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Original Article

In vitro and Computational Studies of Compounds from *Coleus amboinicus* against Osteosarcoma

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ABSTRACT

Background: Osteosarcoma (OS) is among the most prevalent primary bone cancers, predominantly affecting children and adolescents. The average prevalent rate of osteosarcoma is 4.3 per million in males and 3.4 per million in females. Osteosarcoma poses diagnostic challenges due to the often-subtle clinical manifestations. **Objectives:** To investigate bioactive compounds in *Coleus amboinicus* Leaves (CAL) using computational methods and predict, their potential interactions with osteosarcoma cell surface receptors to explore therapeutic possibilities. **Materials and Methods:** The extract obtained from CAL was analyzed using a phytochemical assay, Fourier-Transform Infrared (FTIR) spectroscopy, and Gas Chromatography-Mass Spectrometry (GCMS). Molecular docking studies were conducted using Autodock to assess the interactions between the components of the extract and the cell surface receptors of osteosarcoma. The molecular docking experiments assessed the affinity of the phytochemicals to particular proteins that have a significant impact on the cell surface receptors of osteosarcoma. The pharmacokinetic characteristics of the drugs were assessed by examining their ADMET (absorption, distribution, metabolism, excretion, and toxicity) profiles. **Results:** The computer study identified multiple phytochemicals derived from CAL with substantial binding affinity to the proteins of osteosarcoma cell surface receptors. These substances include beta-D-Glucopyranose, 4-O-beta-D-galactopyranosyl, Hexanoic acid, 1-methylethyl ester, and Paromomycin. The ADMET analysis revealed that most of the discovered drugs possess favorable pharmacokinetic features and are anticipated to have low toxicity. **Conclusion:** Molecular docking revealed bioactive compounds with strong binding affinity to these receptors, indicating their potential inhibitory effects on cancer cell proliferation. Further research is required to provide comprehensive validation and clinical development.

Keywords: Molecular docking, Plant extract, Osteosarcoma, GCMS, Phytochemical test, Bioactive compounds.

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INTRODUCTION

Osteosarcoma (OS) is a cancer that develops in connective tissue, specifically in bones. It is responsible for 20% of all primary bone tumors worldwide. It is the most prevalent type of malignant bone tumour among adolescents.[1] Males are more likely to be affected than females. It can develop at any age, despite peaking in the second and third decades of life.[2] OS is widespread in the metaphysis of long tubular bones (e.g., the proximal tibia, proximal humerus, and distal femur), but is uncommon in the spine, pelvis, and sacrum. Clinically, swelling and localized pain are the primary symptoms at the beginning of the illness, with joint dysfunction appearing less frequently.[1] Currently, available curative radical therapy consists of surgery along with three or four cytotoxic drugs (doxorubicin, cisplatin, and high-dose methotrexate/ifosfamide). These drugs are used in a multimodal approach, both before and after surgery. Nevertheless, survival rates have remained virtually constant over the past 20 years despite several attempts with various chemotherapy regimens, and no effective targeted treatments have been identified for osteosarcoma so far.[3] OS treatment in the future will need to depend on the "bench to bedside" concept.[2]

Hyaluronic Acid (HA) stimulates OS cell proliferation and invasion via intracellular signal transduction. Inhibiting HA

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accumulation decreased OS cell proliferation, motility, and invasiveness. It can also reduce cell viability and induce apoptosis. HA engages with cells via cell surface receptors, such as Cluster of Differentiation 44 (CD44), regulating cell-cell interactions, adhesion, and migration.[4]

Researchers are investigating the use of Tumor-Associated Macrophages (TAMs) for treating OS. By using a liposome that eliminates macrophages, TAMs were ablated in human OS implantation mice, therefore inhibiting tumor formation. Tumor development is inhibited by silencing epidermal growth factor receptors in implanted cells. Drug research can use TAMs-specific surface molecules, such as CD47R and PD-1, to create immune checkpoint inhibitors.[5] Every manifestation of cancer has been linked to a change in a distinctive molecular fingerprint. However, invasive development and unchecked proliferation are traits that all malignancies share.[6] Cancers are treated using a variety of treatment modalities, including radiation, chemotherapy, and/or surgery.[7]

Incorporating natural substances is a viable method treatment for cancer. Plant-based products are very effective in this way because their extract contains different kinds of phenols, flavonoids, and other secondary metabolites that are far safer to use than synthetic substances because they are less hazardous to humans.[6] Bioactive substances such as curcumin, myricetin, geranin, tocotrienol, quercetin, resveratrol, berberine, and genistein exhibit anti-cancer properties.[2,3] Curcumin-mediated alterations in ITPR1(Inositol 1,4,5-Trisphosphate Receptor type 1) increased apoptosis and inhibited proliferation, migration, and invasion, indicating curcumin's potential for osteosarcoma treatment. Findings showed that genistein suppressed OS cell development. Without impacting normal osteoblast cells, Resveratrol (Res) inhibits the regeneration of OS cells to regenerate.[8]

