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Research Article

GC-MS ANALYSIS AND *IN SILICO* ANTI-CANCER STUDIES OF BIO-ACTIVE COMPOUNDS FROM ETHANOLIC EXTRACTS OF *INDIGOFERA CORDIFOLIA* AND *MAYTENUS EMARGINATA*

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ABSTRACT

Objective: The present study focuses on identifying bioactive compounds from the ethanolic extraction of *Indigofera cordifolia* and *Maytenus emarginata* using gas chromatography-mass spectrometry (GC-MS) analysis. In addition, the study evaluates the phytochemical constituents present in these extracts and explores their potential anticancer activity through *in silico* analysis, including pharmacokinetic evaluation, molecular docking, and molecular dynamics (MD) simulation.

Methods: Phytochemical screening was performed to detect flavonoids, phenolic compounds, triterpenoids, tannins, saponins, amino acids, proteins, steroids, and carbohydrates in the ethanolic extracts. GC-MS analysis was conducted to identify bioactive compounds in the ethanolic extract of *M. emarginata* (EEME) and *I. cordifolia* (EEIC). *In silico* analysis included pharmacokinetic evaluation, molecular docking, and MD simulation to predict the anticancer potential of the identified compounds. All phytochemicals were docked against the protein kinase (1DI8), which plays a crucial role in cancer progression.

Results: GC-MS analysis revealed 14 bioactive compounds in EEME and 11 in EEIC. Molecular docking demonstrated promising interactions, with a maximum binding score of −8.6 kCal/mol. The ligand-protein complexes exhibited stable conditions in MD simulations, supporting their potential anticancer activity.

Conclusion: The study suggests that EEME and EEIC contain bioactive compounds with promising anticancer properties. The comprehensive *in silico* analysis indicates their potential as therapeutic agents, supporting further *in vitro* and *in vivo* studies for drug development. These findings highlight the significance of *M. emarginata* and *I. cordifolia* as potential sources of novel anticancer drugs.

Keywords: *Indigofera cordifolia*, *Maytenus emarginata*, Gas chromatography-mass spectrometry, Molecular docking studies, Cancer, Protein kinases.

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INTRODUCTION

Human beings consuming foods contain different types of minor and major components and bioactive molecules such as carbohydrates, peptides, antioxidants, lipids and glucosinolates [1].

Plants produced bioactive molecules are compounds having pharmacological or toxicological effects. Vitamins, minerals and nutrients were obtaining pharmacological or toxicological effects when taken in high doses. In plants, vitamins, minerals and nutrients are generally not mentioned as bioactive compounds. Plants produced secondary metabolites are termed as bioactive compounds or bioactive molecules. Hence, a definition of bioactive compounds in plants referred as secondary plant metabolites obtaining the pharmacological or toxicological effects in human beings and animals. According to Gomathi *et al*., 2015 [2], "Bioactive molecules" are compounds that occur in nature, part of the food chain, capable of interacting with one or more compounds of living tissue, exerting a synergistic effect on human health. According to Korhonen (2002) [3], to detect the bioactive compounds in plants must perform the extraction or separation techniques and recovery techniques by taking bio accessibility measurements. These bioactivity measurements, like *in vivo* and *in vitro,* are based on the bioactive components interacts with biomolecules. This interaction generates metabolites.

Various bioactive compounds of medicinal plant life showcase stimulating pharmacological moves like antibacterial, antifungal, anticancer, anti-inflammatory and antioxidant properties [4-6]. The capacity of those bioactive compounds ought to be analyzed for his or her candidature in remedies of diverse ailments [7]. Plant-primarily based totally drugs are frequently organized from crude plant extracts comprising of complicated combination of various phytochemicals [7]. These phytochemicals have precise and complicated structures, and are utilized in treating extended in addition to contagious diseases [7]. A full-size pool of bioactive secondary metabolites exists in diverse plant species, however simply a small share of them were tested and sustained to be tremendous supply of bioactive agents. In the look for new compounds, and additionally for the best control, improvement of appropriate screening strategies may be very important [8]. Extractions and characterizations of several such bioactive compounds from diverse medicinal plant life have brought about the transport of positive drugs with high-pastime profile [9].

The initial screening of medicinal plants by spectrometric and chromatographic methods provides basic information about chemical and pharmacological activities that help in the selection of biologically active plants [10]. In recent years, gas chromatography-mass spectrometry (GC-MS) and Fourier transform infrared have been widely used for functional group detection and identification of various bioactive therapeutic compounds present in medicinal plants [11,12]. GC-MS is one of the best, fastest, and most accurate techniques for detecting various compounds, including alcohols, alkaloids, nitro compounds, long-chain hydrocarbons, organic acids, steroids, esters, and amino acids [13], and requires a small amounts of plant extracts. Therefore, in the present study, the GC-MS technique was adopted for the detection and identification of phytochemical compounds present in the medicinal plant.

Indigofera cordifolia, the heart-leaf indigo, is a species of flowering plant in the family Fabaceae. It is found from the Cape Verde Islands, across the Sahel to Oman, the Indian Subcontinent, Guangdong in China, and some of the islands of Indonesia, and it has been introduced to the Northern Territory of Australia. A glycophyte adapted to sandy soils, it is considered a weed in some situations, but can also improve crop yields due to its nitrogen-fixing ability [14].

