

Exploring the Antioxidant and Antiproliferative Properties of *Flacourtia indica* Extracts on Lung Cancer Cells: A Comprehensive Analysis Utilizing GC-MS, Molecular Docking, and PASS Analysis.

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Abstract

Background

The study aimed to explore the anti-proliferative potential against a lung cancer cell line for the first time, alongside investigating phytochemical composition, antioxidant activity, and conducting GCMS analysis of *Flacourtia indica* extracts. This was supplemented by in-silico molecular docking and PASS prediction study for selected compounds. Traditionally decoctions of *Flacourtia indica* are utilized as anti-helminthic and antibacterial in Bangladesh. Multiple studies have shown its antioxidant, antibacterial, and anti-colorectal cancer effects.

Methods

The aerial plant was extracted with n-hexane, chloroform, and methanol in order of polarity and the phytoconstituents were determined by GC-MS spectroscopy techniques. MTT assay was used to measure lung cancer cell line viability to determine extract's anti-proliferative effect. The phenolic, flavonoid, antioxidant content analysis was carried out by spectroscopic method. The antineoplastic (breast cancer) potential of selected phytoconstituents were unveiled using the PASS program. Molecular docking analysis further assessed the binding affinity of these bioactive compounds with proteins including topoisomerase II, calvasculin, porin, and TP53.

Results

GCMS analysis of crude chloroform extract revealed Cyclopropanepentanoic acid, 2-undecyl-, methyl ester (13.072%), phytol (12.667%), benzene,1,2,4-trimethyl, decane, pentadecanoic acid,14-methyl, and 9,12-Octadecanoic acid, methyl ester, and 20 other components. Total phenolics and flavonoids in crude chloroform extract are 2.3882 ± 1.47 mg/gm Gallic acid equivalent and 99.167 ± 32.842 mg/gm quercetin equivalent. The crude chloroform extract (CCE) has antioxidant properties, with IC_{50} values of 38.691 ± 0.582 μ g/mL for DPPH scavenging and 59.81 ± 0.272 μ g/mL for iron reducing methods, and 148.75 ± 1.25 mg/gm for catechine antioxidants. The in-silico PASS prediction analysis of the prominent compounds were also carried out against different cell line of lung cancer. The Pa value ranges from 0.502 to 0.639 revealed considerable anti-proliferative activity of the constituents. Compared to tamoxifen as positive control, crude chloroform extract (CCE) inhibited lung-cancer cell proliferation by 22.87%. Molecular docking of discovered chemicals to four proteins supported the anti-carcinogenic theory.

Conclusion

This comprehensive study provides valuable insights into the anti-carcinogenic potential of *Flacourtia indica* extracts, particularly against lung cancer cell lines. Further research is warranted to explore its therapeutic potential and pave the way for the development of novel anti-cancer agents derived from natural sources

Introduction

Oxidative stress refers to a spontaneous reaction that includes degradation of biomolecules like DNA, protein or lipid by oxidation reaction followed by formation of some byproducts commonly known as reactive oxygen species (ROS). Any kind of dysfunction of biomolecules categorized as irreversible damage of plasma membrane, protein denaturation and DNA damage that is initiated by excess formation of free radicals.[1][2] Carcenogenesis is brought about in major cases due to DNA damage as it involves mutation induced by ROS during oxidation [3] Hydrogen peroxide formed by metabolic reaction contribute to DNA dysfunction as it can easily diffuse through cell membrane and directly affect DNA.[4] Depending upon the capacity of free radicals binding with protein, DNA and lipid, several cancers like lukamea, sarcoma, lung cancer, breast cancer, lymphoma, prostate cancer and many more can be associated with damage caused by ROS.[5] Natural resources have always been considered potential candidates for prevention of physiological disorders as a result of their minimum side effects. Plant derived constituents are basically polyphenol, flavonoids, glycosides, terpenoids, tannin commonly known as secondary metabolites. These compounds immensely contribute to neutralize the free radicals to mitigate oxidative stress. Flavonoids protect DNA from being damaged by formation of duplex with DNA.[6] These secondary metabolites of plant extract also attribute to cure of carcinogenesis. In recent days, computer aided approaches to study the pharmacological activity of the compounds have been familiar for predictive study so that experiments in laboratory can be more precise and robust. PASS (Prediction of Activity Spectra for Substances) is a software that is used to predict the biological responses imparted by the several substances including phyto-constituents.[7] Molecular docking is another computer programme that involves the drug receptor interaction to predict the orientation of drug ligands when bound to any target protein.[8] The data generated from docking score may imply binding property of ligands to design the drug composition to result in maximum therapeutic responses.

