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Research Article

PHYTOCHEMICAL CHARACTERIZATION BY GAS CHROMATOGRAPHY-MASS SPECTROMETRY AND SCREENING OF ANTICANCER AGENTS FROM MEDICINAL STEM BARK: A STUDY ON *DALBERGIA LANCEOLARIA* BY *IN VITRO* AND *IN SILICO* STUDIES

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ABSTRACT

Objectives: The objectives of the study are to evaluate *Dalbergia lanceolaria* (DL) bark extract for anticancer activity by *in vitro* and *in silico* methods

Methods: The dried bark of DL was extracted by different solvents of order of their polarity, starting with petroleum ether, followed by dichloromethane, ethyl acetate, and methanol. Preliminary screening for the presence of phytochemicals was performed for all the extracts showed various secondary metabolites in all the extracts. Methanolic extract of DL was evaluated for anticancer activity on HepG2 and MCF-7 cell lines by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay. Extracts were analyzed by gas chromatography-mass spectrometry (GC-MS) analysis. From the GC-MS report, five compounds from each plant were selected and studied against cyclin-dependent kinase (CDK) and epidermal growth factor receptor (EGFR) through molecular docking studies.

Results: Phytochemical screening by thin-layer chromatography and GC-MS showed the presence of various classes of medicinally important constituents and methanolic extract of DL exhibited significant anticancer activity by *in vitro* by MTT assay with half maximal inhibitory concentration values of 69.4 µg/mL, against MCF7 cells, and 61.1 µg/mL, against HepG2 cells, and by *in silico* studies, the test compounds, β-amyrin and hinesol, have shown similar interactions of standard sorafenib with good binding affinity with target CDK8 (β amyrin −7.4 Kcal/mole, hinesol −7 Kcal/mole) whereas docking studies on protein EGFR using erlotinib as the reference inhibitor of EGFR (binding energy of −7.8 kcal/mol) and *agarospirol* showed strong binding energy of −6.2 kcal/mol.

Conclusion: From the study, it was concluded that methanolic stem bark extract of DL exhibited anticancer potential against HepG2 cells and MCF-7 cell lines, and few of the phytochemicals from this extract can help in developing natural anticancer agents.

Keywords: Cancer, *Dalbergia lanceolaria*, *In silico* analysis, Molecular docking, Gas chromatography-mass spectrometry, 3-(4,5-dimethythiazol- 2-yl)-2,5-diphenyl tetrazolium bromide assay.

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INTRODUCTION

Cancer remains a major global cause of death despite advances in treatment. Plant-derived phytochemicals have gained attention for their anticancer potential, as they can block carcinogen activation, enhance detoxification, regulate the cell cycle, reduce oxidative stress, induce apoptosis, and modulate immune responses. The Fabaceae family, one of the largest groups of flowering plants, is widely distributed and rich in bioactive compounds such as flavonoids, isoflavonoids, alkaloids, saponins, lectins, carotenoids, and phenolic acids. Many of these metabolites exhibit antioxidant, anti-inflammatory, anti-proliferative, and pro-apoptotic activities, making them promising candidates for cancer prevention. Carcinogenesis is a multistep process involving genetic and epigenetic alterations, chronic inflammation, and oxidative damage. Phytochemicals can interfere at different stages by targeting key enzymes, cell cycle regulators, and signaling pathways such as nuclear factor-kappa B, mitogen-activated protein kinase (MAPK), and phosphoinositide 3-kinase/Akt. Integrating ethnobotanical knowledge with pharmacological research may help identify Fabaceae species with potential chemopreventive properties and support the development of novel anticancer agents [1].

Cell cycle regulatory kinases

Mitosis is a tightly coordinated process that allows a cell to duplicate its DNA and divide it evenly between two daughter cells. This sequence of events depends on several regulatory proteins, many of which are now considered important targets in cancer therapy. Key among them are checkpoint kinases (CHKs), cyclin-dependent kinases (CDKs), and mitotic regulators such as Aurora and polo-like kinases. CHKs help preserve genomic stability by responding to DNA damage. When damage is detected, they pause the cell cycle to provide time for repair; if the damage cannot be corrected, they promote programmed cell death. Since checkpoint pathways are often disrupted in cancer cells, CHKs have become promising targets for drug development. CDKs, which function through their association with cyclins, regulate critical transitions in the cell cycle. In many tumors, mutations lead to persistent activation of these cell cycle drivers, resulting in uncontrolled cell division. This has encouraged the development of selective CDK and mitotic kinase inhibitors, including natural and synthetic compounds such as staurosporine, alkaloids, and flavonoids, several of which have been evaluated in clinical research [2-6].

Receptor tyrosine kinases (RTKs), members of the ErbB family, are membrane receptors that regulate key cellular processes such as growth, survival, and differentiation. Their abnormal activation is strongly linked to cancer development. Epidermal growth factor receptor (EGFR), a major RTK, is a critical therapeutic target in cancers such as lung cancer because it drives tumor cell proliferation and progression. EGFR is composed of three main structural regions: An extracellular domain responsible for ligand binding, a hydrophobic transmembrane segment that anchors the receptor

within the cell membrane, and an intracellular tyrosine kinase domain. The intracellular domain contains the ATP-binding site, where phosphorylation is initiated, activating downstream signaling pathways such as MAPK, Akt, and signal transducer and activator of transcription. In many epithelial malignancies, particularly non-small cell lung cancer (NSCLC), breast cancer, and glioblastoma, EGFR is frequently overexpressed or carries activating mutations. These gain-of-function alterations result in continuous receptor activation, driving uncontrolled cell proliferation and tumor progression. In molecular docking studies, the ATP-binding pocket of the tyrosine kinase domain is a primary target, as it is the binding site for tyrosine kinase inhibitors such as gefitinib and erlotinib [7-9].