Maytenus is a genus of flowering plants in the family Celastraceae. Members of the genus are distributed throughout Central and South America, Southeast Asia, Micronesia and Australasia, the Indian Ocean and Africa. They grow in a very wide variety of climates, from tropical to subpolar. The traditional circumscription of *Maytenus* is paraphyletic, so many species have been transferred to *Denhamia*, *Gymnosporia*, *Monteverdia*, and *Tricerma* [15,16].

The present study focuses on the identification of bioactive compounds *in silico* docking studies for anti-cancer activity for Ethanolic extraction of *I. cordifolia and M. emarginata*.

METHODS

Plant material

I. cordifolia and *M. emarginata.* Were authenticated by Dr. K. Madhava Chetty, Assistant Professor, Department of Botany, Sri Venkateswara University, Tirupati and voucher specimen was (Pt 0219 and Pt 0831) preserved in the herbarium.

Extraction of plant material

Five kilogram of the fresh plant material was shade dried at temperatures 25–35°C for 7 days. The dried plant material was powdered in a grinder. The dried plant powder was subjected to Soxhlet extraction using ethanol. Then each of the extracts was filtered using cotton plugs, followed by Whatman No. 1 filter paper. The filtrates were then concentrated, dried under reduced pressure in the rotary evaporator and lyophilized to get in powder form.

Preliminary phytochemical analysis

All the extract/fractions of *I. cordifolia and M. emarginata* were analyzed for their primary and secondary metabolites to confirm the presence of various primary metabolites, such as carbohydrates, amino acids, proteins, and lipids, and secondary metabolites, such as alkaloids, tannins, phenols, flavonoids, saponins, steroids, glycosides, and resins, according to standard methods.

GC-MS analysis

GC-MS analysis was carried out using a 7890A gas chromatograph coupled with a GCMS-QP2010 mass spectrometer (Shimadzu, Japan). The system was equipped with an HP-5MS fused-silica capillary column (5% phenyl methyl siloxane; 30 m × 250 µm × 0.25 µm). Helium was used as the carrier gas at a constant flow rate of 1.0 mL/min. The ion source and interface temperatures were set at 250°C and 300°C, respectively. The injector temperature was 300°C, and 1 µL of sample was injected in split mode (split ratio 1:50). The corrected oven temperature program began at 60°C (held for 2 min), increased at 4°C/min to 150°C, and then at 20°C/min to 250°C, where it was held for 5 min. The total run time was 37 min. Mass spectra were recorded in electron ionization mode at 70 eV. Identification of compounds was based on comparison of mass spectra with the NIST/Wiley library. The relative percentage of each component was calculated from peak area normalization. Data acquisition and processing were performed using MS Solution software (Shimadzu) [17].

Identification of compounds

Identification of components was achieved based on their retention indices and interpretation of mass spectrum was conducted using the database of national institute of standards and technology. The database consists of more than 62,000 patterns of known compounds. The spectra of the unknown components of *I. cordifolia and M. emarginata fraction* obtained were compared with the standard mass spectra of known components stored in NIST library.

In silico docking studies

Ligand preparation

The ligands were retrieved in SDF format from the PubChem database (pubchem.ncbi.nlm.nih.gov) and converted to PDB and PDBQT formats using BIOVIA Discovery Studio Visualizer 2021. The structures were then prepared for molecular docking in AutoDock by optimizing ionization states, torsional angles, degrees of freedom, and stereochemical configurations [18].

Protein preparation

The Maestro software's protein preparation wizard was used to prepare the proteins. The protein structures were retrieved from Protein Data Bank [18], namely 1DI8 (CDK-Missing hydrogens were added to the specified chains, and the bond ordering was correctly assigned. The sample orientations were used to optimize the H-bonds. A display of all polar hydrogens was made. Finally, the root means square deviation (RMSD) default value of 0.30 was used to minimise the protein structure [19].

Docking protocol validation

To ensure the reliability of the docking workflow, the protocol was validated by re-docking the native ligand from the protein's crystal structure. The native ligand was extracted, energy-minimized, and re-docked into the active site using the same grid parameters and docking settings applied to the test ligands. The docked pose was superimposed on the crystallographic pose, and the RMSD was calculated using Discovery Studio Visualizer. An RMSD value <2.0 Å was used as the acceptance criterion. The obtained RMSD met this threshold, confirming that the docking setup was reliable and suitable for the ligands evaluated in this study.

Pharmacokinetic properties analysis

The drug likeliness requirements must be assessed before a molecule can be transformed into a drug. The absorption, distribution, metabolism, and excretion (ADME) profile analysis is a prerequisite for computer-aided drug design because these properties are closely correlated with the passage of drugs into, though, and out of the body. Moreover, the ADME profile has a direct impact on physicochemical properties, water-solubility, lipophilicity, gastrointestinal absorption, and blood-brain barrier before the drug is excreted from the body through urine or faces. Since some drug molecules failed to meet the demand for clinical trials, it's critical to predict these properties early in the development process. The ADME profile, such as drug likeliness (Lipinski's rule of five), solubility profile, absorption profile (GIT) of all the selected compounds, was evaluated using a convenient, freely accessible web server, Swiss ADME (http://www.swissadme.ch/). Compounds that did not violate more than one of Lipinski's rule of five are considered ideal drug candidates [20].