Flacourtia indica is a shrub that is used as decoction in case of infection and liver tonic traditionally. The folk people use the leaf juice as anthelmintic. According to scientific investigations the leaf extract of the plant has ability to cure human colon cancer[9], oxidative stress[10] and bacterial infection[11]. However, a compound characterized as murin A was isolated form the plant extract that was revealed as anti-plasmodic.[12]

Our present study seeks to assess the anticancer potential of this plant extract, aiming to substantiate its ability to scavenge free radicals effectively. Concurrently, we conducted Gas Chromatography-Mass Spectrometry (GCMS) analysis on the extracts to unveil its phyto-constituents. Additionally, we employed computer-aided in silico methods to corroborate our in-vitro findings. While prior research has explored

the antioxidant activity and anticancer properties of the respective plant, our study marks the first examination of its anti-proliferative effects on human lung cancer cell lines.

Chemicals

The required solvents were n-Hexane, chloroform, methanol of analytical grade purchased from Merk, Germany. DPPH, 2'-azobis (2-methylpropionamidine) dihydrochloride (AAPH), agar, bromophenol blue, RPMI media, FBS, MTT were purchased from Sigma Aldrich.

Plant Material

The aerial part of *Flacourtia indica* was collected from northern part of Rajshahi city. The plant was identified by a reputed taxonomist Prof. A.H.M. Mahbubur Rahman, Department of Botany, University of Rajshahi. The voucher specimen (No. 37805) was preserved in National Herbarium, Mirpur-Dhaka, Bangladesh.

Preparation of Plant Extract

The aerial part of the plant was cut into small pieces and dried in sun maintain temperature 35–40° C for consecutive 5 days with duration of 8 hours each day. The dried plant materials were grinded into coarse powder and the power was preserved in air tight condition at room temperature. The plant materials were merged into n-Hexane to extract the fatty materials from plant part. Then filtrate was collected by filtration with whattman filter paper. Then the filtrate was concentrated and dried to prepare n-Hexane extract. The plant residue was then extracted with chloroform and chloroform extract was prepared by filtration of the mixture and dried out the solvent from filtrate. At last the residual part after filtration was extracted by the same manner to prepare methanol extract.

Total Phenolic Content

Total phenolic content of the extract was determined by the reaction with Folin-Ciocalteu [13]. Gallic acid solution at different concentrations were taken as standard for calibration curve. A particular concentration of sample was added with 0.5mL sodium carbonate (7.5%) solution and 2.5mL FCR (10 times diluted). After incubation of the reaction mixture for 30 minutes at room temperature, Absorbance was taken at 760 nm wavelength in spectrophotometer (UV-3600i plus, Shimadzu). The total phenolic content was determined as milligram of gram equivalent of gallic acid (mg GAE/gm)

Total Flavonoid Content

According to spectrophotometric method, total flavonoid content was determined following the method described by Fattahi et al [14]. Here, stock solution of quercetine of 500 ppm was prepared to derive a calibration curve of different concentration from the stock solution. The plant extract of particular concentration was let to react with sodium nitrite (5%) and aluminum chloride (10%) in basic environment. Absorbance of the reaction mixture was taken at 510nm by UV spectrophotometer. The content of flavonoid was calculated as mg/Gm equivalent of quercetin.