Plectranthus amboinicus, formerly known as *Coleus amboinicus*, is a perennial semi-succulent plant belonging to *Lamiaceae* family that has a strong smell and aroma reminiscent of oregano. The plant is also known as Indian mint or borage. The plant contains β-selinene, thymol, α-γ-terpinene, humulene, α-terpineol, caryophyllene oxide, undecanal, ρ-cymene, and carvacrol, along with flavonoids and phenolics in its ethanolic extract.[9] Numerous flavonoids, including chrysoerial, cirsimaritin, eriodictyol, luteolin, rutin, salvigenin, thymoquinone, quercetin, apigenin, and 5-O-methyl-luteolin, have been found in the leaves of *P. amboinicus* (Lour.) *Spreng.*[10] *C. amboinicus* Leaves (CAL) had higher percentages of the monoterpene chemical carvacrol and thymoquinone than at the other time points, according to the GCMS data. For many years, researchers have been analyzing the functional characteristics and bioactivities of carvacrol and thymoquinone, including their effect on oxidative stress and antioxidants activities.[11]

According to these findings, VO(CQ)2 (VO-Clioquinol) is a viable option that may have antimetastatic action against OS cells; therefore, it would be worthwhile to investigate this complex in additional *in vivo* tests for the treatment of cancer.[12] Vascular Endothelial Growth Factor (VEGF) is a key player in angiogenesis and primarily acts on endothelial cells; inhibition of VEGF signaling in OS results in cell growth arrest and apoptosis; VEGFR-2 and Programmed Death-Ligand 1 (PD-L1) are expressed in approximately 64.5% and 35.5% of cases, respectively, of OS.[13]

Similarly, *Moringa oleifera* Leaf (MOL) extract promotes cell growth at low concentrations, inhibits proliferation at high concentration, enhances osteoblast activity, and interacts with BMP2 and Runx2 proteins with good binding affinity and pharmacokinetics as demonstrated by *in silico* studies.[14] This study aims to investigate bioactive compounds in CAL using computational methods and predict their potential interactions with osteosarcoma cell surface receptors to explore therapeutic possibilities.

MATERIALS AND METHODS

Preparation of aqueous extract

The CAL plant was acquired from Chennai, Tamil Nadu, India. The samples were verified by the Centre for Advanced Studies in Botany at the University of Madras, Chennai, India. The preparation of the CAL powder included several procedures, including the removal of solid particles and dust by washing with double distilled water, followed by shade drying for 3 days. Once the CAL dried up, it was powdered using a mechanical grinder and then filtered using a sieve. The obtained biosorbent was stored for future purposes.

For the experiment to prepare *C. amboinicus* Leaf Extract (CALE), 25 g of dried CAL powder was added to 100 mL of distilled water and stirred carefully. The solution was left overnight, and then heated, for 20 min at 60°C. After cooling, the solution was filtered using Whatman No. 1 filter paper to remove any leftover particles. The resulting liquid extract was stored at 4°C for future experiments.[15]

FTIR

FTIR spectroscopy is an analytical method used for the identification of organic, polymeric, and occasionally inorganic substances. The process operates by quantifying the absorption of infrared radiation by a specimen at various wavelengths, resulting in a spectrum that accurately depicts the unique molecular characteristics of the specimen. After the extraction process, the CALE was analyzed by Fourier-Transform Infrared (FTIR) spectroscopy in the range of 4000-400 cm^{-1}. This analysis effectively identified several functional presents in CALE.[15]

GCMS

GCMS operates by initially separating the chemical constituents of a sample by Gas Chromatography (GC) and subsequently determining the identity of each constituent through Mass Spectrometry (MS). This analysis was performed using a Shimadzu Gas chromatograph mass spectrometer, Model GC/MS-QP2O2O EI. 100 µL of an aqueous solution of CALE was dissolved in 1 mm of methanol. The solution was agitated firmly using a vortex stirrer for 20 sec and then filtered through a 0.2-micron membrane filter. Subsequently, this clear extract was used for GCMS examination. The following methods are outlined.[15]

Phytochemical analysis

Phytochemical analysis is the scientific procedure of identifying and analyzing bioactive compounds found in plants. Phytochemicals, such as alkaloids, flavonoids, tannins, saponins, glycosides, terpenoids, phenols, carbohydrates, proteins, and fatty acid compounds, comprise a diverse range of substances found in plants. These compounds have a role in the therapeutic, nutritional, and poisonous qualities of plants. Qualitative analysis of the aqueous extract of CAL was conducted using a variety of chemical tests.[16]

In silico study

The protein structures were acquired from the Research Collaboratory for Structural Bioinformatics (RCSB) Protein Data Bank (PDB) (accessed: May 10, 2024: https://www.rcsb.org/) and validated via a build/check/repair model to ensure integrity, using AutoDock Tools for pdbqt file preparation. Ligands from PubChem were optimized in Avogadro and were converted for docking. AutoDock4 was used for molecular docking with a fine-tuned grid, followed by cluster analysis to determine the optimal binding poses. Binding interactions and affinities were analyzed in Biovia Discovery Studio (accessed: May 10, 2024: https://www.3ds.com/products/biovia/discovery-studio). *In silico* ADMET studies were performed using Swiss ADME (accessed: May 10, 2024: http://www.swissadme.ch/), assessing drug-likeness based on Lipinski's rule of five and interaction with biological components to predict therapeutic efficacy and safety. Figure 1 shows the graphical abstract of the *in silico* study on CALE.[15]