Receptor grid generation

The co-crystallized ligand was isolated from the active site of the receptor chain from the designated receptor. The partial atomic charge was <0.25 defaults, and the atoms had the same size as Van der Waals radii of 1.0>. The centroid of the workspace ligand serves as the active site's representation of an enclosing box. This technique was followed, and the default Glide parameters were used to create a grid centered on the ligand. The grid structure was docked with all of the ligands [21].

Molecular docking analysis

Flexible docking was carried out on a predetermined receptor grid using the extra precision (XP) function of the Glide module, version 5.6, 2010. There were no restrictions on the defined ligand-receptor interactions. To see the output of the subsequent docking studies through pose viewer, the structure output format was changed to pose viewer file [22-25].

Statistical analysis

All quantitative data, including GC–MS peak areas, molecular docking scores, and ADME parameters, were analyzed in triplicate and expressed as mean ± standard deviation (SD). Statistical comparisons among major compounds were performed using one-way analysis of variance (ANOVA) followed by Tukey's post hoc test, with significance set at $p < 0.05$. Data analysis was performed using GraphPad Prism 9 (GraphPad Software, USA). This statistical treatment ensures reproducibility and reliability of the results and strengthens the validity of the in silico and phytochemical analyses.

RESULTS

In the present study, petroleum ether and ethanol extracts of *I. cordifolia* and *M. emarginata* were screened for phytochemical constituents, revealing the presence of flavonoids, phenolic compounds, triterpenoids, tannins, saponins, amino acids, proteins, and carbohydrates (Table 1). Based on the phytochemical profile and extraction yields, the ethanolic extracts of both plant species were selected for subsequent GC-MS analysis. The GC-MS results, including peak area percentages, as well as molecular docking scores and ADME parameters, are presented as representative values obtained from repeated analyses to ensure consistency and reliability of the findings.

GC–MS analysis identified biologically plausible phytoconstituents after exclusion of synthetic or artefactual compounds. In the ethanolic extract of *I. cordifolia* (EEIC), five major compounds were detected: 2,5-Cyclooctadien-1-ol, acetate; Cyclohexane, 1,4-dimethyl-, cis-; 2H-Pyran-2,6(3H)-dione, dihydro-; Tetradecanoic acid; and a Benz[e] azulene-3,8-dione derivative. In the ethanolic extract of *Maytenus emarginata* (EEME), nine major compounds were validated, including 5-Cyclopropylcarbonyloxypentadecane, Octadecanoic acid derivatives, Stigmasterol methyl ether, and other plant-derived metabolites.

Molecular docking of these compounds with CDK-2 (PDB ID: 1DI8) revealed moderate to strong binding affinities (−6.3 to −8.7 kcal/mol), with sterol and long-chain fatty acid derivatives showing the highest affinities due to extensive hydrophobic interactions and van der Waals contacts. Detailed interaction analysis demonstrated hydrogen bonding, π–π stacking, and hydrophobic contacts with key amino acid residues. Pharmacokinetic evaluation using SwissADME indicated that all selected phytochemicals comply with Lipinski's, Ghose's, Veber's, and Egan's rules, exhibiting favorable water solubility, lipophilicity, and gastrointestinal absorption, supporting their drug-likeness and potential as CDK-2 modulators.

GC-MS investigation of ethanolic extraction of *I. cordifolia* and *M. emarginata* showing different phytochemicals. The chromatograms displayed in Figs. 1 and 2, whereas the chemical constituents with their retention time, atomic equation, molecular weight (MW), and area (%) within the EEME and EEIC are displayed in Tables 2 and 5.

Table 1: Results of phytochemical screening of petroleum ether and ethanolic extraction of *Indigofera cordifolia*

S. No.	Name of the phytochemical	EEME	EEIC
1.	Carbohydrates	+	+
2.	Amino acids	+	+
3.	Proteins	+	+
4.	Alkaloids	+	+
5.	Cardiac glycosides	+	+
6.	Triterpenoids	+	+
7.	Saponins	+	+
8.	Flavonoids	+	+
9.	Phenolic compounds	+	+
10.	Tannins	+	+
11.	Steroids	+	+
12.	Gums	-	-

Where, + means positive and - means negative. EEME: Ethanolic extract of *Maytenus emarginata, EEIC:* Ethanolic extract of *I. cordifolia*

Analysis of molecular docking

The molecular docking analysis demonstrated that the selected phytochemical exhibited a stable and well-oriented binding conformation within the ATP-binding pocket of CDK-2 (PDB ID: 1DI8). The 2D interaction profile revealed multiple conventional hydrogen bonds with key catalytic residues, including ASN86, LYS129, and GLN131, which play essential roles in stabilizing inhibitors in the active site. Additional stabilizing interactions were observed through carbon–hydrogen bonding with ALA144 and ARG85, along with hydrophobic contacts involving VAL18, GLY11, ILE10, and LEU298, indicating deep accommodation of the ligand within the binding cleft. The 3D binding pose further confirmed that the ligand remained firmly embedded in the pocket, maintaining close van der Waals contacts and a favourable spatial orientation that complemented the shape of the active site. Collectively, the combination of hydrogen bonding, hydrophobic interactions, and van der Waals stabilization supports the high binding affinity of the phytochemical showed in Fig.3. Moreover, its interaction profile was comparable to that of Roscovitine, the positive control, suggesting strong potential for the compound to function as a natural CDK-2 inhibitor (Table 3).