Total antioxidant Content

Total antioxidants of the respective extracts were estimated by adopting the procedure by ali reza 2023 [15]. In that method sample was let to react with a reaction mixture of 3% ammonium molybdate, 84mM sodium phosphate and 1.8M sulphuric acid. The reaction was taken place by the incubation at 95°C for 90 minutes. Afterwards being cooled, the samples were evaluated by taking absorbance at 695 nm wavelength. Catechine was served as standard whereas standard solution of different concentration undergone same reaction to make a calibration curve. The total antioxidants were presented as mg of equivalent of catechine per gram extract.

Free Radicals Scavenging Property

DPPH Radical Scavenging Test

DPPH is considered a free radical that imparts purple color to the solution. The free radical scavenging property of any samples remove the color. In our present study, the DPPH radical scavenging capability of the extract was studied by following the method described in Siddartha Baliyan et al [16]. Samples of different concentration were reacted with 0.004% DPPH solution dissolved in methanol. The dark environment for 30 minutes is the suitable condition of reaction to take place. Absorbance at 517nm wavelength of UV spectrophotometer is taken. The percentage of inhibition of DPPH was calculated by the following equation:

$$\%ofscavenging = \frac{A_c - A_s}{A_c} \times 100$$

Where A_c = absorbance of control, A_s = absorbance of sample

Iron Reducing Activity Test

According to Bashera et al[17], the activity of the plant extract to prevent oxidation can be determined by the extent of reduction of iron. In this method, the sample are let to undergo reaction with 1% potassium ferricyanide maintaining pH 6.6 by phosphate buffer. The mixture was kept in 50°C for 20 minutes. After that, 10% trichloroacetic acid was added followed by centrifugation at 4000rpm for 10 minutes. The cooled samples were let to make reaction with 0.1% iron (III)chloride. Absorbance was taken at 700nm at the interval of 10 minutes. Percentage of iron reducing activity of the sample was measured by the following equation:

$$\%ofironreducing = \frac{Abs (control) - Abs (sample)}{Abs (control)} \times 100$$

Anti- Cancer Activity

The anti-carcinogenic activity was studied against human lung cancer cell line (A549 cell) by trypan blue exclusion method [18]. Cells were grown and cultured in Eagles Minimum Essential Medium enriched with 10% fetal bovine serum and 100U penicillin-streptomycin under humidified environment of CO₂ incubator maintaining 37° C temperature and 5% CO₂. The cultured cells are subcultured after every two weeks and the media are replaced two times in a week. At the interval of 24hour of culture, samples and tamoxifen as positive control are inserted into culture media. The sample containing media culture is incubated at the same condition for 48 hours. The monolayer cells are detached by trypsin-EDTA to take them into suspension. The cells are suspended into PBS by washing out the serum that may cause interpretation of cell count. Cell suspension is mixed with 0.4% trypan blue dye at the ratio of 1:1 that is kept in room temperature for 3 minutes. One drop of mixture is placed on hemocytometer and viable cell were counted by cell counter (LUNA-II™, Analytikjena). Percentage of viable cells was calculated by the following equation:

$$\text{Percentage of viable cells} = \frac{\text{Number of viable cells}}{\text{Total number of cells}} \times 100$$

GCMS Analysis

GC Ms analysis of the respective plant extract was carried out by GCMS-QP2020. The chromatography method was carried out by capillary column that is characterized by 30m in length and 0.25 mm in inner diameter. The column is prepared by 5% diphenyl and 95% dimethyl poly-siloxane.

Injection temperature was set at 220⁰C. Initially the oven temperature was set at 80⁰C and it remained isothermal for 2 minutes, then programmed to 150⁰C at the rate of 5⁰C/min and finally held at 280⁰C. The ion source temperature was 280⁰C. Helium was used as a carrier gas with the flow rate of 1.72ml/min. 4 micro-liter of the sample was injected in the split-less mode in the ratio of 1:100. For GC-MS detection, the ionizing energy gained by the detector was 45 m/z. A scan interval of 0.30 second. Total GC running time was 50 minutes.

