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ORIGINAL ARTICLE

Phytochemical and In-silico Screening for Anti-inflammatory Action of Major Phytochemicals Present in the Alcoholic Extract of *Clerodendrum thomsoniae* Balf.f.

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ABSTRACT

Clerodendrum thomsoniae Balf.f., Lamiaceae family has its traditional medicinal uses. This study used the molecular docking technique to examine the phytochemicals and their anti-inflammatory effect in silico. PyRX, a docking program using the Vina Wizard tool, was employed. For the investigation, the plant's Ariel parts were utilized. Soxhlet extraction using ethanol was carried out. Proteins, alkaloids, carbohydrates, flavonoids, glycosides, saponins, and tannins were all detected in the initial phytochemical analysis. The plant ethanolic extract was subjected to GCMS analytics, which revealed the presence of some significant Phyto compounds Bis (2-ethylhexyl) phthalate, 4-Biphenyl carboxylic acid, Do doxylamine, "N-(2,3,4,6 Tetra –o- acetyl beta- alpha-glucose pyrantel) glycine "which was used as ligands for docking study. The receptor used was 5KIR, Vivoxx bound to human COX-2 enzyme. PyRX docking revealed that the best active compound, 4-Biphenyl carboxylic aid, scored 7.7 compared to the standard Rofecoxib 6.7 drug docking score. Further, in-vivo, in-vitro, and clinical studies may be carried out to validate these results and for further SAR modification in drug development. The antioxidant activity established by in-vitro methods adds to the anti-inflammatory activity predicted.

Keywords: *Clerodendrum*; Extraction; Docking; Anti-inflammatory

INTRODUCTION

Originally belonging to the Verbenaceae family, *Clerodendrum* is currently included under the Lamiaceae family. The ethno-medical uses reported for *Clerodendrum* genus plants are anti-hypertensive, anti-inflammatory, and analgesic[1–3] *Clerodendrum thomsoniae* Balf.f. is an ornamental plant with a rich source of polyphenols. It is a vine native to western Africa, widely cultivated in the tropics and subtopics. The Phyto compounds in the plant were used to treat depression, skin rashes, blisters, neurological abnormalities, and oxidative stress[4]. Inflammation is a complex defense mechanism of the human body against harmful foreign substances. After inflammation, wound healing begins[5]. Targeting cyclooxygenase -2 (Cox – II) receptors using anti-inflammatory drugs is the critical process in drug design for treating inflammation. COX –II enzyme produces prostaglandins, which cause inflammation[6]. Through in vitro techniques, the antioxidant activity of this plant's components has already been determined, and these activities are related because excess free radicals can cause inflammation[7]. Free radicals can cause other illnesses like cancer, atherosclerosis, and neurodegenerative disorders. Natural antioxidants are safer and cheaper compared to the cost of synthetic antioxidants. The prolonged use of synthetic anti-inflammatory drugs may lead to GIT and cardiovascular and renal adverse effects[8]. Herbs with high polyphenolic content possess antioxidant activity. Anti-inflammatory agents act by modulating the activation of pro-inflammatory factors and cytokines[9].

MATERIALS AND METHODS

Soxhlet Extraction

The Soxhlet apparatus, which included a thimble, a condenser, and a round-bottom flask, was put together. The powdered plant stuff was in the thimble [Figure 1]. The

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solvent, methanol, was added to the flask with a circular bottom. The extraction procedure was started by heating the equipment. The solvent was cycled through the apparatus to efficiently dissolve the desired compounds from the plant material and collect them in the round-bottom flask. The extraction procedure was continued for a certain amount of time or until the solvent in the siphon showed no discernible color change, signifying that not much more extraction occurred. The obtained extract was then concentrated using a rotary evaporator to remove the solvent and create a crude extract.[10]

Fig. 1: Powdered plant Ariel parts of *Clerodendrum thomsoniae* Balf.f.