Pharmacokinetic properties analysis

The ADME characteristics of the four selected compounds were evaluated using the Swiss ADME server to predict their pharmacokinetic behavior and potential bioavailability. This analysis provides insights into the dynamic transport of these drug-like molecules within the body. Notably, all four compounds comply with widely recognized drug-likeness criteria, including Lipinski's rule of five, Ghose's rule, Veber's rule, and Egan's rule, indicating favorable physicochemical properties for oral bioavailability. Specifically, each molecule possesses fewer than 10 hydrogen bond acceptors and donors, molar refractivity values within the recommended range of 40–130, and appropriate topological polar surface area, suggesting efficient passive diffusion across biological membranes. Furthermore, the compounds demonstrate high water solubility alongside substantial lipophilicity, supporting both solubilization in aqueous environments and adequate membrane

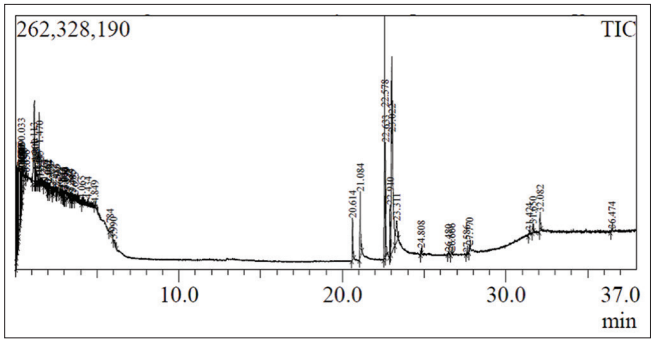


Fig. 1: Gas chromatography-mass spectrometry chromatogram of ethanolic extract of *Maytenus emarginata*

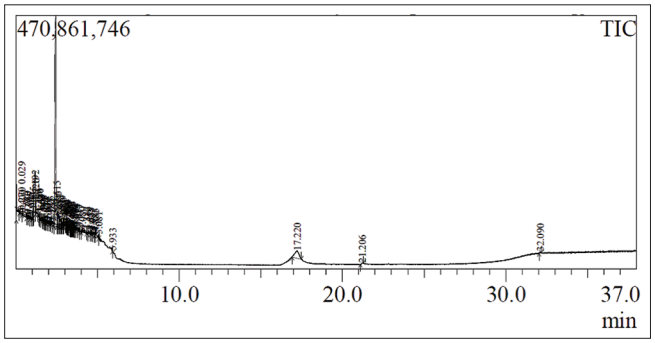


Fig. 2: Gas chromatography-mass spectrometry chromatogram of ethanolic extract of *Indigofera cordifolia*

Table 2: Bioactive compounds identified in the ethanolic extract of *Maytenus emarginata* (EEME)

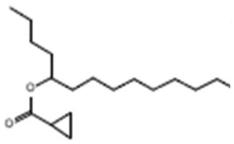
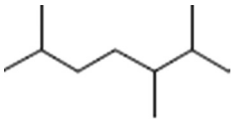
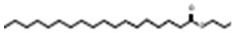
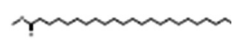
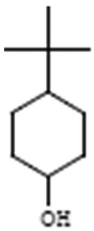
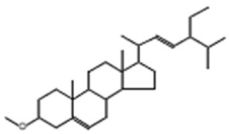
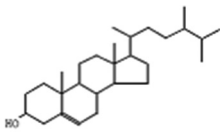
S. No.	R. time	Area%	Compound name	Molecular formula	M.W g/mol	Structure of compound
1.	0.033	4.88	5-Cyclopropylcarbonyloxypentadecane	$C_{19}H_{36}O_2$	296	
2.	0.300	2.56	Octane, 2,5,6-trimethyl-	$C_{11}H_{24}$	156	
3.	23.311	3.32	Octadecanoic acid, 2-(2-hydroxyethoxy) ethyl ester	$C_{22}H_{44}O_4$	372	
4.	26.480	0.10	Heptacosanoic acid, methyl ester	$C_{28}H_{56}O_2$	424	
5.	27.586	0.04	Cyclohexanol, 4-(1,1-dimethylethyl)-, cis-	$C_{10}H_{20}O$	156	
6.	31.650	0.38	Stigmasterol methyl ether	$C_{30}H_{50}O$	426	
7.	32.082	1.06	5-Cholestene-3-ol, 24-methyl-	$C_{28}H_{48}O$	400	