Improvements in analytical and computational methods, including different forms of column chromatography, liquid chromatography-mass spectrometry, GC-MS, *in silico* screening, and pharmacokinetic modeling, have significantly strengthened the study of medicinal plants. These approaches have made it easier to isolate, characterize, and evaluate individual compounds, resulting in the identification of many new and promising plant-derived products [10].

Antioxidant-rich plants are considered valuable in cancer prevention and treatment. During normal metabolism, the body produces reactive oxygen species (ROS), including superoxide, singlet oxygen, peroxides, nitric oxide, and hydroxyl radicals, which can contribute to cancer development. The body's natural antioxidants help neutralize these harmful molecules, and additional antioxidants from foods or medicinal plants can further limit ROS accumulation, reducing the risk of cancer [11,12].

Natural antioxidants such as phenolic compounds, flavonoids, and carotenoids are known for their anti-inflammatory, anti-aging, and anticancer effects. To evaluate their potential as therapeutic agents, these plant-derived compounds are tested for cytotoxicity against cancer cells while ensuring they cause minimal harm to normal cells, which is essential for their use in chemotherapy [13,14].

Plants of the genus *Dalbergia* have been used in traditional medicine for a long time, including for anticancer purposes. Studies have identified several bioactive compounds in these plants, such as flavonoids, benzophenones, styrenes, and terpenoids. Many plants, however, still need to be explored to discover new compounds for treating chronic diseases like cancer, as well as conditions such as osteoarthritis, gonorrhea, and rheumatism [15,16]. *Dalbergia lanceolaria* (DL) shows a variety of biological activities depending on the plant part used. The root bark is used for dyspepsia; the whole plant for rheumatoid arthritis, anorexia, and edema; and the seed oil for rheumatism. Leaf extracts have been applied as antidiabetic, antioxidant, anticancer, analgesic, antipyretic, and jaundice treatments, while flowers are used for skin disorders, blood purification, and boosting immunity. This highlights the plant's rich phytochemical content and wide-ranging medicinal uses [17,18].

The research aims to investigate the use of *Dalbergia* species to identify bioactive compounds and evaluate their potential therapeutic effects, including anticancer, antioxidant, and other health benefits through successive solvent extraction and phytochemical identification from the bark extracts. It also aimed to identify novel anticancer compounds from the bark extract by using *in vitro* and *in silico* approaches.

METHODS

Collection of plant materials

The bark from stems of DL was collected in December 2023, following taxonomic verification by a taxonomist (Dr. M. Murali Krishna Kumar) at Andhra University, Visakhapatnam, Andhra Pradesh, India. Voucher specimen of plant sample having number 22450AUV was deposited and preserved at the University College of Pharmaceutical Sciences, Acharya Nagarjuna University, Guntur.

Preparation of extract

1 kg of dried, plant bark material was powdered, then it was extracted by maceration using each solvent according to their polarity from low to high – first using petroleum ether, then extract is filtered, and marc is dried and extracted with dichloromethane, then ethyl acetate, and finally methanol. The resulting extracts were concentrated by rotary evaporator, removing solvents efficiently. The concentrated semisolid extracts were then stored in a desiccator to eliminate any residual moisture. Subsequently, the dried extracts were weighed, labeled, and stored in airtight containers at temperatures below 0°C. The percentage yield of methanolic extract was found to be 1.3%. This crude methanolic extract was used for *in vitro* studies.

Preliminary phytochemical screening

The therapeutic activities of medicinal plants are majorly due to their secondary metabolites. In this study, the bark extracts of DL were subjected to preliminary phytochemical evaluation for identifying both primary and secondary constituents. Standard test tube reactions and thin-layer chromatography (TLC) were employed to find the existence of different phytochemicals, including alkaloids, glycosides, proteins, terpenes, carbohydrates, flavonoids, tannins, and saponins [19]. Preliminary phytochemical screening: Test tube reactions conducted on bark extract of *Dalbergia lanceolaria*, in various solvents like petroleum ether, dichloromethane, ethyl acetate and methanol extract revealed that these plants contains flavonoidal glycosides, saponins, steroidal/triterpenoidal glycosides, phenolic compounds and tannins and the TLC profile of all the extracts of plant revealed that the extracts contains a number of flavonoids and steroidal/triterpenoidal compounds. TLC studies of DL showed green coloured spots in methanol and dichloromethane extracts and purple coloured and yellow coloured spots in ethyl acetate, methanol and dichloromethane extracts which have potential anticancer activity (Fig. 1) .

TLC analysis of different extracts of DL

After successive solvent extraction, the different extracts are subjected to TLC analysis in various solvent systems for preliminary phytochemical screening. Various solvent systems used were ethyl acetate: methanol:water in a ratio of 81:11:8 and other solvent systems, chloroform: methanol:water (70:30:4, 80:15:1), and chloroform: methanol (60:40). Developed chromatograms were seen in ultraviolet (UV) light at wavelengths 254 nm and 366 nm to detect separated components based on fluorescence and quenching patterns. In addition, the plates were coated by spraying with vanillin-sulfuric acid reagent and gently heated to enhance the detection of phytoconstituents, which appeared as colored spots, confirming the presence of different compounds.