RESULTS

FTIR

C. amboinicus's FTIR spectrum shown in Figure 2 has peaks at 3245 cm^{-1} for spreading O-H/N-H, 1574 cm^{-1}, 1386 cm^{-1} for C=C stretching and 1060 cm^{-1} for extending from C to O. This implies that to establish appropriate communication with OS cell receptors and alter cell signaling and receptor activity, hydrogen bonding, polar interactions and metal-ligand interactions must occur. Thus, such interaction assessment is crucial for evaluating the binding/action of CAL constituents with OS cell surface receptors that may influence the function of the receptors or signal transduction events in the pathway. These may have metal ions as co-factors or receptor activation may depend on the metal-oxygen bones of the coordination complex.

GC-MS

CAL underwent GC-MS, as investigation shown in Figure 3, which produced numerous important peaks representing various chemicals. While the peaks at 10.067 and 12.007 min most likely indicate important constituents, the peaks at 7.841 and 9.130 min suggest minor, volatile chemicals like aldehydes or ketones. Peaks

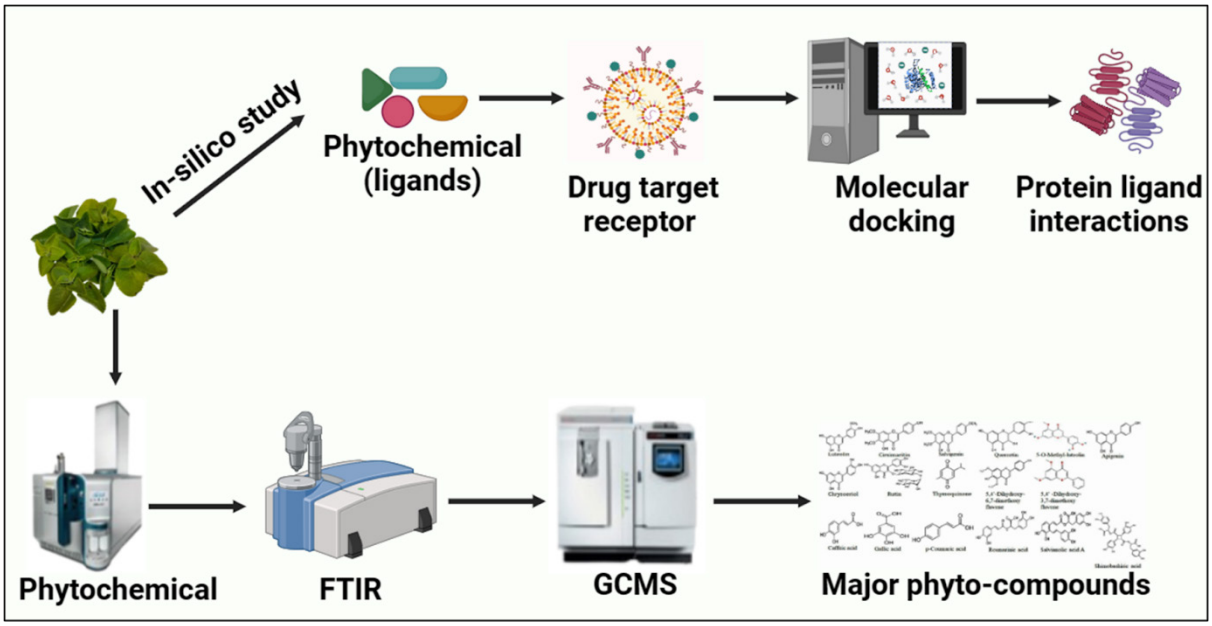

Figure 1: Graphical abstract of *in silico* study of *Coleus amboinicus* leaf extract.

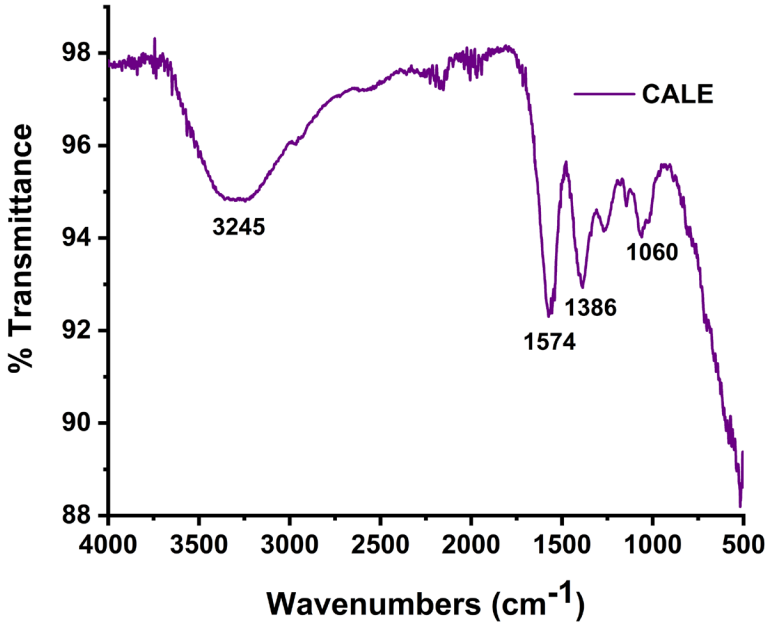

Figure 2: FTIR spectrum of *Coleus amboinicus* leaf extract.

occurring at or after 18.860 min indicate larger or less volatile molecules. The spectrum points to a complicated composition that may be effective against OS cell receptors, indicating the need for more research in the hopes of finding therapeutic uses.