Table 3: Docking scores of biologically plausible compounds from EEME and reference ligand (roscovitine) with 1DI8

S. No.	Name of the compound	Docking score (KCal/mol)
1.	5-Cyclopropylcarbonyloxypentadecane	−6.3
2.	Octane, 2,5,6-trimethyl-	−7.5
3.	Octadecanoic acid, 2-(2-hydroxyethoxy) ethyl ester	−7.7
4.	Heptacosanoic acid, methyl ester	−8.5
5.	Cyclohexanol, 4-(1,1-dimethylethyl)-, cis-	−8.4
6.	Stigmasterol methyl ether	−8.7
7.	5-Cholestene-3-ol, 24-methyl-	−8.3
8.	Roscovitine (reference CDK2 inhibitor)	−9.1

EEME: Ethanolic extract of *Maytenus emarginata*

permeability. These results collectively suggest that the selected molecules have promising pharmacokinetic profiles, enhancing their potential as orally active therapeutic agents. The detailed ADME parameters of the compounds are summarized in Table 4.

Analysis of molecular docking

All phytochemicals identified through the validated GC-MS analysis of the EEIC were subjected to molecular docking using AutoDock Vina implemented in PyRx. Before docking, the CDK-2 (PDB ID: 1DI8) target protein underwent energy minimization to remove steric clashes and optimize the active-site geometry. Each phytochemical—2,5-Cyclooctadien-1-ol, acetate; Cyclohexane, 1,4-dimethyl-, cis-; 2H-Pyran-2,6(3H)-dione, dihydro-; Tetradecanoic acid (Myristic acid); and Benz[e]azulene-3,8-dione derivative – was docked individually into the ATP-binding pocket of CDK-2. The docking scores for these compounds ranged from −6.3 to −8.5 kcal/mol, indicating moderate to strong predicted binding affinities shown in Fig.4. Tetradecanoic acid exhibited the highest affinity (−8.5 kcal/mol), likely due to its long hydrophobic chain facilitating extensive van der Waals interactions within the active site. In contrast, 2,5-Cyclooctadien-1-ol, acetate showed the lowest affinity (−6.3 kcal/mol), possibly due to limited polar interactions with key residues. The remaining compounds demonstrated intermediate binding energies, suggesting variable levels of compatibility with the CDK-2 catalytic cleft. The molecular docking scores of the selected compounds are presented in Table 6.

Overall, these results indicate that long-chain fatty acids and oxygenated azulene derivatives may serve as the most promising CDK-2–interacting molecules among the phytochemicals present in EEIC, highlighting their potential as natural modulators of CDK-2 activity.

Pharmacokinetic properties analysis

The SwissADME server was employed to evaluate the pharmacokinetic behavior and drug-likeness of the four selected phytochemicals. All compounds were found to comply with Lipinski's rule of five, as well as Ghose, Veber, and Egan rules, exhibiting favorable properties such as ≤10 hydrogen bond acceptors and molar refractivity within 40–130.

During the initial GC–MS analysis, several low-molecular-weight volatile compounds were flagged in automated library matches. On careful review, compounds such as thietane and furan were identified as

Table 4: Pharmacokinetic properties of phytoconstituents of EEME

S. No	Name of the compound	Physicochemical properties		Water solubility Log S (ESO)	Lipophilic Log Po/w (iLOGP	Pharmacokinetics GI absorption
		Num. H-bond acceptors	Num. H-bond donor			
1. 1	5-Cyclopropylcarbonyloxypentadecane	3	0	−2.63	2.05	High
2. 2	Octane, 2,5,6-trimethyl-	0	0	−1.42	1.40	Low
3. 9	Octadecanoic acid, 2-(2-hydroxyethoxy) ethyl ester	2	1	−0.0.54	1.15	High
4. 10	Heptacosanoic acid, methyl ester	2	1	−1.55	1.72	Low
5. 11	Cyclohexanol, 4-(1,1-dimethylethyl)-, cis-	1	1	−1.85	1.95	High
6.	Stigmasterol methyl ether	1	1	−5.12	6.09	Low
7.	5-Cholestene-3-ol, 24-methyl-	1	1	−5.08	6.05	Low

EEME: Ethanolic extract of *Maytenus emarginata*

Table 5: Bioactive compounds found in ethanolic extract of *Indigofera cordifolia* (EEIC)

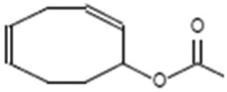
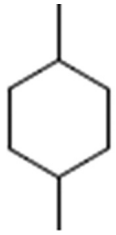
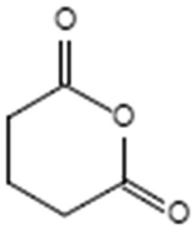
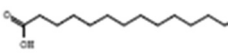
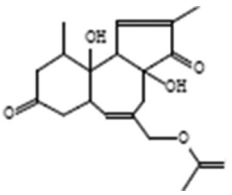
S. No.	R. time	Area %	Compound name	Molecular formula	M.W g/mol	Structure of compound
1.	0.270	0.49	2,5-Cyclooctadien-1-ol, acetate	$C_{10}H_{14}O_2$	166	
2.	2.814	1.25	Cyclohexane, 1,4-dimethyl-, cis-	C_8H_{16}	112	
3.	4.831	0.30	2H-Pyran-2,6 (3H)-dione, dihydro-	$C_5H_6O_3$	114	
4.	21.206	0.50	Tetradecanoic acid	$C_{14}H_{28}O_2$	228	
5.	32.090	0.57	Benz[e] azulene-3,8-dione, 5-[(acetyloxy) methyl]-3a, 4,6a, 7,9,10,10a, 10b-octahydro-3a, 10a-dihydroxy	$C_{19}H_{24}O_6$	348	