Total phenolic concentration

The content of phenolic compounds present in the methanolic bark extract was estimated using Folin–Ciocalteu methodology. The standard phenol used was gallic acid to prepare a calibration curve which was dissolved in 50% concentration of ethanol. For the assay, 1 mg/mL solution of plant extract was prepared from which 1 mL was added to 1 mL of Folin–Ciocalteu reagent and mixed. Then, 10 mL of 7% sodium carbonate (Na_2CO_3) solution, 13 mL of distilled water were mixed, and then, it was thoroughly stirred and maintained under incubation conditions in dark area for 60 min, reaction mixture absorbance was then measured with UV-visible spectrophotometer at 750 nm. Phenolic content present in the bark extract was measured in amounts of gallic acid equivalents in 1 gram of dry bark extract (mg GAE/g) [20].

Total flavonoid concentration

The content of flavonoids of the bark extract was measured using quercetin as the standard reference. A calibration plot was created with various concentrations of quercetin dissolved in 50% ethanol. For the assay, 1 mL bark extract (1 mg/mL) was mixed in 0.5 M sodium nitrite ($NaNO_2$) and 0.3 M aluminum chloride hexahydrate. After 5 min, 1 m sodium hydroxide was added, and solution was properly mixed. Absorbance readings were measured at 506 nm, and the total amount of

flavonoid present in bark extract was expressed in amount of quercetin present in 1 g of dried bark extract, mg of QE/g [21].

Free radical scavenging potential of bark extract by 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay

DL methanolic bark extract antioxidant capacity was measured by DPPH inhibition of free radicals. 100 mg of DPPH was dissolved in 100 mL of ethanol, and from this, 0.2 mg in 1 mL was prepared using ethanol, from which 2 mL was added to 1 mL of bark extract in varying strengths (0.5/mL) in ethanol. These mixtures were vigorously mixed and kept under incubation for 30 min in the dark at room temperature. A spectrophotometer was used to measure absorbance in solutions at 517 nm. Ascorbic acid was employed as a standard antioxidant, and all measurements were taken three times for accuracy [22,23].

***In vitro* anticancer activity**

HepG2 and MCF-7 cell lines were obtained from the National Centre for Cell Science (NCCS), Pune, and used to determine anticancer activity of DL at Osmania University, Department of Microbiology, Hyderabad, Telangana, using 3-(4,5-dimethylthiazol- 2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay.

MCF-7 and HepG2 cells were cultured in Dulbecco's modified Eagle medium supplemented with 10% fetal bovine serum and 1% penicillin-streptomycin solution. For cytotoxicity evaluation, cells were exposed to different concentrations of DL extract for 48 h. This assay measures the mitochondrial succinate dehydrogenase reduction of yellow MTT. Five different concentrations of extract and doxorubicin in triplicate were tested on MCF-7 and HepG2 in this assay. Viable cells in cell suspension were determined by trypan blue assay in which cells were trypsinized. Cells were seeded in such a way that they were at density of 5.0×10^3 cells/well in 100 µL media, and hemocytometer was used for counting cells in a 96-well plate culture medium which was incubated overnight at 37°C. After completion of incubation time, 100 µL of fresh media was added to replace old media with different concentrations of test compound in wells in 96 plates. The drug solution was removed after 48 hrs. Plates were incubated at 37°C for 3 h again after adding fresh medium and MTT solution. Cells with metabolically active mitochondria reduce MTT salt to chromophore formazan crystals which results in the formation of precipitates after incubation time. A microplate reader was used to measure the optical density of solubilized crystals in DMSO at 570 nm. Using the following formula, the inhibition of growth in percentage was calculated [24].

$$\% \text{ Inhibition } = \frac{100(\text{Control} - \text{Treatment})}{\text{Control}}$$

Linear regression equation, i.e., Y=mx+C, was used to estimate half-maximal inhibitory concentration (IC_{50}) values where Y=50, m and C values are derived from %viability graph [25].

A graph plotted by taking the concentration of the extract and percentage cytotoxicity by half of the initial cell population was used to determine IC_{50} values for the extracts.

Characterizing the DL extract by GC and MS analysis

GC and MS of the DL plant extract were conducted at the Department of Analytical and Structural Chemistry, CSIR-Indian Institute of Chemical Technology, Hyderabad. For sample preparation, 10 mg of extract was dissolved in methanol (1000 µL). From the prepared solution, 1 µL was injected in capillary connected to gas chromatograph, which is equipped with a mass spectrometer (Thermo Scientific TSQ 8000). The ion source was kept at a steady temperature of 230°C, while the transfer line was maintained at a temperature of 300 °C. Electron impact was used for ionization. Helium was used as a carrier gas with a constant flow rate of 1 mL/min. The oven program began at 60°C, held for 5 min, then to 240°C at a rate of 10°C in 1 min, held for more 5 min. Finally, the temperature increased to 300°C at the same ramp rate and held for a further 5 min. Spectra of the analytes were recorded and compared to entries of the National Institute of Standards and Technology library of mass spectra, which contains over 62,000 reference spectra, for the tentative identification of compounds [26].