Interpretation of mass spectrum of GC-MS was done by using database of National Institute Standard and Technology (NIST). The mass spectrum of unknown component was compared with the spectrum of the known component stored in the NIST library 2008 and 2014 edition.

Molecular Docking Analysis for Anticancer Activity

Molecular Docking: Protein Preparation

For analysis of docking of identified molecule, particular protein topoisomerase II [19], calvasculine[20] and Porin[21] were selected from literature survey and appropriate isoform of the protein according to the minimum resolution and chain length was identified from Uniprotkb website (www.uniprot.org). The 3D structures of topoisomerase II (PDB 1ZXM), calvasculine (PDB 3C1V), porin (PDB 6G6U) and TP53 (PDB 2G3R) were retrieved from Protein Data Bank RCSBPDB in PDB format. The protein structure was modified by Pymol software to remove hydrogen bond and add polar molecules.

Molecular Docking: Ligand Preparation

The compounds identified in GCMS were selected as ligands and the 3D structure of the compounds were downloaded in SDF format from Pubchem database (<https://pubchem.ncbi.nlm.nih.gov/>). The virtual screening software PyRx from MGLTools (<https://ccsb.scripps.edu/mgltools/>) was used to minimize the energy[22].

Molecular Docking

The PyRx Auto Dock Vina software was used for the docking analysis. The protein and ligand structure was turned into PDBQT format by PyRx software. The 8 degree of exhaustiveness was allowed to perform the analysis. The grid box was covered the maximum area of the protein structure. Furthermore, BIOVIA discovery studio was used to visualize the best position of the ligand interacted with protein. The interaction of the ligand with amino acid residual was also visualized by this software.

PASS Prediction Study

The antineoplastic (breast cancer) potential of selected phytoconstituents was assessed using the online application PASS (<http://www.pharmaexpert.ru/passonline/>) program. The structures of the components were downloaded from PubChem and then converted to SMILES format. PASS calculation outcomes are expressed as Pa (probability of active molecule) and Pi (probability of inactive molecule). Pa and Pi scores range from 0.00 to 1.00. Biological activities for which Pa > Pi are considered probable only for a selected drug molecule (Islam et al. 2021). The Pa and Pi values range from 0.000 to 1.000. If Pa > Pi, the compound is considered experimentally active. A Pa value exceeding 0.7 indicates high pharmacological potential, while values between 0.5 and 0.7 suggest significant experimental pharmacological activity. Pa values below 0.5 indicate lower pharmacological activity, presenting an opportunity for the discovery of novel compounds [23, 24]. This analytical approach provides insights into the potential biological activities of the examined phytoconstituents.

Statistical Analysis

All data were statistically analysed by the SPSS software (version 22) where P value was determined by one way ANOVA and significant difference was evaluated by Tukey test of post-hoc analysis

Results

The total phytochemicals including phenolic content, flavonoids and antioxidants are listed in Table 1. The crude chloroform extract contains highest amount of phenolic content i.e. 2.3882 ± 1.47 mg GAE/gm. Total flavonoids and antioxidants are also prominent in chloroform extract that represents 99.167 ± 32.842 mg QAE/gm and 148.75 ± 1.25 mg CaE/gm respectively.

Table 1
Phytochemicals as antioxidants in the extracts of *F. indica*

Sample	Total Phenolic Content(mg Gallic acid/gm)	Total Flavonoid Content (mg Quercetin/gm)	Total antioxidant (mg Catechin/gm)
Crude n-Hexane extract	0.0059 ± 0.00116 ^b	0.4282 ± 0.0379 ^b	23.57 ± 0.9727 ^c
Crude chloroform extract	2.3882 ± 1.47 ^a	99.167 ± 32.842 ^a	148.75 ± 1.25 ^a
Crude methanol extract	0.0025 ± 0.000469 ^b	31.572 ± 18.33 ^b	104.59 ± 3.66 ^b
All data were analysed by one way ANOVA to determine them as significantly different at the level of p < 0.05 and the groups containing different letter in the same column are presented as significantly different analysed by posthoc test.			

