal amino acids in 3D protein active regions. Androst-11-en-17-one, 3-formyloxy-, (3α, 5α), 3β-Hydroxy-21-oxoolean-12-en-28-oic acid methyl ester, Lanosterol, trimethylsilyl ether, and Cholesta-5,17(20)-dien-3-ol, acetate, (3β,17E)-show bioactive properties, and the hydrogen bonds 2 to 3 have strong interactions with target proteins. The drugs exhibited strong inhibitory efficacy against Leptin, GDF15, Adiponectin (strong interaction), and Ghrelin (moderate interaction). As a result, we infer that bioactive chemicals found in Centella asiatica have synergistic effects on many proteins involved in lipid metabolism.

Abbreviations:

ABTS :2,2-azino-bis-3-ethylbenzothiazoline-6-sulphonic acid:
DPPH : 2,2-Diphenyl-1-picrylhydrazyl
BHT : Butylated hydroxytoluene ()
miLogP: Octanol-water partition coefficient.
TPSA : Topological polar surface area.
nOHNH : number of nonhydrogen atoms.
MW : Molecular weight.
Natoms : Number of Atoms.
nON: hydrogen bond acceptor.
nOHNH: hydrogen bond donor.
Nrotb: Number of Rotatable Bonds.
GDF15: Growth/differentiation factor 15
FABP2: Fatty acid-binding protein 2
IGF1: Insulin-like growth factor-1

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Authors' contributions

SBR, KG, SM, AS performed experiments. VDR and RN designed the work. KG and VDR and SM wrote the paper. All authors read and approved the final manuscript.

Conflict of interests

None

References

1. Panuganti KK, Nguyen M, Kshirsagar RK. Obesity. In: StatPearls. Treasure Island (FL): StatPearls Publishing; August 8, 2023.
2. Fruh SM. Obesity: Risk factors, complications, and strategies for sustainable long-term weight management. J Am Assoc Nurse Pract. 2017;29(S1):S3-S14. doi:10.1002/2327-6924.12510.