Phytochemical analysis

The preliminary phytochemical survey of CAL is shown in Table 1. It has a favorable content of flavonoids and fatty acids that might show appreciable antioxidant and cardiovascular activities. Thus, low tannins, phenols, terpenoids, alkaloids, and saponin levels have anti-inflammatory, antibacterial, and anti-cancer characteristics. Nutritional value incorporates proteins and carbohydrates at the same time, making it more valuable. This report also shows the potential for health gain when treating inflammation-related diseases as well as oxidative stress in addition to the supportive potential of conventional medical use.

Molecular docking

Paromomycin showed the greatest binding affinity (-7.0) and strong interactions among the ligands tested against the osteosarcoma cell surface receptor, particularly through numerous H bonds and diverse amino acid residues. Considerable hydrogen and Van der Waals interactions supported the mild affinity (-6.2) of 4-O-beta-D-galactopyranosyl and beta-D-glucopyranose. With an affinity of -4.6, hexanoic acid, 1-methylethyl ester mostly relied on hydrophobic interactions, as shown in Table 2.

Table 1: **Phytochemical analysis of *Coleus amboinicus* leaf extract.**

Phytochemicals	*Coleus amboinicus*
Alkaloid	++
Flavonoid	+++
Tannins	++
Saponin	++
Glycoside	+
Terpenoids	++
Phenol	++
Carbohydrates	+
Proteins	+
Fatty acids	+++

Note: (+++): High present, (++): Low present, and (+): Present.

Interaction of ligands with the surface receptor of osteosarcoma cell

Different interaction characteristics of ligands against the osteosarcoma cell surface receptor are shown in Figure 4. Stability is increased by the moderate binding of 4-O-beta-D-galactopyranosyl and beta-D-glucopyranose, which exhibit strong hydrogen bonding and van der Waals interactions. Lower affinity is correlated with easier interactions, mainly via van der Waals and alkyl forces, in hexanoic acid, and 1-methylethyl ester. With several hydrogen bonds and a sophisticated interaction network, paromomycin exhibits strong, selective binding, suggesting potential therapeutic benefits. Because of its superior binding properties, paromomycin appears to be a promising option for further therapeutic research against OS.

ADME properties of drug

Paromomycin

The examined result in Figure 5 shows a complex configuration with several amine and hydroxyl groups, suggesting strong hydrogen bonding interactions under aqueous conditions and high polarity. The low lipophilicity, high polarity, and modest size were all confirmed by the radar plot and were consistent with its structure. These properties point to the molecule's possible applicability in biology, possibly as a bioactive substance with the ability to interact with biological targets in certain ways. The therapeutic potential of this agent in pharmaceutical or biological research might be validated by further investigation.

Physiochemical properties

With a molecular weight of 615.63 g/mol and formula $C_{23}H_{45}N_5O_{14}$, the molecule exhibits low GI absorption and a high polar surface area (TPSA 347.32 Å²), demonstrating that it is not easily absorbed when taken orally and cannot pass through the blood-brain barrier, as shown in Table 3. These results point to possible restrictions on drug use when taken orally, necessitating changes to the formulation or delivery systems to achieve successful therapeutic use.

DISCUSSION

There are various studies on the use of CAL against OS, and its diverse chemical structure and thinking ability have been analyzed through different approaches. When compared with related studies, our further work of FTIR analysis of the ZNO/CHIT nanocomposite revealed more profound peaks related to hydrogen bonding, polar interactions and metal-ligand interactions (3245, 822, 1574, 1386, 1060 cm^{-1}) which are highly effective in altering OS cell receptors and signaling.[17] Thus, this finding supports previous studies that indicated that the phytochemical content, flavonoids, and fatty acids, are responsible for the antioxidant and potentially anti-cancer effects, which can be attributed to tannins, phenols, terpenoids, alkaloids, and saponins (GCMS peaks at 10. 067 and 12. 007 min).[18] However, for the knowledge of author, the present research has filled a gap by providing information concerning the nutritional content, here protein and carbohydrates that buttress traditional medicinal uses and hence post positive linkages. There was a suggestion from the molecular docking simulation that paromomycin and other compounds from CAL were potentially therapeutically important; The binding became particularly stronger with the osteosarcoma receptor (-7. 0) through hydrogen bonds and amino acid interactions, as supported by structural analysis. These findings support the complex role of CAL activities in OS, and call for further investigations regarding the application and prominence of its biochemical constituents in cancer medicine.[19]