Molecular docking study

The binding affinity of bioactive compounds from GC-MS data of barks of DL on two anticancer target receptors – including (CDK8) (PDB ID: 5CEI), EGFR (PDB ID: 3POZ) – was evaluated using AutoDock. The 3D structures of bioactive molecules, Agarospirol, Hinesol, Phytol, Mellein, and β-Amyrin were drawn using ChemDraw software. The proteins were energy-minimized, and ligands converted to PDBQT format. The grid parameters were optimized and molecular docking was performed following the protocol by Trott and Olson (2009). From the GC MS data, a few molecules were selected and their 3D structures were downloaded from PubChem and the energies were minimized. Then, docking was performed by following the earlier published procedure. Co-crystal ligand Sorafenib and Erlotinib and protein structures of EGFR-PDBID: 3POZ and CDK-8 PDBID: 5CEI were downloaded from the protein data bank. The co-crystallized ligand and water molecules were first removed from the refined protein structure, and appropriate charges were assigned before generating the corresponding PDBQT file. Molecular docking was then carried out at the active site by defining a three-dimensional grid box along the X, Y, and Z axes with suitable dimensions. All compounds were docked using the Lamarckian Genetic Algorithm, with up to ten successful runs performed for each molecule. The final docking results were recorded as binding energy values expressed in kcal/mol. For all scoring purposes, lower scores indicate a more favorable mode of interaction. The binding energies of molecules taken from GC-MS data were compared with sorafenib which was taken as a standard for CDK8 target and erlotinib to identify potential anticancer molecules on EGFR target [27].

RESULTS

Preliminary phytochemical screening

Test tube reactions conducted on bark extracts of DL, *Derris scandens* (DS) in various solvents such as petroleum ether, dichloromethane, ethyl acetate, and methanol extract revealed that these plants contain flavonoidal glycosides, saponins, steroidal/triterpenoidal glycosides, phenolic compounds, and tannins, and the TLC profile of all the extracts of both plants revealed that the extracts contains a number of flavonoids and steroidal/triterpenoidal compounds. TLC studies of DL showed green colored spots in methanol and dichloromethane extracts and purple colored and yellow colored spots in ethyl acetate, methanol, and dichloromethane extracts, and TLC profile of DS showed orange colored spots in dichloromethane and pet ether extracts, yellow colored spots in methanol and ethyl acetate extracts, and green and purple colored spots in dichloromethane and pet ether extracts in different solvent systems which indicates that these extracts contain steroids, terpenoids, and flavonoids which have potential anticancer activity.

Total content of phenolic and total content of flavonoid

Phenolic content in the methanolic extract of DL was 75.8±2 mg in amounts of gallic acid in 1 g of extract. The flavonoid content was shown as 68.5±1.6 mg in equivalents of quercetin per 1 g of methanolic extract for DL

Measurement of antioxidant activity by DPPH Assay

Antioxidant capacity of ascorbic acid and the methanolic bark extract of DL was measured by using the DPPH assay. This assay estimated free radical inhibition of bark extract of DL and ascorbic acid, using multiple concentrations from 20 to 100 µg/mL for both test extract and standard ascorbic acid, providing an indication of their antioxidant activity as given in Table 1.

GC-MS analysis of the crude methanol extract

Analysis of the bark extract of DL by GC-MS helped in the identification of 41 important phytochemical constituents. Among the detected compounds, few important constituents are listed in Table 2; the mass spectra of those were compared with data from standard libraries and published literature (Fig. 2).

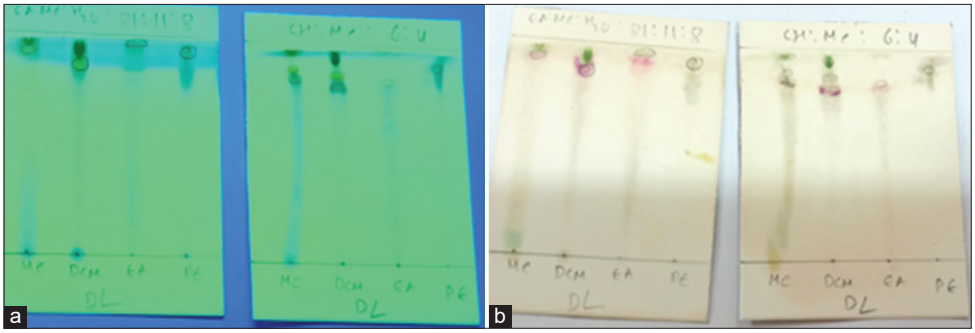

Fig. 1: Thin-layer chromatography chromatograms of *Dalbergia lanceolaria* extracts. (a) Visualization under ultraviolet light (254 nm); (b) Visualization after derivatization with vanillin-sulfuric acid reagent. Solvent system: (Ethylacetate: methanol:water [81:11:8] chloroform: methanol [60:40]). Track 1: Methanolic extract; Track 2: Dichloromethane extract; Track 3: Ethyl acetate extract; Track 4: Petroleum ether extract

Table 1: Percentage radical scavenging activity (%RSA) of *Dalbergia lanceolaria* (DL) methanolic extract and ascorbic acid by 2,2-diphenyl-1-picrylhydrazyl assay

Concentration (µg/mL)	%RSA of ascorbic acid	%RSA of DL
20	41.2±0.3	27.4±0.2
40	47.6±0.2	38.5±0.4
60	60.8±0.2	44.3±0.3
80	65.3±0.3	57.2±0.2
100	78.6±0.3	68.3±0.5

Values are expressed as mean±standard deviation of three replicates (n=3)

Table 2: Major phytochemical compounds identified in the methanolic stem bark extract of *Dalbergia lanceolaria* (DL) by gas chromatography-mass spectrometry analysis