Journal of Chemical Health Risks

www.jchr.org

JCHR (2024) 14(6), 116-129 | ISSN:2251-6727

3. Lin X, Li H. Obesity: Epidemiology, Pathophysiology, and Therapeutics. Front Endocrinol (Lausanne). 2021;12:706978. doi:10.3389/fendo.2021.706978.
4. Huai P, Liu J, Ye X, Li WQ. Association of Central Obesity with All Cause and Cause-Specific Mortality in US Adults: A Prospective Cohort Study. Front Cardiovasc Med. 2022;9:816144. doi:10.3389/fcvm.2022.816144.
5. World Obesity Atlas 2023 published by the World Obesity Federation, on 2-03-2023.
6. Yeo GSH, Chao DHM, Siegert AM, et al. The melanocortin pathway and energy homeostasis: From discovery to obesity therapy. Mol Metab. 2021;48:101206. doi:10.1016/j.molmet.2021.101206.
7. Bernotiene E, Palmer G, Gabay C. The role of leptin in innate and adaptive immune responses. Arthritis Res Ther. 2006;8(5):217. doi:10.1186/ar2004.
8. Yang L, Chang CC, Sun Z, et al. GFRAL is the receptor for GDF15 and is required for the anti-obesity effects of the ligand. Nat Med. 2017;23(10):1158-1166. doi:10.1038/nm.4394.
9. Achari AE, Jain SK. Adiponectin, a Therapeutic Target for Obesity, Diabetes, and Endothelial Dysfunction. Int J Mol Sci. 2017;18(6):1321. doi:10.3390/ijms18061321.
10. Turkovic LF, Pizent A, Dodig S, Pavlovic M, Pasalic D. FABP2 gene polymorphism and metabolic syndrome in elderly people of croatian descent. Biochem Med (Zagreb). 2012;22(2):217-224. doi:10.11613/bm.2012.024.
11. Bailes J, Soloviev M. Insulin-Like Growth Factor-1 (IGF-1) and Its Monitoring in Medical Diagnostic and in Sports. Biomolecules. 2021;11(2):217. doi:10.3390/biom11020217.
12. Lewitt MS, Dent MS, Hall K. The Insulin-Like Growth Factor System in Obesity, Insulin Resistance and Type 2 Diabetes Mellitus. J Clin Med. 2014;3(4):1561-1574.doi:10.3390/jcm3041561.
13. Halpern B, Oliveira ES, Faria AM, et al. Combinations of drugs in the Treatment of Obesity. Pharmaceuticals (Basel). 2010;3(8):2398-2415.doi:10.3390/ph3082398.
14. de Freitas Junior LM, de Almeida EB Jr. Medicinal plants for the treatment of obesity: ethnopharmacological approach and chemical and biological studies. Am J Transl Res. 2017;9(5):2050-2064.
15. Sofowora A, Ogunbodede E, Onayade A. The role and place of medicinal plants in the strategies for disease prevention. Afr J Tradit Complement Altern Med. 2013;10(5):210-229.
16. Kumar P, Bhandari U. Common medicinal plants with antiobesity potential: A special emphasis on fenugreek. Anc Sci Life. 2015;35(1):58-63. doi:10.4103/0257-7941.165629.
17. Gohil KJ, Patel JA, Gajjar AK. Pharmacological Review on *Centella asiatica*: A Potential Herbal Cure-all. Indian J Pharm Sci. 2010;72(5):546-556. doi:10.4103/0250-474X.78519.
18. Orhan IE. *Centella asiatica* (L.) Urban: From Traditional Medicine to Modern Medicine with Neuroprotective Potential. Evid Based Complement Alternat Med. 2012;2012:946259. doi:10.1155/2012/946259.
19. Parham S, Kharazi AZ, Bakhsheshi-Rad HR, et al. Antioxidant, Antimicrobial and Antiviral Properties of Herbal Materials. Antioxidants (Basel). 2020;9(12):1309. doi:10.3390/antiox9121309.
20. Maulidiani, Abas F, Khatib A, et al. Metabolic alteration in obese diabetes rats upon treatment with *Centella asiatica* extract. J Ethnopharmacol. 2016;180:60-69. doi:10.1016/j.jep.2016.01.001.
21. Hasimun, Patonah, Mulyani, et al. *Centella Asiatica* Effect in A High Fat and Fructose Diet-Induced Model of Metabolic Disorder in Rats. Journal Of Biochemical Technology.2021;12.1-5. 10.51847/GzeDdExlEH.
22. Chang, YB, Ahn Y, et al. *Centella asiatica* Lowers Body Fat Accumulation via Regulating Cholesterol Homeostasis- and Lipid Metabolism-Related Genes in Mice with High-Fat, High-Sugar Diet-Induced Obesity. Preprints.org 2023; 2023(06)0254.
23. Kumari S, Deori M, Elancheran R, Kotoky J, Devi R. *In vitro* and *In vivo* Antioxidant, Anti-hyperlipidemic Properties and Chemical Characterization of *Centella asiatica* (L.) Extract. Front Pharmacol. 2016;7:400. doi:10.3389/fphar.2016.00400.
24. Redfern J, Kinninmonth M, Burdass D, Verran J. Using soxhlet ethanol extraction to produce and test plant material (essential oils) for their antimicrobial properties. J Microbiol Biol Educ. 2014;15(1):45-46. doi:10.1128/jmbe.v15i1.656.
25. Kancherla N, Dhakshinamoothi A, Chitra K, Komaram RB. Preliminary Analysis of Phytoconstituents and Evaluation of Anthelminthic Property of *Cayratia auriculata* (*In Vitro*). Maedica (Bucur). 2019;14(4):350-356. doi:10.26574/maedica.2019.14.4.350.
26. Auwal MS, Saka S, Mairiga IA, Sanda KA, Shuaibu A, Ibrahim A. Preliminary phytochemical and elemental analysis of aqueous and fractionated pod extracts of *Acacia nilotica* (Thorn mimosa). Vet Res Forum. 2014;5(2):95-100.
27. Das BK, Al-Amin MM, Russel SM, Kabir S, Bhattacherjee R, Hannan JM. Phytochemical Screening and Evaluation of Analgesic Activity of Oroxylum indicum. Indian J Pharm Sci. 2014;76(6):571-575.