Phytochemical analysis [Table 1]

Alkaloids are detected by phytochemical screening using a variety of reagents, such as Dragondroff's (orange-red precipitation), Wagner's (reddish-brown residue), Mayer's (white precipitate), and Hager's (yellow precipitate), carbohydrates by the Molisch test (purple/violet interface), Fehling's (red brick residue), and "Benedict's " (red precipitate); proteins and amino acids by the Biuret test (violet color); steroids by a brown ring formation with sulfuric acid; glycosides by the Legal test (pink-red in pyridine) and Keller- Killiani test (reddish-brown at the sulfuric acid junction); saponins by the formation of foam when shaken with water; flavonoids by the red color with magnesium and hydrochloric acid; and tannins and phenolic compounds by the dark blue or greenish-black with ferric chloride and white precipitate with lead acetate, respectively.[10]

GCMS analysis

Using an "Agilent Technologies 7890 GC system" with a 5975C inert MSD and helium as the carrier gas, the "GC-MS analysis" of the plant extracts revealed five critical analytes. The data was compared to NIST11 and RTLPEST3 libraries. The chromatographic process separates molecules according to their chemical properties for mass detection. HPLC with a 5975C inert MSD and helium as the carrier gas, the GC-MS analysis of the plant extract revealed five critical analytes. The data was compared to NIST11 and RTLPEST3 libraries. The chromatographic process separates molecules according to their chemical properties for mass detection.[11–14]

Determination of antioxidant activity

Twenty milligrams of dry plant extract were dissolved in one milliliter of 98% methanol to create a stock solution (20 mg/ml).

- **DPPH Free Radical Scavenging Assay:** The "DPPH scavenging activity" was measured using a one mM DPPH solution in methanol. Mixing different amounts of the stock (20, 40, 60, 80, and 100 μl) with 100 μl of DPPH solution produced 300 μl of methanol. The control included only DPPH and methanol. After 30 minutes of dark incubation, the absorbance of the samples was measured at 517 nm.
- **The FRAP experiment:** It was conducted using a solution of 200 mM acetate buffer (pH 3.6), TPTZ (15 mM), and ferric chloride (20 mM) in a 1:1:1 ratio. 300 μl of distilled water was added after this was mixed with" 20 μl, 40 μl, 60 μl, 80 μl, and 100 μl" of stock solution. After 30 minutes in the dark, absorbance at 593 nm was measured, and the FRAP value was obtained using a standard curve using ferrous sulfate.
- **Nitric Oxide Scavenging Assay:** Griess reagent was mixed with a six mM sodium nitroprusside solution in different stock quantities (10, 20, 30, 40, and 50 μl). After a 20-minute dark incubation period, absorbance was measured at 546 nm.

Molecular Docking using PyRX[15]

Ligand Preparation

The ligands dodecyl amine, 4-biphenyl carboxylic acid, bis (2-ethyl hexyl) phthalate, "n-(2, 3, 4, 6 tetra-o-acetyl beta-alpha glucopyranosyl) glycine", identified by PubChem IDs 13583, 66724, 8343, 536074 respectively, were expertly synthesized. They were obtained from the "PubChem "database in Sdf format, and their structures were accurately constructed using Chem Sketch software. The system was successfully opened, and using Biovia Drug Discovery Studio, polar hydrogens and charges were systematically incorporated, with torsions precisely configured before saving in PDBQT format.

Receptor Preparation [Figure 2]:

The enzyme designated as the target, PDBID: 5 KIR, was downloaded in PDB format from the RCSB and maintained in PDB format we efficiently removed all water molecules and Heteroatoms added polar hydrogens and Kolman charges and saved the results in PDBQT format.

Simulation of Molecular Docking

Four ligands and the reference ligand Rofecoxib were docked with the constructed COX-2 receptor using the Auto dock Vina docking software. Auto Dock Vina determined each ligand's most likely binding position within the receptor's binding site. The binding affinity of each ligand-receptor complex was calculated using Vina's scoring function.

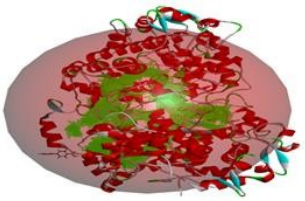


Fig. 2: Receptor COX-2 with the binding site (from RCSB protein data bank visuals by Biovia Discovery Studio)

Re-docking and Validation

The control Rofecoxib was separated from the enzyme and stored in a separate molecular window in PDB format, which was then utilized for Redocking and validation.