S. No.	DL	Retention time (min)	Molecular formula
1	Dihydrocoumarin	11	$C_9H_8O_2$
2	Coumarin	12	$C_9H_6O_2$
3	Mellein	14	$C_{10}H_{10}O_3$
4	Agarospirol	19	$C_{15}H_{26}O$
5	Hinesol	24	$C_{15}H_{26}O$
6	Mome inositol	21	$C_6H_{12}O_6$
7	Pentadecanoic acid	25	$C_{15}H_{30}O_2$
8	Hexadecanoic acid	27	$C_{16}H_{32}O_2$
9	Trans-sinapyl alcohol	28	$C_{11}H_{14}O_4$
10	Octadecadienoic acid	31	$C_{18}H_{32}O_2$
11	Octadecatrienoic acid	29	$C_{18}H_{30}O_2$
12	Phytol	31	$C_{20}H_{40}O$
13	Linoelaidic acid	33	$C_{18}H_{32}O_2$
14	Eicosatrienol	35	$C_{21}H_{36}O_2$
15	Squalene	39	$C_{30}H_{50}$
16	stigmasterol	46	$C_{29}H_{48}O$
17	β-Sitosterol	48	$C_{29}H_{50}O$
18	Γ-Sitosterol	49	$C_{29}H_{50}O$
19	β-amyrin	51	$C_{30}H_{50}O$

***In vitro* anticancer activity**

TLC analysis of bark extract of DL showed the presence of considerable amounts of flavonoids, terpenoids, and especially steroids in the extract. Hence, based on phytochemical composition, it was assumed that the extract may possess good cytotoxic potential, and hence, the MTT assay was performed. The methanolic extract of DL exhibited IC_{50} values of 69.4 µg/mL against MCF7 cells and 61.1 µg/mL against HepG2 cells, demonstrating significant anticancer activity. This effect may be associated with the bioactive phytochemical constituents present in the extract. The percentage inhibition of the cells is shown in Tables 3 and 4.

Methanol extract of the bark of DL by GC-MS analysis revealed the identification of 41 compounds. Several key compounds identified in this analysis, as listed in Table 4, were characterized by matching their retention times and mass spectral data with previously published reports and database libraries. From these identified constituents, selected compounds from each plant were further considered for molecular docking studies against relevant anticancer targets.

Table 3: Cytotoxic effect of *Dalbergia lanceolaria* (DL) methanolic extract on MCF-7 breast cancer cell lines using 3-(4,5-dimethythiazol- 2-yl)-2,5-diphenyl tetrazolium bromide assay

DL		
Concentration (µg/mL)	**% Inhibition**	**IC_{50}**
Control	0	IC_{50}=69.4
10	15	
25	29	
50	43	
100	61	

IC_{50}: Half-maximal inhibitory concentration

Table 4: Cytotoxic effect of *Dalbergia lanceolaria* (DL) methanolic extract on HepG2 liver cancer cell lines using 3-(4,5-dimethythiazol-2-yl)-2,5-diphenyl tetrazolium bromide assay

DL		
Concentration (µg/mL)	**% Inhibition**	**IC_{50}**
Control	0	IC_{50}=61.1
10	20	
25	32	
50	48	
100	57	

The IC_{50} value (µg/mL) is the concentration required to inhibit 50% of cell growth. The experiment was performed in triplicate. Values are expressed as mean ± SD. $p < 0.05$ was considered statistically significant. IC_{50}: Half-maximal inhibitory concentration

Molecular docking study

Docking studies on protein CDK8, among all tested compounds, β-amyrin and hinesol showed good binding affinity, and all these compounds' binding energies and interactions were compared to Sorafenib, the standard drug. Sorafenib interacts by forming two hydrogen bonds with Glu885, by one more hydrogen bond with Asp1046, and with Val916 and Lys868 interactions, which were found to be hydrophobic. The test compounds β-amyrin and hinesol have shown similar interactions with good binding affinity with the target (β amyrin −7.4 Kcal/mole, hinesol −7 Kcal/mole) and also exhibited similar interactions (Table 5) (Figs. 3 and 4). Whereas docking studies of phytoconstituents from DL methanolic extract on protein EGFR using Erlotinib as the reference inhibitor of EGFR showed a binding

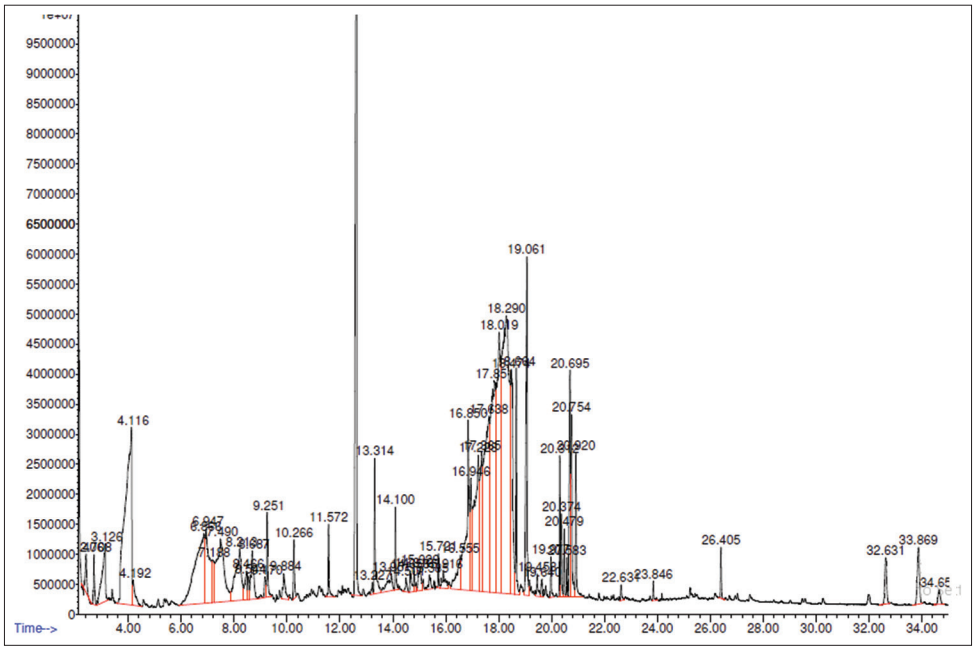

Fig. 2: Gas chromatography-mass spectrometry chromatogram of *Dalbergia lanceolaria*