Among the physicochemical properties and ADME properties of CAL, the molecule carries a complex arrangement (molecular weight of 615.63 g/mol formula $C_{23}H_{45}N_5O_{14}$), which consequently influences the bioactivity and pharmacokinetics of the plant. These results are also consistent with other studies regarding the nature of its highly polar, low lipophilicity, and relatively large surface area (347.32 Å²), which limits its oral bioavailability and ability to cross the blood-brain barrier.[20] This is similar to research on natural products that interact with cancer receptors. The development of new drug delivery systems or formulations might be useful for enhancing the therapeutic efficiency of natural products targeting cancer.[21] However, our study contributes to the existing literature by providing additional evidence regarding CAL's potential as a bioactive substance, and its suitability as a treatment in clinical practice, which requires further research into increasing its biological availability and overcoming natural barriers. Our results, when compared with those of previous

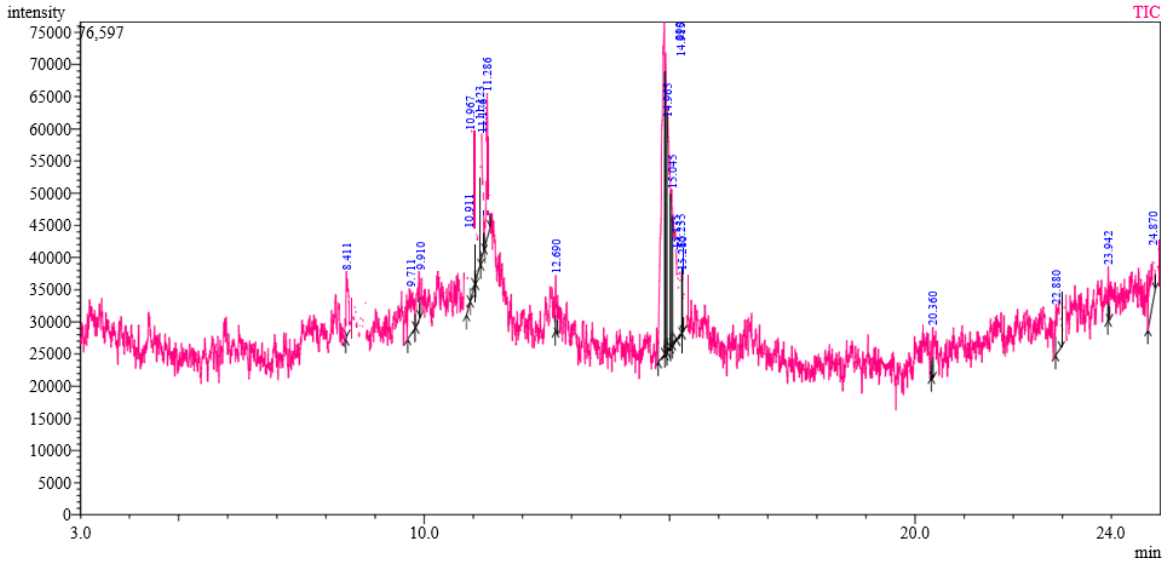

Figure 3: GCMS spectra of *Coleus amboinicus* leaf extract.

Table 2: **Molecular docking analysis.**

Ligand Name	Binding Affinity Value (kcal/mol)	Distance (Å)	Hydrogen Interaction	Amino acid residues
beta-D-Glucopyranose, 4-O-beta-D-galactopyranosyl	-6.2	3.0 GLY 133) 3.0 (ALA 208)	1. Van der Waals 2. Conventional Hydrogen Bond 3. Carbon HHydrogen Bond 4. Unfavorablr Donor-Donor Bond	1. LEU B:207, LEU B:259, ALA B:156, THR B:205, ASP B:134, GLY B:135, ASP B:270, VAL B:140, LYS B:158 2. GLY B:133 3. ALA B:208, LEU B:132
Hexanoic acid, 1-methylethyl ester	-4.6	-	1. Van der Waals 2. Alkyl	1. GLU B:206, VAL B:140, THR B:205, ASP B:270, GLY B:269 2. LEU B:132, LEU B:259, ALA B:156, ALA B:208, LYS B:158, ILE B:190
Paromomycin	-7.0	2.2(GLU 130) 2.6 (GLY 211) 2.3 (LEU 132) 2.3 (SER 212) 2.6 (PRO 209)	1. Van der Waals 2. Conventional Hydrogen Bond 3. Carbon hydrogen bond	1. ARG B:256, ASP B:134, GLY B:133, LYS B:131, ARG B:142, ASP B:215, LEU B: 207, GLY B: 211, LE B:259, VAL B:140, ASP B:270 2. LEU B:132, SER B:212, ALA B:215, PRO B:209, GLU B:130 3. PRO B:209