Swiss ADME Bioavailability

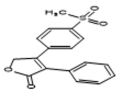
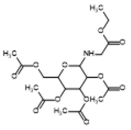
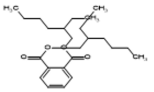
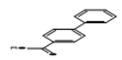
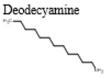
The drug-likeness RADAR for the compounds demonstrating substantial activity was generated, and thorough ADME predictions were the drug-likeness RADAR for the compounds exhibiting significant activity was generated, and comprehensive ADME predictions were conducted. [16,17]

RESULTS AND DISCUSSION

Table 1: Preliminary

Chemical constituents	Result with ethanolic extract
Alkaloids	++
	+
	+
	+
Carbohydrate	+
	+
Protein	++
Steroids	-
Glycosides	+
Saponins	+
Flavonoids	+
	++
Tannins	+
	++

The material responded well to the DPPH free radical scavenging experiment and demonstrated antioxidant activity. It has strong ferric-reducing antioxidant capacity, according to the FRAP investigation. Compared to the control, it displayed less nitric oxide scavenging activity. The primary substances found by GC-MS analysis included "N-(2, 3, 4, 6-Tetra-O-acetyl-.beta.-d-glucopyranosyl)"-glycine, ethyl ester, 1-Dodecanamine, N-methyl-N-nitroso, Biphenyl-4-carboxylic acid, Indole-2-one derivative, and Bis (2- ethylhexyl) phthalate [Table 2].

Ligand Name	PubChem ID	*Score as binding energy	RMSD/UB
Rofecoxib (re-docking) 	5090	-6.7	0.00
"N-(2, 3, 4, 6 tetra-o-acetyl beta-alpha - glucopyranosyl)" glycine 	536074	-6.9	0.00
Bis (2-ethyl hexyl) phthalate 	8343	-6.5	0.00
4-biphenyl carboxylic acid 	66724	-7.7	0.00
Deodecyamine 	13583	-3.9	0.00

*n=9, a maximum negative score indicates best-predicted activity

Fig. 3: Results of Pyrex Docking

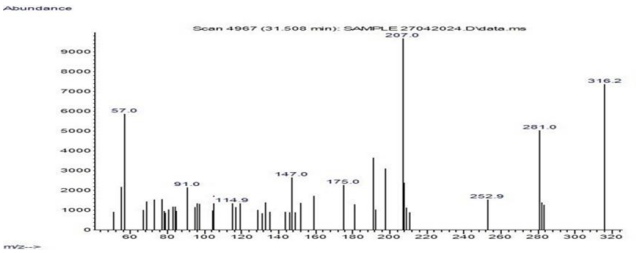


Fig. 4: GCMS spectra of extract (CUSAT SAIF KOCHI Kerala, India)

Five peaks were seen throughout the analysis [Figure 4]. The Phyto-compound 4- biphenyl carboxylic acid has demonstrated encouraging anti-inflammatory activity, as indicated by its high docking score. 4-biphenyl carboxylic acid received a docking score of -7.7 according to the PyRX technique. The standard drug used to compare docking scores was Rofecoxib, which had a docking score of -6.7. All the other phytoligands had similar binding energies

Table 2: GCMS analysis results of *Cleodendrum thomsoniae*

Peak #	Retention Time (min)	Area (%)	Library/ID	CAS Number	Quality Score	Putative Identification
1	16.711	30.41	NIST11. L	1000286-43-3	17	"N-(2,3,4,6-Tetra-O-acetyl-.beta.-d-glucopyranosyl)-glycine, ethyl ester (with lower quality matches for Acetaldehyde derivative and Benzene derivative)"
2	23.955	39.42	NIST11. L	055090-44-3	47	1-Dodecanamine, N-methyl-N-nitroso (with lower quality matches for Benzothiazole derivative and 4-Amino-4'-hydroxytoluene)
3	26.352	14.66	NIST11. L	2	58	Biphenyl-4-carboxylic acid (with lower quality matches for 2-Biphenylcarboxylic acid and 4- Methylnaphtho [1,2-b]theophany)
4	31.508	5.55	NIST11. L	1000129-52-1	25	Indole-2-one derivative (with lower quality matches for Hexahydropyridine derivative and Dibenzo [b, E]-8-azabicyclo [3, 2, 1] octane dvt.)
5	32.612	9.96	NIST11. L	000117-81-7	64	Bis(2-ethylhexyl) phthalate (with lower quality matches for Phthalic acid ester and Didecan-2-yl phthalate)

[Figure 3].