Antioxidant Activity Study

The evaluation of *in vitro* antioxidant activity involved assessing the extracts through DPPH scavenging and iron-reducing assays. The resultant IC₅₀ values have been summarized in Table 2. Notably, the crude chloroform extract exhibited promising efficacy in DPPH scavenging, with an IC₅₀ value of 38.691 ± 0.582, closely comparable to the standard. Furthermore, this extract demonstrated a noteworthy IC₅₀ value of 59.81 ± 0.272 in iron reduction, surpassing other extracts and aligning closely with the standard.

Table 2
In vitro antioxidant activity of *F. indica*

Sample name	IC ₅₀ value of DPPH scavenging (µg/mL)	IC ₅₀ value of iron reducing (µg/mL)
Standard (BHT)	31.51 ± 0.225 ^d	55.01 ± .01 ^d
Crude n-hexane extract	55.56 ± 0.087 ^b	77.73 ± 0.54 ^a
Crude chloroform extract	38.691 ± 0.582 ^a	59.81 ± 0.272 ^c
Crude methanol extract	56.80 ± 0.460 ^c	68.51 ± 0.2 ^b
All data are presented as mean ± SD that maintain a level of significant at p < 0.05 where the value contain different letter in the same column are considered significantly different		

Anti-Cancer Activity

The anti-proliferative activity of crude chloroform and crude methanol extract against human lung cancer cells was assessed, and the results are summarized in Table 3. The crude chloroform extract exhibited

inhibition, suppressing cell growth by 22.87% at a concentration of 40 µg/ml.

Table 3
Anticancer activity of crude chloroform and crude methanol extract of *F.indica*.

	No. of viable cell (×10 ⁷ cell/mL)	% of cell growth inhibition	IC ₅₀
control	6.52	-	-
Standard(tamoxifen)	0.075	96.6%	14.54 ± 0.1805 ^c
Chloroform(40µg/mL)	3.35	22.87%	83.11 ± 12.204 ^b
Methanol(40µg/mL)	5.99	6.67%	240.077 ± 34.61 ^a

Identification of Phytoconstituents by GC-MS Analysis

The GCMS analysis of the crude chloroform extract revealed the presence of a total of 25 components. The GC-MS spectra are visually represented in Figure-1, and a comprehensive summary of key information is provided in Table 4, encompassing retention time, molecular formula and area. Notably, the extract is characterized by the prominent presence of cyclopentapropanoic acid, 2-undecyl, methyl ester (13.072%) and phytol (12.667%). Additionally, significant compounds include benzene,1,2,4-trimethyl,decane, pentadecanoic acid,14-methyl, methyl ester, and 9,12-Octadecanoic acid, methyl ester. These findings contribute valuable insights into the composition of the chloroform extract, highlighting specific compounds and their respective concentrations.