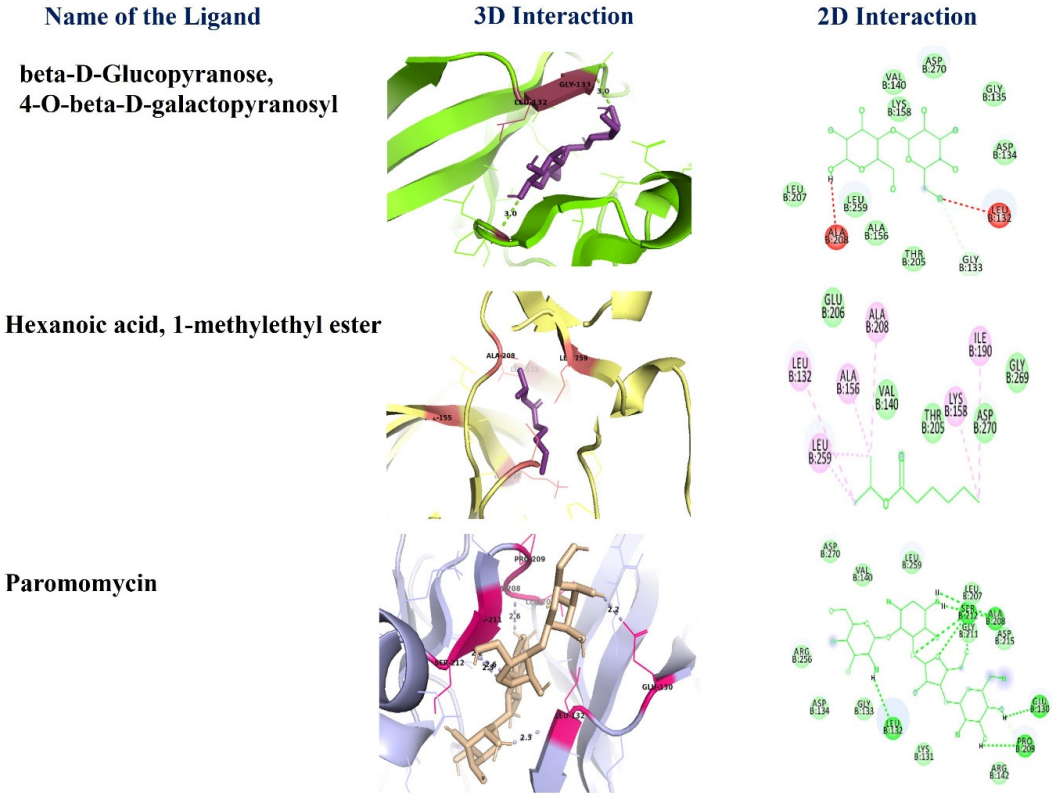

Figure 4: Interactions of ligands with the surface receptor of osteosarcoma cell.

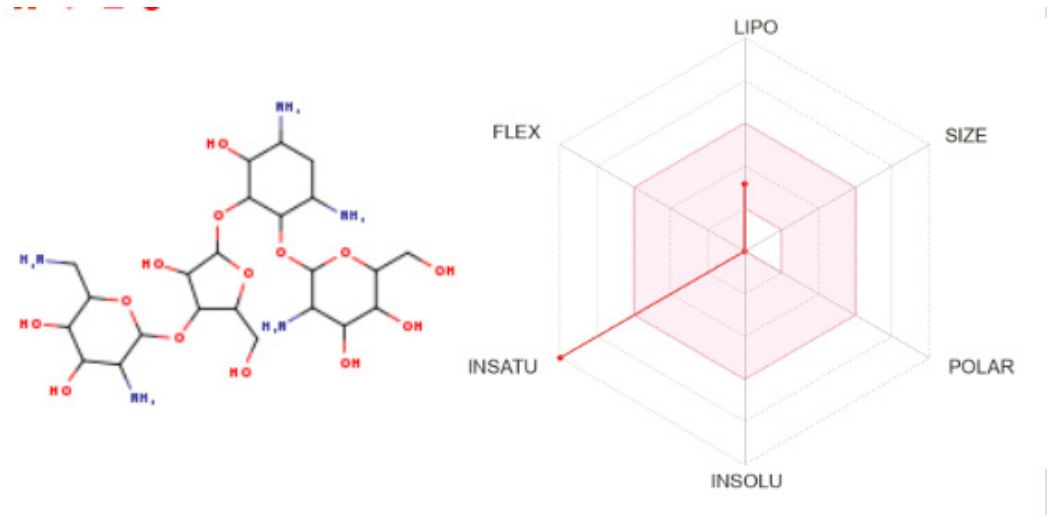

Figure 5: The molecular configuration of the compound.

Table 3: Physiochemical analysis of the extract.

Physiochemical Properties	
Mol wt (g/mol)	615.63 g/mol
Formula	$C_{23}H_{45}N_5O_{14}$
Canonical SMILES	OCC1OC(C(C1OC1OC(CN)C(C(C1N)O)O)O)OC1C(O)C(N)CC(C1OC1OC(CO)C(C(C1N)O)O)N
TPSA	347.32 Å²
BBB permeant	No
GI absorption	Low
Lipinski violations	No; 3 violations: MW> 500, NorO> 10, NH or OH> 5
Bioavailability Score	0.17
Synthetic Accessibility	7.37
Water solubility	Moderately soluble

research, demonstrate the significance of CAL in the overall context of studies investigating natural product-based cancer treatments. Additionally, our study's findings encourage more research into the potential applications of CAL for treating OS and possibly other cancers.