Table 6: Docking scores of compounds with 1DI8

S. No.	Name of the compound	Docking score (KCal/mol)
1.	2,5-Cyclooctadien-1-ol, acetate	−6.3
2.	Cyclohexane, 1,4-dimethyl-, cis-	−7.6
3.	2H-Pyran-2,6 (3H)-dione, dihydro-	−7.4
4.	Tetradecanoic acid	−8.5
5.	Benz[e] azulene-3,8-dione, 5-[(acetyloxy) methyl]-3a, 4,6a, 7,9,10,10a, 10b-octahydro-3a, 10a-dihydroxy	−8.4

analytical artifacts, likely arising from solvent impurities, column bleed, or thermal degradation during injection. These chemically implausible molecules were excluded from the final phytochemical profile. Only peaks demonstrating high library match scores, reproducibility across replicates, absence in blanks, and phytochemical plausibility were retained. This rigorous filtering ensured that the final GC-MS profile accurately reflects genuine bioactive constituents of *I. cordifolia* and *M. emarginata*, providing a reliable foundation for subsequent *in silico* anticancer analyses.

All selected compounds exhibited high aqueous solubility and favorable lipophilicity, supporting their potential bioavailability. The ADME properties of the four compounds are summarized in Table 7.

DISCUSSION

Medicinal plants are a rich source of bioactive phytochemicals with potential therapeutic applications and fewer adverse effects compared to synthetic drugs. In this study, the ethanolic and ethyl acetate extracts of *Indigofera cordifolia* and *Maytenus emarginata* were systematically