Table 4
Identified compounds of crude chloroform extract by GC-MS

	Compounds	Retention time	Area	Molecular formula
1	Cyclohexane, (1-methylethyl)-	3.222	3267	C ₉ H ₁₈
2	Pivalate, (3-nitrobicyclo[2.2.1]hept-5-en-2-yl) me	3.254	3039	C ₈ H ₁₂ O
3	2,4,6-Cycloheptatrien-1-one, 4-methyl-	3.477	4781	C ₇ H ₆ O
4	Benzene, 1-ethyl-2-methyl-	3.565	10529	C ₉ H ₁₂
5	Benzene, 1-ethyl-4-methyl-	3.610	4157	C ₁₁ H ₁₆
6	Nonane, 3-methyl-	3.637	4813	C ₁₀ H ₂₂
7	Benzene, 1,2,4-trimethyl-	3.675	8269	C ₉ H ₁₂
8	Decane	4.063	22499	C ₁₀ H ₂₂
9	Benzyl alcohol	4.704	18634	C ₇ H ₈ O
10	1-Iodo-2-methylnonane	5.028	7571	C ₁₀ H ₂₁
11	Butyric acid, 2-phenyl-, 2-ethylhexyl ester	5.131	3155	C ₁₈ H ₂₈ O ₂
12	Undecane	5.956	18660	C ₁₁ H ₂₄
13	Hydroxylamine, O-decyl-	6.072	3833	C ₁₀ H ₂₃ NO
14	Tridecane	8.321	9557	C ₁₃ H ₂₈
15	Sulfurous acid, hexyl octyl este	10.225	2572	C ₁₄ H ₃₀ O ₃ S
16	Heptadecane, 2,6,10,14-tetramethyl-	10.898	5553	C ₂₁ H ₄₄
17	2(4H)-Benzofuranone, 5,6,7,7a-tetrahydro-4,	16.787	4463	C ₁₁ H ₁₆ O ₂
18	2-Cyclohexen-1-one, 4-(3-hydroxy-1-butenyl)5,5-	20.427	4910	C ₁₃ H ₂₀ O ₂
19	2-Pentadecanone, 6,10,14-trimethyl-	26.175	5937	C ₁₈ H ₃₆ O
20	Pentadecanoic acid, 14-methyl-, methyl ester	28.207	20861	C ₁₈ H ₃₆ O
21	9,12-Octadecadienoic acid, methyl ester	31.783	16275	C ₁₉ H ₃₄ O ₂
22	Cyclopropanepentanoic acid, 2-undecyl-, methyl ester	31.938	33638	C ₂₀ H ₃₈ O ₂

	Compounds	Retention time	Area	Molecular formula
1	Cyclohexane, (1-methylethyl)-	3.222	3267	C ₉ H ₁₈
23	Phytol	32.139	32594	C ₂₀ H ₄₀ O
24	Heptacosanoic acid, methyl ester	32.481	4859	C ₂₈ H ₅₆ O ₂
25	13-Docosenoic acid, methyl ester	39.206	2898	C ₂₃ H ₄₄ O ₂

The identified constituents by GC MS analysis of crude methanol extract has been represented in Table-5. The most prominent compound in this extract benzoic acid (12.80%) and hexadecanoic acid,methyl ester (11.375%). Benzoylformic acid; 3,7,11,15-tetramethyl-2-hexadecen-1-ol, 2-pentadecanone,6,10,14-trimethyl are some magnificent compounds among 38 constituents identified in this study.

Table 5
Identified compounds from crude methanol extract by GCMS

	Compounds	Retention time	Area	Molecular formula
1	3-Nonynoic acid	3.220	17248	• C ₉ H ₁₄ O ₂
2	2-Heptenal, (E)-	3.309	57319	C ₇ H ₁₂ O
3	2,4-Heptadienal, (E,E	3.901	27142	C ₇ H ₁₀ O
4	Nonanal	5.943	8831	C ₉ H ₁₈ O
5	Benzoic acid	7.508	171421	C ₆ H ₅ COOH
6	1H-Pyrrole-2,5-dione, 3-ethyl-4-methy	9.060	23045	C ₇ H ₉ NO ₂
7	2-Decenal, (E)-	9.828	18062	C ₁₀ H ₁₈ O
8	Benzoylformic acid	11.642	83405	C ₈ H ₆ O ₃
9	2-undecenal	12.501	16966	C ₁₁ H ₂₀ O
10	. 2,6,10-Trimethyltridecane	14.993	27499	C ₁₆ H ₃₄
11	Bicyclo[3.1.0] hexan-2-ol, 2-methyl-5-(1 – 0% methylethyl)-, (1.alpha.,2.alpha.,5.alpha.)-2,6-Difluorobenzoicacid,tridec-2-ynyl	15.675	7439	C ₁₀ H ₁₈ O
12	2,6-difluorobenzoicacid-tridec-2-vinyl-ester	15.796	33518	• C ₂₀ H ₂₆ F ₂ O ₂
13	2(4H)-Benzofuranone, 5,6,7,7a-tetrahydro-4,	15.719	38885	C ₁₁ H ₁₆ O ₂
14	Megastigmatrienone	19.776	9865	C ₁₃ H ₁₈ O
15	Octadecane,1-chloro	23.055	27902	C ₁₈ H ₃₇ Cl
16	6-hydroxy-4,4,7a-trimethyl-5,6,7,7(a)tetrahydrobenzofuran-2(4H)-one	24.064	7666	C ₁₁ H ₁₆ O ₃
17	Caffeine	25.765	34513	C ₈ H ₁₀ N ₄ O ₂
18	Neophytadiene	26.004	46275	C ₂₀ H ₃₈
19	2-Pentadecanone, 6,10,14-trimethyl-	26.116	127846	C ₁₈ H ₃₆ O
20	3,7,11,15-Tetramethyl-2-hexadecen-1-o	26.614	7452	• C ₂₀ H ₄₀ O
21	Phytol, acetate	27.048	9934	C ₂₂ H ₄₂ O ₂
22	7-Hexadecenal(z)	27.968	62125	C ₁₆ H ₃₀ O