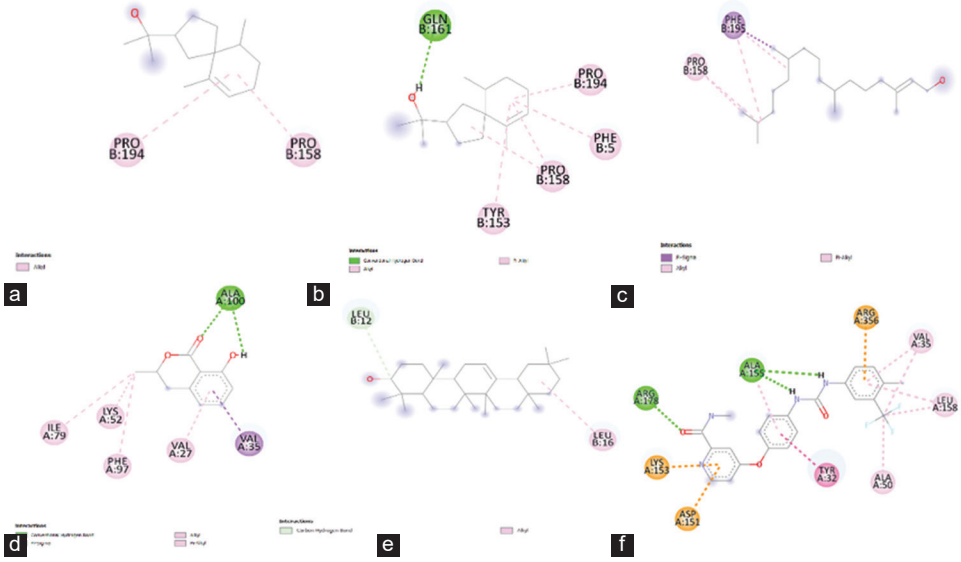

Fig. 3: 2D interaction diagrams of ligand-protein complexes showing hydrogen bonding and hydrophobic interactions between the selected ligands and the active site residues. (a) Agarospirol, (b) Hinesol, (c) Phytol, (d) Mellein, (e) Beta Amyrin, (f) Sorafenib

Table 5: Molecular docking binding energies (kcal/mol) of selected phytocompounds from *Dalbergia lanceolaria* with cyclin-dependent kinase 8 (CDK8, PDB ID: 5CEI)

Ligand	Dock score against−5CEI in kcal/mol
Sorafenib (standard drug)	−9.02
Agarospirol	−6.8
Hinesol	−7.0
Beta amyrin	−7.4
Mellein	−5.3
Phytol	−5.2

Binding energy is expressed in kcal/mol. Lower (more negative) values indicate a stronger predicted binding affinity. Sorafenib was used as a reference standard for CDK8

energy of −7.8 kcal/mol. Erlotinib formed key interactions such as H-bonds and hydrophobic interactions with the ATPbinding pocket, including MET793 (hinge), LYS745, VAL726, and LEU718. Among the phytoconstituents selected, *Agarospirol* showed a strong binding energy of −6.2 kcal/mol, by hydrogen bonds to MET793 and hydrophobic contacts with VAL726 and LEU718, suggesting competitive binding at the ATP site. Hinesol, Phytol, Mellein, and β-Amyrin also demonstrated favorable docking scores and multiple hydrophobic and hydrogen bond interactions with key EGFR residues, indicating their potential as EGFR modulators (Figs. 5 and 6). All the compounds from the selected have shown a binding energy above −5 Kcal/mole, which clearly indicates that all the compounds have significant potential for anticancer property and which reveals they have the ability to develop as natural drug for treating cancer. Therapeutic ability of the phytoconstituents of DL methanolic extract as anticancer agents was revealed by *in silico* studies as well as *in vitro* methods. 3D interactions and 2D interactions of proteins with phytoconstituents are shown in Tables 6 and 7. Binding affinities are shown in Tables 5 and 8.