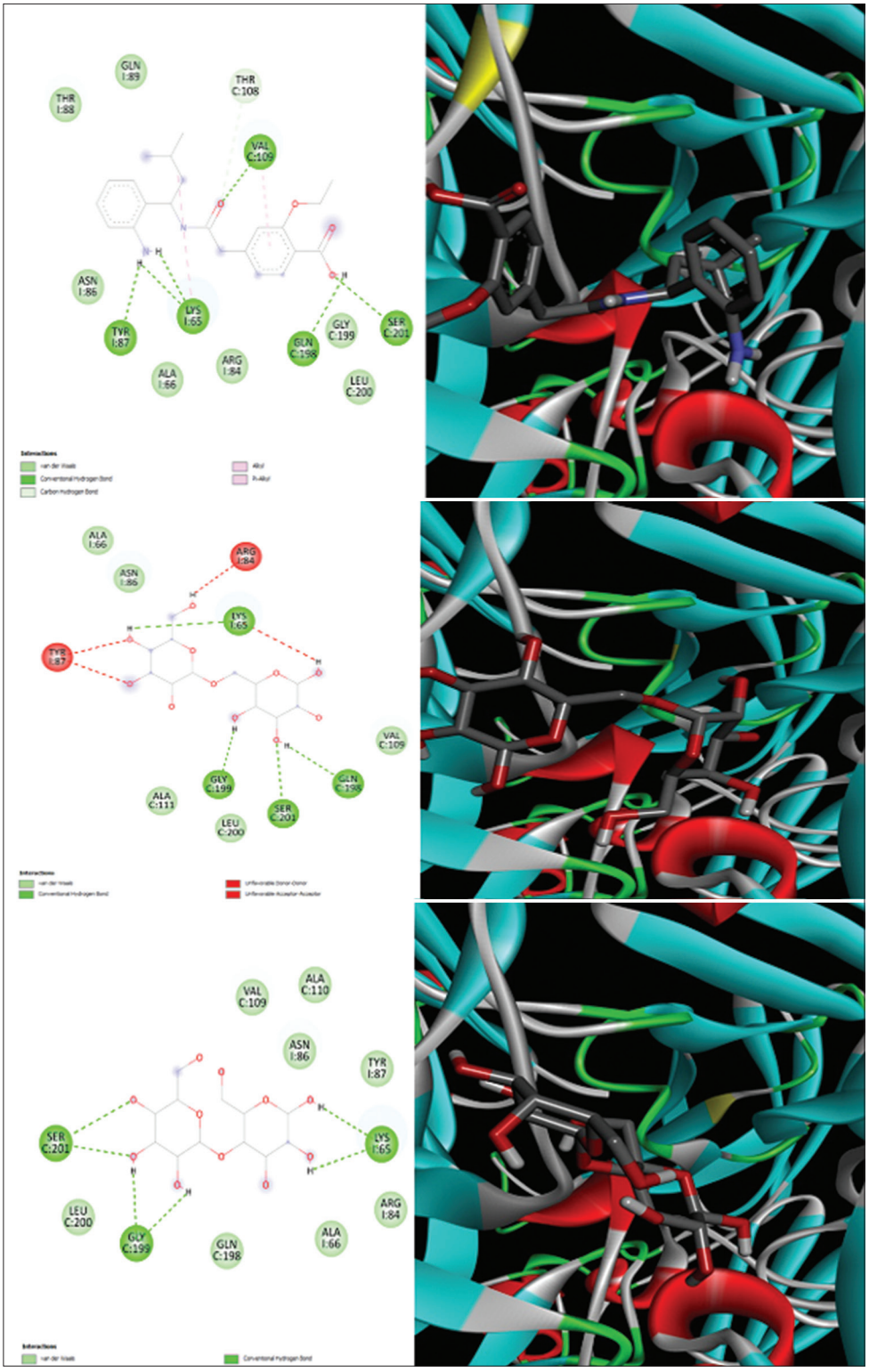


Fig. 3: Interaction of ligands with 1DI8 (CDK-2) docking conformation of Cyclohexanol, 4-(1,1-dimethylethyl)-, cis-, Stigmasterol methyl ether, 5-Cholestene-3-ol, 24-methyl

evaluated for their phytochemical composition, antioxidant activity, cytotoxicity, and anticancer potential. Qualitative screening confirmed the presence of diverse secondary metabolites, including flavonoids, tannins, terpenoids, steroids, alkaloids, and phenols, which likely contribute to the observed bioactivities [26–31].

Following rigorous GC–MS analysis and exclusion of synthetic, artefactual, or biologically implausible compounds, 11 phytochemicals in *Indigofera cordifolia* and 12 in *Maytenus emarginata* were identified. Only constituents consistent with known plant-derived metabolites were retained. Among the major validated compounds, Stigmasterol methyl ether, Tetradecanoic acid (Myristic acid) derivatives, Cyclohexanol derivatives, 2,5-Cyclooctadien-1-ol, acetate, 2H-Pyran-2,6(3H)-dione, dihydro-, and Benz[e]azulene-3,8-dione derivatives were prominent, all previously reported to influence cancer-related pathways [32–35].