Researchers frequently use the molecular docking technique to identify possible ligand binding patterns with approved pharmaceutical proteins. This respectable technique has successfully investigated the interactions between different ligands and proteins. The COX-2 enzyme and Rofecoxib, a COX-2 receptor essential for inflammation and activated in the inflammatory pathway, were used in the current study's molecular Docking. The COX-2 enzyme and the medication Rofecoxib combine to produce a complex 5KIR. The literature indicates that the 5KIR receptor is broadly distributed because the COX-2 enzyme is a part of the inflammatory pathway that generates prostaglandins. It is a possible target for therapeutic study because it is active in inflammation. As a result, therapeutic action may be directed towards it using Rofecoxib for these people.

CONCLUSION

The application of in-silico testing using a variety of computer algorithms that forecast a compound's biological activity has been made possible by recent developments in computational biology. The analysis of the chemicals' binding energies with their corresponding biological receptors forms the primary basis of this predictive modeling. This study evaluated the anti-inflammatory properties of key chemicals isolated from the *Clerodendrum thomsoniae* Balf.f. using two different in-silico docking software packages. Rofecoxib, a drug with ID 5KIR, forms a complex with the COX-2 enzyme. Since the COX-2 enzyme contributes to the inflammatory pathway that produces prostaglandins, literature data show that the 5KIR receptor is widely distributed. Active in inflammation, it is a potential target for therapeutic research. Therefore, it may be the target of therapeutic intervention. Rofecoxib, a COX-2 inhibitor, is an excellent choice for lowering inflammation for these individuals. To create novel medications and treat pain and inflammation, it is essential to understand how Rofecoxib binds to the COX-2 enzyme. The molecular docking technique used in this study predicts the potential binding mechanism of new drugs that target the COX-2 enzyme. This lead chemical has a significant potential for additional structural changes. Designing and developing even more potent medications to reduce inflammation and pain may be more manageable using Quantitative Structure-Activity Relationship (QSAR) approaches. A significant turning point in the history of medicine is the creation of any new medication, particularly one that addresses human health concerns. These developments can help patients receive better treatment options and advance the general objective of improving healthcare outcomes. We are tapping into a wealth of possible new medications by continuing to investigate and validate the therapeutic qualities of chemicals originating from plants, eventually opening the door for creative solutions to various health issues.

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REFERENCES

1. Abbaszadeh G, Srivastava C, Walia S. Insect Growth Inhibitory Activity of Clerodane Diterpenoids Isolated from Clerodendron