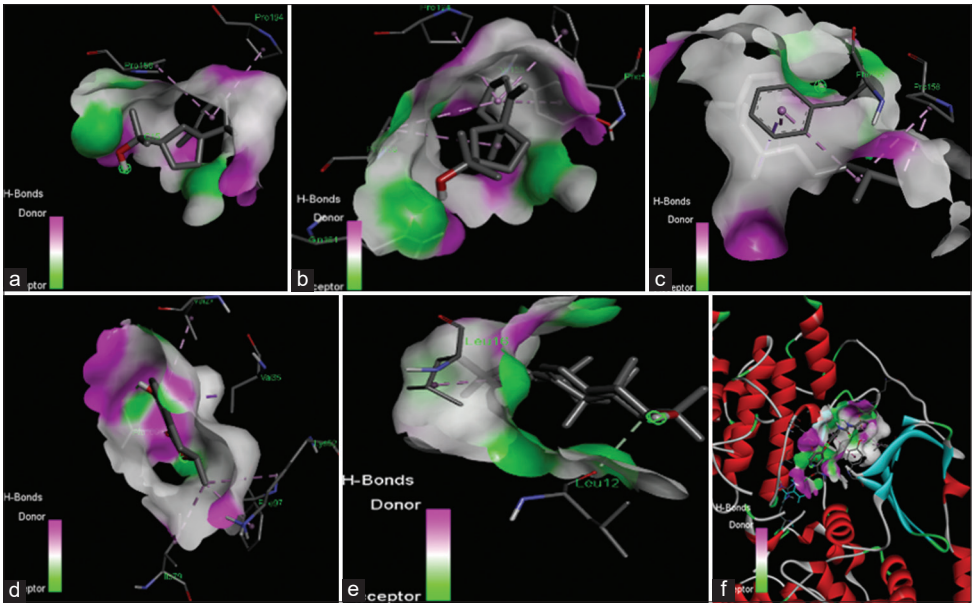

Fig. 4: 3D representations of ligand–protein complexes illustrating the spatial orientation and binding conformations of the ligands within the active site of the target protein. (a) Agarospirol, (b) Hinesol, (c) Phytol, (d) Mellein, (e) Beta amyrin, (f) Sorafenib

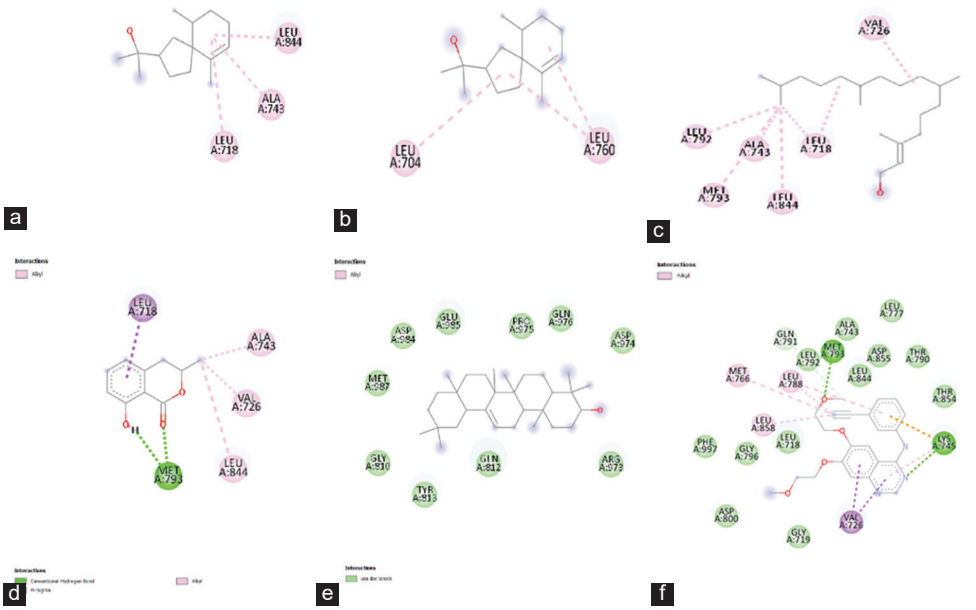

Fig. 5: 2D interaction diagrams of ligand-protein complexes showing hydrogen bonding and hydrophobic interactions between the selected ligands and the active site residues. (a) Agarospirol, (b) Hinesol, (c) Phytol, (d) Mellein, (e) Beta amyrin, (f) Sorafenib

Table 6: Molecular interaction of plant derivatives with cyclin-dependent kinase-8 (PDB ID-5CEI)

Ligand	Key interaction types
Agarospirol	Alkyl interactions
Hinesol	Conventional hydrogen bonding, alkyl, π–alkyl.
Phytol	π–alkyl, alkyl, π– sigma
Mellein	Carbon hydrogen bond, π–alkyl, alkyl, π– sigma
Beta amyrin	Carbon-hydrogen bond
Sorafenib	Conventional H bonding, π–π , π– alkyl, π– cation, π– anion.

Table 7: Molecular interactions of plant derivatives with epidermal growth factor receptor (PDB ID-3POZ)

Ligand	Key interaction types
Agarospirol	Alkyl interactions
Hinesol	Alkyl interactions
Phytol	Alkyl interactions
Mellein	Carbon hydrogen bonding, alkyl, π-sigma
Beta amyrin	Van der Waals interactions
Erlotinib	Conventional hydrogen bonding, π-sigma, π-alkyl, π-cation, Van der Waals interactions

DISCUSSION

The present study demonstrated that the methanolic extract of DL exhibits significant anticancer activity against MCF and HepG2 cell lines, as evidenced by MTT assay. The observed cytotoxicity may be attributed to the presence of diverse bioactive phytoconstituents identified through GC-MS analysis. Previous studies have reported that plant-derived