CONCLUSION

In conclusion, the *in silico* investigation of CALE against osteosarcoma cell surface receptors highlights its potential as a promising therapeutic agent. Through molecular docking and computational analysis, several bioactive compounds in CALE exhibited significant binding affinity to osteosarcoma receptors, suggesting possible inhibitory effects on cancer cell proliferation. These findings provide a foundation for further experimental validation and the development of CALE-derived compounds for osteosarcoma treatment. Future studies should focus on *in vitro* and *in vivo* assessments to fully establish the efficacy and safety of these natural compounds in cancer therapy.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

CAL: *Coleus amboinicus* leaves; **FTIR:** Fourier-transform infrared; **GCMS:** Gas Chromatography-Mass Spectrometry; **ADMET:** Absorption, Distribution, Metabolism, Excretion, and Toxicity; **OS:** Osteosarcoma; **HA:** Hyaluronic Acid; **CD44:** Cluster of Differentiation 44; **TAMs:** Tumor-Associated Macrophages; **VO(CQ)2:** VO-Clioquinol; **VEGF:** Vascular Endothelial Growth Factor; **PD-L1:** Programmed Death-Ligand 1; **MOL:** *Moringa oleifera* leaf; **CALE:** *C. amboinicus* leaf Extract; **RCSB:** Research Collaboratory for Structural Bioinformatics; **PDB:** Protein Data Bank.

AUTHORSHIP CONTRIBUTIONS

Archana Behera: Conceptualization, Data curation, Formal analysis; Methodology, Writing-original draft, and Writing-review and editing. **Raeesha Rahman:** Writing-original draft, Writing-review and editing. **Benjamin Vinodh Joshua:** Investigation, Visualization. **Iadalin Ryntathiang:** Conceptualization, Data curation, Formal analysis; Methodology, Writing-original draft, and Writing-review and editing.

Monisha Prasad: Investigation, Visualization. **Mukesh Kumar Dharmalingam Jothinathan:** Investigation, Visualization.

SUMMARY

Osteosarcoma (OS) is a prevalent bone malignancy mostly impacting children and adolescents, with an average incidence rate of approximately 4.3 per million in men and 3.4 per million in women. Diagnosing osteosarcoma might be difficult due to its frequently understated symptoms. This study sought to investigate the medicinal potential of bioactive chemicals present in the leaves of CAL as medicines against osteosarcoma.

The study entailed the analysis of CAL extract by multiple methodologies, including phytochemical tests, FTIR spectroscopy, and GC-MS. Molecular docking analyses were performed to anticipate the interactions of bioactive chemicals with the surface receptors of osteosarcoma cells, which are pivotal in cancer growth. The findings indicated that multiple chemicals in CAL, including as beta-D-Glucopyranose and Paromomycin, exhibit substantial binding affinity to these receptors, implying their potential to impede cancer cell proliferation.

Furthermore, the pharmacokinetic analysis (ADMET) indicated that these compounds possess advantageous properties for medication development and are expected to be safe with little toxicity. Although these findings are encouraging, additional research is required to comprehensively evaluate these molecules and advance toward clinical application.