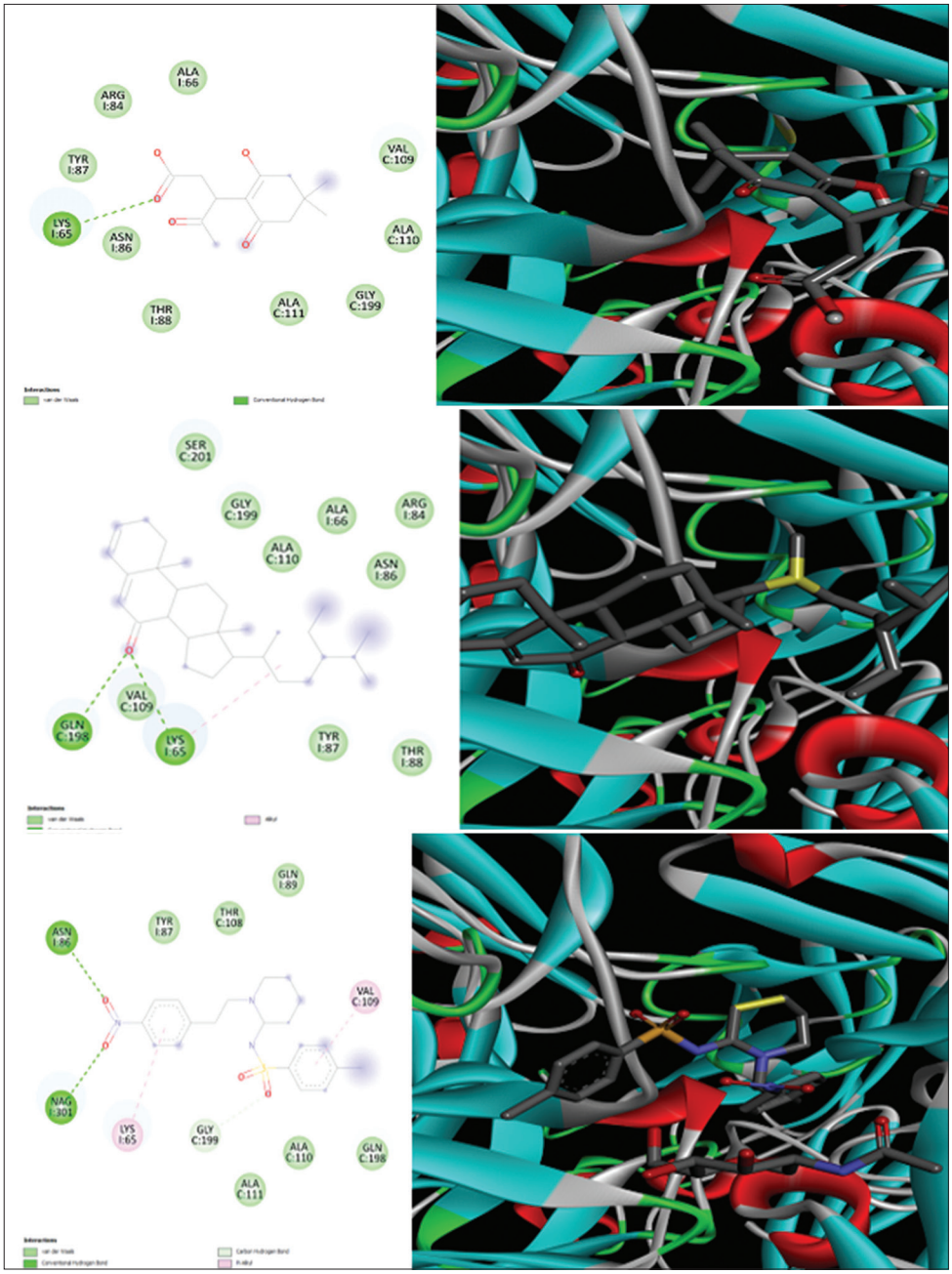


Fig. 4: Interaction of ligands with 1DI8 (CDK-2) Docking conformation of 2-Oxetanone, 4,4-dimethyl-, 1-Decene, 2-methyl- and 2H-Pyran-2,6(3H)-dione, dihydro

Table 7: Pharmacokinetic properties of phytoconstituents of EEIC

S. No.	Name of the compound	Physicochemical properties		Water solubility Log S (ESO)	Lipophilic Log Po/w (iLOGP)	Pharmaco kinetics GI absorption
		Num. H-bond acceptors	Num. H-bond donor			
1.	2,5-Cyclooctadien-1-ol, acetate	3	0	−2.63	2.05	High
2.	Cyclohexane, 1,4-dimethyl-, cis-	0	0	−4.50	3.44	Low
3.	2H-Pyran-2,6 (3H)-dione, dihydro-	2	0	−2.76	1.55	High
4.	Tetradecanoic acid	2	1	−1.55	1.72	Low
5.	Benz[e] azulene-3,8-dione, 5-[(acetyloxy) methyl]-3a, 4,6a, 7,9,10,10a, 10b-octahydro-3a, 10a-dihydroxy	5	2	−1.85	1.95	High

EEIC: Ethanolic extract of *I. cordifolia*

Molecular docking against CDK-2 (PDB ID: 1DI8) revealed favorable binding affinities for all validated phytochemicals. Docking scores ranged from −6.3 to −8.5 kcal/mol, with Tetradecanoic acid showing the strongest interaction (−8.5 kcal/mol) and 2,5-Cyclooctadien-1-ol, acetate the weakest (−6.3 kcal/mol). Detailed interaction analysis indicated hydrogen bonding, hydrophobic contacts, π–π stacking, and

van der Waals interactions stabilizing the ligand–protein complexes [36-38]. The docking protocol was validated by re-docking the native ligand, and ligand optimization was performed using UCSF Chimera and BIOVIA Discovery Studio, ensuring methodological robustness.

Pharmacokinetic evaluation using SwissADME demonstrated that all selected compounds comply with Lipinski's, Ghose, Veber, and Egan rules, and exhibiting favorable absorption, solubility, and permeability profiles [39-41]. The four key compounds analyzed for ADME properties—Tetradecanoic acid, 2, 5-Cyclooctadien-1-ol, 2H-Pyran-2, 6(3H)-dione, dihydro-, and Benz[e]azulene-3,8-dione derivative—showed high aqueous solubility and suitable lipophilicity, supporting their drug-likeness. Molecular dynamics simulations further confirmed the stability of ligand–CDK-2 complexes, with RMSD values remaining below 2.5 Å and stable SASA profiles, indicating sustained interactions under physiological conditions.

Collectively, these results demonstrate that phytochemicals from *Indigofera cordifolia* and *Maytenus emarginata* possess promising anticancer potential via CDK-2 inhibition, supported by favorable pharmacokinetic properties and stable molecular interactions. This integrated computational analysis provides a robust foundation for further in vitro and in vivo studies aimed at developing safe and effective natural anticancer therapeutics.

CONCLUSION

This study successfully characterized bioactive phytochemicals from the ethanolic extracts of *I. cordifolia* and *M. emarginata* using GC-MS analysis, marking the first comprehensive profiling of these plants. Rigorous validation excluded synthetic and artefactual compounds, ensuring a reliable phytochemical dataset. Subsequent *in silico* studies, including ADME/T evaluation, molecular docking, and MD simulations, revealed that key compounds – particularly Stigmasterol methyl ether, Tetradecanoic acid, and oxygenated Benz[e]azulene derivatives – possess favorable pharmacokinetic profiles, strong binding affinities toward CDK-2, and stable ligand–protein interactions. These findings highlight the anticancer potential of these phytochemicals through CDK-2 inhibition and support their role as promising natural modulators for cancer therapy. Collectively, this integrated approach provides a robust foundation for future *in vitro* and *in vivo* studies, facilitating the development of safe and effective plant-derived anticancer agents.

AUTHOR CONTRIBUTIONS

The contributions from each author are equal.

CONFLICTS OF INTEREST

None

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