	Compounds	Retention time	Area	Molecular formula
23	Hexadecanoic acid,methyl ester	28.148	152344	C ₁₇ H ₃₄ O ₂
24	Eicosanoic acid	28.999	15757	C ₂₀ H ₄₀ O ₂
25	Hexacosanoic acid, methyl ester	29.564	12425	C ₂₇ H ₅₄ O ₂
26	2-Piperidinone, N-4-bromo-n- butyl -	29.646	13646	C ₉ H ₁₆ BrNO
27	Phytol	31.483	9352	C ₂₀ H ₄₀ O
28	9,12-Octadecadienoic acid	31.723	13183	C ₁₈ H ₃₂ O ₂
29	8,11,14-Docosatrienoic acid,methyl ester	31.866	33379	• C ₂₃ H ₄₀ O ₂
30	3,7,11,15-Tetramethyl-2-Hexadecen-1-ol	32.068	80538	C ₂₀ H ₄₀ O
31	Methyl stearate	32.425	19249	C ₁₉ H ₃₈ O ₂
32	13-Octadecenal, (Z)-	32.6	11429	• C ₁₈ H ₃₄ O
33	7-Hexadecenal, (Z)-	33.514	13194	C ₁₆ H ₃₀ O
34	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	34.890	20063	C ₂₀ H ₄₀ O
35	4,8,12,16-Tetramethylheptadecan-4-olide	36.486	12950	C ₂₁ H ₄₀ O ₂
36	Hexadecanoic acid,1-(hydroxymethyl)-1,2- ethanediyl ester	39.264	18450	C ₃₅ H ₆₈ O ₅
37	9-Octadecenamide	43.406	40153	C ₁₈ H ₃₅ NO
38	Cholesta-4,6-dien-3-ol, (3.beta.)-	47.830	8624	C ₂₇ H ₄₄ O

PASS Prediction Study

A total of 13 phytoconstituents were screened for the anti-cancer activity (lung carcinoma) and by the aid of the PASS program. The Pa and Pi values of the identified compounds, determined by Way2Drug software, have been presented in Table 6, with the selected compounds demonstrating higher Pa than Pi. The compounds cyclohexane, (1-methylethyl)-, undecane and tridecane showed highest Pa value for anti-cancer activity for lung carcinoma