REFERENCES

1. Zhao X, Wu Q, Gong X, Liu J, Ma Y. Osteosarcoma: a review of current and future therapeutic approaches. Biomed Eng OnLine. 2021; 20(1): 24. doi: 10.1186/s12938-021-00860-0, PMID 33653371.
2. Smrke A, Anderson PM, Gulia A, Gennatas S, Huang PH, Jones RL. Future directions in the treatment of osteosarcoma. Cells. 2021; 10(1): 172. doi: 10.3390/cells10010172, PMID 33467756.
3. Czarnecka AM, Synoradzki K, Firlej W, Bartnik E, Sobczuk P, Fiedorowicz M, *et al.* Molecular biology of osteosarcoma. Cancers. 2020; 12(8): 2130. doi: 10.3390/cancers12082130, PMID 32751922.
4. Cui J, Dean D, Hornicek FJ, Chen Z, Duan Z. The role of extracellular matrix in osteosarcoma progression and metastasis. J Exp Clin Cancer Res. 2020; 39(1): 178. doi: 10.1186/s13046-020-01685-w, PMID 32887645.
5. Zhu T, Han J, Yang L, Cai Z, Sun W, Hua Y, *et al.* Immune microenvironment in osteosarcoma: components, therapeutic strategies and clinical applications. Front Immunol. 2022; 13: 907550. doi: 10.3389/fimmu.2022.907550, PMID 35720360.
6. Roy A, Datta S, Bhatia KS, Bhumika P, Jha P, Prasad R. Role of plant derived bioactive compounds against cancer. S Afr J Bot. 2022; 149: 1017-28. doi: 10.1016/j.sajb.2021.10.015.
7. Subramaniam S, Selvaduray KR, Radhakrishnan AK. Bioactive compounds: natural defense against cancer? Biomolecules. 2019; 9(12): 758. doi: 10.3390/biom9120758, PMID 31766399.
8. Tobeiha M, Rajabi A, Raisi A, Mohajeri M, Yazdi SM, Davoodvandi A, *et al.* Potential of natural products in osteosarcoma treatment: focus on molecular mechanisms. Biomed Pharmacother. 2021; 144: 112257. doi: 10.1016/j.biopha.2021.112257, PMID 34688081.
9. Arumugam G, Swamy MK, Sinniah UR. *Plectranthus amboinicus* (Lour.) Spreng: botanical, phytochemical, pharmacological and nutritional significance. Molecules. 2016; 21(4): 369. doi: 10.3390/molecules21040369, PMID 27043511.
10. Manurung K, Sulastri D, Zubir N, Ilyas S. *In silico* anticancer activity and *in vitro* antioxidant of flavonoids in *Plectranthus amboinicus*. Pharmacogn J. 2020; 12(6s):1573-7. doi: 10.5530/pj.2020.12.215.
11. Ashaari NS, Mohamad NE, Afzinizam AH, Ab, Lai KS, Ong Abdullah J. Chemical composition of hexane-extracted *Plectranthus amboinicus* leaf essential oil: maximizing contents on harvested plant materials. Appl Sci. 2021; 11(22): 10838. doi: 10.3390/app112210838.
12. Balsa LM, Quispe P, Baran EJ, Lavecchia MJ, León IE. *In silico* and *in vitro* analysis of FAK/MMP signaling axis inhibition by VO-clioquinol in 2D and 3D human osteosarcoma cancer cells. Metallomics. 2020; 12(12): 1931-40. doi: 10.1039/d0mt00176g, PMID 33107537.
13. Velayutham NK, Thamaraikani T, Wahab S, Khalid M, Ramachawolran G, Abullais SS, *et al.* Stylopine: A potential natural metabolite to block vascular endothelial growth factor receptor 2 (VEGFR2) in osteosarcoma therapy. Front Pharmacol. 2023; 14: 1150270. doi: 10.3389/fphar.2023.1150270, PMID 37056983.
14. Khan MI, Siddiqui S, Barkat MA, Alhodieb FS, Ashfaq F, Barkat HA, et al. *Moringa oleifera* leaf extract induces osteogenic-like differentiation of human osteosarcoma SaOS2 cells. J Tradit Complement Med. 2022; 12(6): 608-18. doi: 10.1016/j.jtcme.2022.08.006, PMID 36325245.
15. Sudhakar Rao SV, Ryntathiang I, Behera A, Saravanan S, Prasad M, Dharmalingam Jothinathan MK. Comparative *in vitro* and *in silico* Analyses of Phytochemicals from *Butea monosperma* for Wound-Healing Potential in Human Cells. Cureus. 2024; 16(6): e62078. doi: 10.7759/cureus.62078, PMID 38989336.
16. Behera A, Dharmalingam Jothinathan MK, Ryntathiang I, Saravanan S, Murugan R. Comparative Antioxidant Efficacy of Green-Synthesised Selenium Nanoparticles from *Pongamia pinnata*, *Citrus sinensis*, and *Acacia auriculiformis*: an *in vitro* Analysis. Cureus. 2024; 16(4): e58439. doi: 10.7759/cureus.58439, PMID 38765437.
17. Saravanan K, Alarfaj AA, Hirad AH, Ravindran B, Narasimhamoorthi SP. Antibacterial and *in silico* analysis of biogenic fabrication of CuO nanoparticles employing *Coleus amboinicus* leaf extract. Biomass Convers Biorefin. 2023; 13: 1-13.
18. Pinheiro GP, Ferreira da Silva G, Frankland Sawaya AC. Effect of leaf development on the composition of *Coleus amboinicus* Lour. hydroethanolic extracts. Biochem Syst Ecol. 2023 Dec 1 ; 111: 104738. doi: 10.1016/j.bse.2023.104738.
19. Haryadi W, Gurning K, Astuti E. Molecular target identification of two *Coleus amboinicus* leaf isolates toward lung cancer using a bioinformatic approach and molecular docking-based assessment. J Appl Pharm Sci. 2024; 14(5): 203-10. doi: 10.7324/JAPS.2024.164753.
20. Al-Ghamdi AY, Fadlelmula AA, Abdalla MO, Zabin SA. Phytochemical screening, chemical composition, antimicrobial activity and *in silico* investigation of the essential oil of *Coleus forskohlii* L. collected from the southwestern region of Saudi Arabia. J Essent Oil Bear Pl. 2021; 24(1): 120-33. doi: 10.1080/0972060X.2021.1901613.
21. Rajkumar M, Kirubakaran D, Selvam K, Prathap N, Thangaraj R, Vimala K, *et al.* Green synthesis of gelatin-loaded magnesium hydroxide nanocomposite biomaterial using *Coleus amboinicus* leaf extract for enhanced antibacterial, antioxidant, anticholinergic, and wound healing activities. J Mater Res. 2024; 39(4): 548-64. doi: 10.1557/s43578-023-01249-6.

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