Journal of Chemical Health Risks

www.jchr.org

JCHR (2024) 14(6), 116-129 | ISSN:2251-6727

28. Oncho DA, Ejigu MC, Urgessa OE. Phytochemical constituent and antimicrobial properties of guava extracts of east Hararghe of Oromia, Ethiopia. Clin Phytosci 7, 37 (2021). https://doi.org/10.1186/s40816-021-00268-2.
29. Grace MH, Xiong J, Esposito D, Ehlenfeldt M, Lila MA. Simultaneous LC-MS quantification of anthocyanins and non-anthocyanin phenolics from blueberries with widely divergent profiles and biological activities. Food Chem. 2019;277:336-346. doi:10.1016/j.foodchem.2018.10.101.
30. Loganayaki N, Siddhuraju P, Manian S. Antioxidant activity and free radical scavenging capacity of phenolic extracts from *Helicteres isora* L. and *Ceiba pentandra* L. J Food Sci Technol. 2013;50(4):687-695. doi:10.1007/s13197-011-0389-x.
31. Mahmud ZA, Bachar SC, Hasan CM, Emran TB, Qais N, Uddin MMN. Phytochemical investigations and antioxidant potential of roots of *Leea macrophylla* (Roxb.). BMC Res Notes. 2017;10(1):245. doi:10.1186/s13104-017-2503-2.
32. Zheleva-Dimitrova D, Nedialkov P, Kitanov G. Radical scavenging and antioxidant activities of methanolic extracts from Hypericum species growing in Bulgaria. Pharmacogn Mag. 2010;6(22):74-78. doi:10.4103/0973-1296.62889.
33. Lobo V, Patil A, Phatak A, Chandra N. Free radicals, antioxidants and functional foods: Impact on human health. Pharmacogn Rev. 2010;4(8):118-126. doi:10.4103/0973-7847.70902.
34. Kim S, Thiessen PA, Bolton EE, et al. PubChem Substance and Compound databases. Nucleic Acids Res. 2016;44(D1):D1202-D1213. doi:10.1093/nar/gkv951.
35. DeLano WL.The PyMOL molecular graphics system. Delano Scientific, San Carlos, 2002.
36. McGinnis S, Madden TL. BLAST: at the core of a powerful and diverse set of sequence analysis tools. Nucleic Acids Res. 2004;32(Web Server issue):W20-W25. doi:10.1093/nar/gkh435.
37. Wlodawer A. Stereochemistry and Validation of Macromolecular Structures. Methods Mol Biol. 2017;1607:595-610. doi:10.1007/978-1-4939-7000-1_24.
38. Gray NE, Alcazar Magana A, Lak P, et al. *Centella asiatica* - Phytochemistry and mechanisms of neuroprotection and cognitive enhancement. Phytochem Rev. 2018;17(1):161-194. doi:10.1007/s11101-017-9528-y.
39. Ali BM, Boothapandi M, Sultan Nasar A. Nitric oxide, DPPH and hydrogen peroxide radical scavenging activity of TEMPO terminated polyurethane dendrimers: Data supporting antioxidant activity of radical dendrimers. Data Brief. 2019;28:104972. doi:10.1016/j.dib.2019.104972.
40. Okawa M, Kinjo J, Nohara T, Ono M. DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging activity of flavonoids obtained from some medicinal plants. Biol Pharm Bull. 2001;24(10):1202-1205. doi:10.1248/bpb.24.1202.
41. Mfotie Njoya E, Munvera AM, Mkounga P, Nkengfack AE, McGaw LJ. Phytochemical analysis with free radical scavenging, nitric oxide inhibition and antiproliferative activity of *Sarcocephalus pobeguinii* extracts. BMC Complement Altern Med. 2017;17(1):199. doi:10.1186/s12906-017-1712-5.
42. Benet LZ, Hosey CM, Ursu O, Oprea TI. BDDCS, the Rule of 5 and drugability. Adv Drug Deliv Rev. 2016;101:89-98. doi:10.1016/j.addr.2016.05.007.
43. Tabassum R, Kousar S, Mustafa G, Jamil A, Attique SA. In Silico Method for the Screening of Phytochemicals against Methicillin-Resistant *Staphylococcus Aureus*. Biomed Res Int. 2023;2023:5100400. doi:10.1155/2023/5100400.
44. Waterhouse A, Bertoni M, Bienert S, et al. SWISS-MODEL: homology modelling of protein structures and complexes. Nucleic Acids Res. 2018;46(W1):W296-W303. doi:10.1093/nar/gky427.
45. Forli S, Huey R, Pique ME, Sanner MF, Goodsell DS, Olson AJ. Computational protein-ligand docking and virtual drug screening with the AutoDock suite. Nat Protoc. 2016;11(5):905-919. doi:10.1038/nprot.2016.051.
46. Gouthami K, Veeraraghavan V, Lavanya L, Prashantha CN, "Molecular docking studies for Vitex negundo (L)leaf extract compounds against Wnt- signalling proteins towards the treatment of colon cancer", Chemical DataCollections, 38, pp.100829, 2022. DOI:10.1016/j.cdc.2022.100.
47. Trott O, Olson AJ. AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. J Comput Chem. 2010;31(2):455-461. doi:10.1002/jcc.21334.
48. Prashantha CN, Gouthami K, Lavanya L, et al. Molecular screening of antimalarial, antiviral, anti-inflammatory and HIV protease inhibitors against spike glycoprotein of coronavirus. J Mol Graph Model.2021;102:107769.doi:10.1016/j.jmgm.2020.107769.