infortunatum L. on the Cotton Bollworm, Helicoverpa armigera (Hubner). *National Academy Science Letters*. 2012;35(5):457–464. Available from: https://dx.doi.org/10.1007/s40009-012-0077-z.
2. Wahab SIA, Mohamed AWH, Mohamed OY, Taha MME, Abdul AB, Al-Zubairi AS. Serotonergic Properties of the Roots of Clerodendron capitatum. *American Journal of Biochemistry and Biotechnology*. 2008;4(4):425–430. Available from: https://dx.doi.org/10.3844/ajbbsp.2008.425.430.
3. Ogunlakin AD, Akinwumi IA, Ambali OA. Ethnomedicinal application, phytochemistry and therapeutic effects of genus clerodendrum. *Functional Food Science*. 2023;3(10):228–247. Available from: https://dx.doi.org/10.31989/ffs.v3i10.1151.
4. Bhattacharyya S, Samanta S, Hore M, Barai S, Dash SK, Roy S. Phytochemical compositions, antioxidant, anticholinesterase, and antibacterial properties of Clerodendrum thomsoniae Balf.f. leaves: In vitro and in silico analyses. *Pharmacological Research - Natural Products*. 2024;5:100121. Available from: https://dx.doi.org/10.1016/j.prenap.2024.100121.
5. Rao A, Gupta A, Kain V, Halade GV. Integrative Cardiovascular Physiology and Pathophysiology: Extrinsic and intrinsic modulators of inflammation-resolution signaling in heart failure. *American Journal of Physiology-Heart and Circulatory Physiology*. 2023;325:433–448. Available from: https://doi.org/10.1152/ajpheart.00276.2023.
6. Anwar G, Chen D, Chen Q, Xia C, Yan J. Rofecoxib derivatives as NIR fluorescent probes for mitochondrial viscosity and in vivo imaging of Aβ plaques. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*. 2024;307:123637. Available from: https://dx.doi.org/10.1016/j.saa.2023.123637.
7. Peng Z, Wang Y, He J, Zhang J, Pan X, Ye X, et al. Chemical constituents and their antioxidant and anti-inflammatory activities from edible Cornus officinalis fruits. *European Food Research and Technology*. 2022;248(4):1003–1010. Available from: https://dx.doi.org/10.1007/s00217-021-03940-6.
8. Rahman MM, Hossain R, Herrera-Bravo J, Islam MT, Atolani O, Adeyemi OS, et al. Natural antioxidants from some fruits, seeds, foods, natural products, and associated health benefits: An update. *Food science & nutrition*. 2023;11(4):1657–1670. Available from: https://doi.org/10.1002/fsn3.3217.
9. Ali KA, Maity A, Roy SD, Pramanik SD, Das PP, Shaharyar MA. Insight into the mechanism of steroidal and non-steroidal anti-inflammatory drugs. *How Synthetic Drugs Work*. 2023;p. 61–94. Available from: https://doi.org/10.1016/B978-0-323-99855-0.00004-X.
10. Margret IU, Joy U, Nnaemeka S. Identification, Quantification of Phytochemicals and Elemental Analysis from Ethanolic Leaf Extract of Andrographis paniculata. *South Asian Research Journal of Natural Products*. 2023;6(3):144–156. Available from: https://journalsarjnp.com/index.php/SARJNP/article/view/120.
11. Rudakov YO, Selemenev VF, Shelekhova NV, Rudakov OB, Khorokhordin AM. Method of Gas Chromatography–Mass Spectrometry for the Determination of Free Bisphenol A in Ethanol Extracts. *Journal of Analytical Chemistry*. 2023;23(1):6–17. Available from: https://dx.doi.org/10.17308/sorpchrom.2023.23/10989.
12. Zhi Y, Lu J, Zheng Q, Cao X, Lv M, Xu Q, et al. Evaluating the detection of barbiturates in dried blood spots: A comparative analysis using gas chromatography-mass spectrometry, gas chromatography-tandem mass spectrometry, and liquid chromatography-tandem mass spectrometry with different extraction methods. *Journal of Chromatography A*. 2024;1737:465434. Available from: https://dx.doi.org/10.1016/j.chroma.2024.465434.
13. Ivanova B. Stochastic dynamics mass spectrometric and Fourier transform infrared spectroscopic structural analyses of composite biodegradable plastics. *Pollution Study*. 2024;5(2):1–55. Available from: https://dx.doi.org/10.54517/ps.v5i1.2741.
14. Xu Z, Wang L, Liu H, Tian X, Wang Y, Xu H, et al. Analysis of Herbal Constituents and In Vivo Pharmacokinetics of Gegen–Huangqi Decoction in Rat Plasma Using HPLC-Q-TOF-MS/MS and HPLC-QQQ-MS/MS. *Biomedical Chromatography*. 2025;39(1):6046. Available from: https://dx.doi.org/10.1002/bmc.6046.
15. Houshmand F, Houshmand S. Potentially Highly Effective Drugs for COVID-19: Virtual Screening and Molecular Docking Study Through PyRx-Vina Approach. *Frontiers in Health Informatics*. 2023;12:1–6. Available from: http://dx.doi.org/10.30699/fhi.v12i0.478.
16. Afridi MB, Sardar H, Serdaroğlu G, Shah SWA, Alsharif KF, Khan H. SwissADME studies and Density Functional Theory (DFT) approaches of methyl substituted curcumin derivatives. *Computational Biology and Chemistry*. 2024;112:108153. Available from: https://dx.doi.org/10.1016/j.compbiolchem.2024.108153.
17. Bakchi B, Krishna AD, Sreecharan E, Ganesh VBJ, Niharika M, Maharshi S, et al. An overview on applications of SwissADME web tool in the design and development of anticancer, antitubercular and antimicrobial agents: A medicinal chemist's perspective. *Journal of Molecular Structure*. 2022;1259:132712. Available from: https://dx.doi.org/10.1016/j.molstruc.2022.132712.