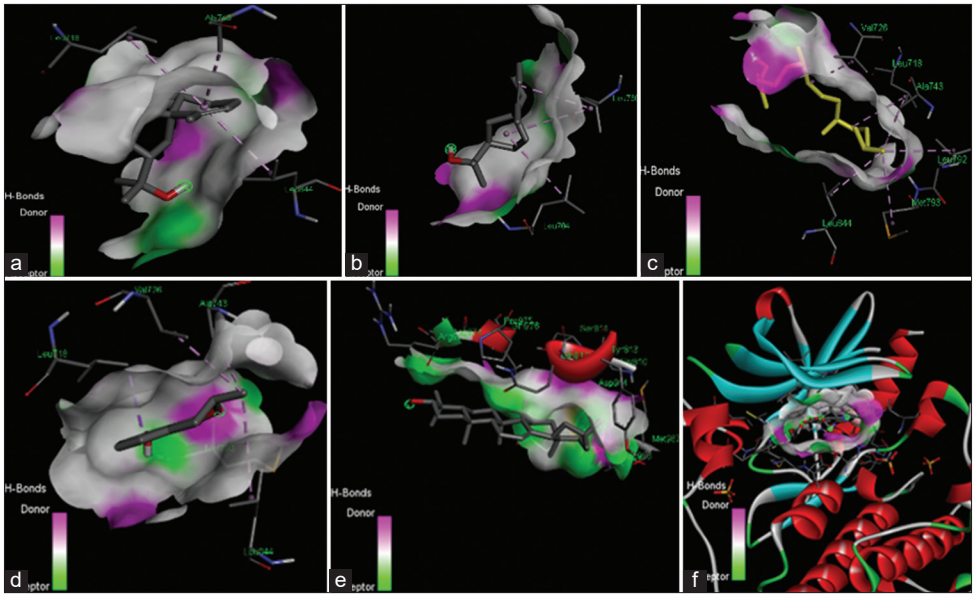

Fig. 6: 3D representations of ligand-protein complexes illustrating the spatial orientation and binding conformations of the ligands within the active site of the target protein. (a) Agarospirol, (b) Hinesol, (c) Phytol, (d) Mellein, (e) Beta amyrin, (f) Sorafenib

Table 8: Molecular docking binding energies (kcal/mol) of selected phytocompounds from *Dalbergia lanceolaria* with epidermal growth factor receptor (EGFR, PDB ID: 3POZ)

Ligand	Dock score against- 3POZ in kcal/mol
Erlotinib (standard drug)	−7.8
Agarospirol	−6.2
Hinesol	−5.0
Beta amyrin	−6.5
Mellein	−6.6
Phytol	−5.4

Binding energy is expressed in kcal/mol. Lower (more negative) values indicate a stronger predicted binding affinity. Erlotinib was used as reference standard for EGFR

extracts rich in secondary metabolites such as flavonoids, terpenoids, and fatty acids exhibit potent anticancer activity through inhibition of cancer cell proliferation and induction of apoptosis. Similar findings have been documented in studies published, where methanolic plant extracts showed significant cytotoxic effects against breast cancer cell lines due to their rich phytochemical composition [28]. GC-MS analysis in the present study revealed compounds belonging to various chemical classes, which are known to possess pharmacological properties. Comparable studies reported in the *literature* have demonstrated that GC-MS identified compounds such as phenolic derivatives and fatty acids contribute significantly to anticancer activity by modulating oxidative stress and apoptotic pathways in cancer cells [29]. Furthermore, molecular docking studies provided insight into the possible mechanism of action of selected compounds by targeting key proteins involved in cancer progression, namely CDK8 and EGFR. CDK8 is a critical regulator of transcription and has been implicated in oncogenic signaling pathways, particularly in breast cancer, while EGFR plays a vital role in cell proliferation, survival, and metastasis in various cancers including liver cancer. In the present study, the selected phytochemicals demonstrated strong binding affinity toward CDK8 and EGFR, with binding energies comparable to standard reference compounds. This suggests that these compounds may inhibit kinase activity and disrupt downstream signaling pathways essential for tumor growth.

These findings are consistent with previous reports where phytochemicals identified through GC-MS exhibited strong binding interactions with cancer-related targets such as EGFR and CDK family proteins. Studies published previously have shown that plant-derived compounds can effectively inhibit EGFR-mediated signaling pathways, thereby reducing cancer cell viability, highlighting the importance of molecular docking in identifying natural inhibitors of CDK proteins, supporting their role in anticancer drug discovery [30]. The correlation between *in vitro* cytotoxicity and *in silico* docking results in the present study strengthens the hypothesis that the anticancer activity of DL may be mediated through inhibition of CDK8 and EGFR pathways. The ability of these phytochemicals to interact with multiple molecular targets suggests a multitarget therapeutic approach, which is advantageous in overcoming drug resistance and improving treatment efficacy.

In the present study, a positive control was not included, as the MTT assay was intended as a preliminary screening to evaluate the cytotoxic potential of DL extract. The primary objective at this stage was to determine whether the extract exhibits measurable cytotoxic activity, rather than to perform a comparative potency assessment against standard anticancer agents. However, the absence of a positive control is recognized as a limitation, as it restricts direct comparison of the observed effects with established cytotoxic compounds and may limit the interpretability of the assay sensitivity. Future studies will address this limitation by incorporating appropriate standard reference drugs, such as doxorubicin or cisplatin, to enable more reliable comparison, validation, and quantification of the extract's cytotoxic efficacy.

However, despite these promising results, the study is limited by the absence of *in vivo* validation and detailed molecular mechanism studies. Further research focusing on isolation of active compounds, evaluation in suitable animal models, and investigation of signaling pathways is necessary to establish the clinical relevance of these findings.

CONCLUSION

DL methanolic extract was subjected to MTT assay which showed good anticancer potential on the tested MCF and HepG2 cell lines. Using GC-MS analysis, phytochemicals of the extract were identified and chemical nature of most of the compounds was noted. Molecular docking studies were performed on specific proteins related to MCF and HepG2 cell lines by taking few compounds from GC-MS data. Binding energies and binding affinity to the selected target proteins revealed that compared to the standard substances, the majority of compounds showed significant anticancer activity.

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AUTHOR CONTRIBUTIONS

Conceptualization, Ravi P; Methodology, Rajeswari CH; Validation, Rajeswari CH; Formal analysis, Rajeswari CH; Investigation, Rajeswari CH and Apoorva S; Data curation, Rajeswari; Writing-original draft preparation, Rajeswari; Visualization, Pravallika KE; Supervision, Ravi P and Pravallika KE.

CONFLICTS OF INTEREST

Authors declare no conflicts of interest.

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