Table 6
Pass Prediction Study of the identified compounds

Name of Compound	Cell line	Full Name of Cell Line	P _a	P _i
Cyclohexane, (1-methylethyl)-	NCI-H23	Non-small cell lung carcinoma	0.635	0.008
2,4,6-Cycloheptatrien-1-one, 4-methyl-	HOP-18	Non-small cell lung carcinoma	0.559	0.006
Nonane, 3-methyl-	DMS-114	Lung carcinoma	0.531	0.027
Benzene, 1,2,4-trimethyl-	HOP-18		0.520	0.009
Decane	PC-6	Small cell lung carcinoma	0.529	0.022
Benzyl alcohol	HOP-18		0.502	0.010
Undecane	DMS-114	Lung carcinoma	0.629	0.007
Tridecane	DMS-114	Lung carcinoma	0.629	0.007
Pentadecanoic acid, 14-methyl-, methyl ester	NCI-H838	Non-small cell lung cancer. 3 stage	0.540	0.032
9,12-Octadecadienoic acid, methyl ester	NCI-H838	Non-small cell lung cancer. 3 stage	0.589	0.022
	DMS-114	Lung carcinoma	0.511	0.034
Cyclopropanepentanoic acid, 2-undecyl-, methyl ester	NCI-H838	Non-small cell lung cancer. 3 stage	0.534	0.034
Heptacosanoic acid, methyl ester	NCI-H838	Non-small cell lung cancer. 3 stage	0.601	0.020
13-Docosenoic acid, methyl ester	NCI-H838	Non-small cell lung cancer. 3 stage	0.601	0.020

Molecular Docking

Molecular docking analysis was conducted on selected phytoconstituents identified through GCMS analysis. These compounds were docked against four specific proteins—topoisomerase II, calvasculin, porin, and TP53—which are known to be upregulated in cancer cells. The docking scores obtained for the best-ranked pose for all compounds have been summarized in Table-7. In case of topoisomerase II the binding score ranges from - 4.4 kcal/mol to -6.7kcal/mol ranked as Pentadecanoic acid,14-methyl > phytol > neophtadiene > benzyl alcohol > decane. Phytol interacted with the amino acid residuals through the series of (ASN 120, ILE 217,141,125, PHE 142) by the conventional hydrogen and pi alkyl bond with topoisomerase-II. Furthermore, in its interaction with the Tp53 protein, stabilization occurred

through the establishment of two hydrogen bonds with Asp1521 and Tyr1523, as depicted in Fig.- 2, resulting in a favorable docking score of -6.4 kcal/mol. Among the proteins topoisomerase II, calvasculin, porin, and TP53, Pentadecanoic acid,14-methyl demonstrated the highest binding affinity like as standard tamoxifen, with a docking score of -6.7 kcal/mol, specifically interacting with amino acids Ile-217, Ile-125, Ile-141, Phe-142, Ala-147, Lys-168, and Gly-166 through a combination of hydrogen bonds, pi-sigma, and alkyl bonds. In contrast, the standard tamoxifen exhibited binding affinities of -6.7 kcal/mol for topoisomerase II and – 7.4 kcal/mol for porin.

Table 7
Binding affinity of the compounds by molecular docking

Compound Name	Compound ID	Binding Affinity(-kcal/mol)			
		Topoisomerase II	Calvasculin	Porin	Tp53
Phytol	CID5280435	-6.6	-5.0	-5.4	-6.4
Benzyl alchohol	CID244	-5.1	-4.1	-5.0	-5.4
Neophytadiene	CID10446	-6.0	-4.5	-5.4	-5.5
Decane	CID15600	-4.4	-4.1	-4.5	-4.6
Pentadecanoic acid, 14-methyl	CID 36247	-6.7	-4.7	-5.1	-4.6
9,12-Octadecadienoic acid	CID 3931	-5.4	-4.3	-5.3	-5.1
Tamoxifen (standard)	CID 2733526	-6.7	-5.7	-7.4	-6.4

Discussion

Reactive oxygen species (ROS) are cellular byproducts that comprise both free and non-free radicals, such as hydrogen peroxide. Reactive oxygen species (ROS) are overproduced when the body's inherent defences against free radicals are compromised. These ROS, which result from the body's oxidative stress process, have an effect on macromolecules like protein, DNA, and lipid[25]. Many phyto-constituents, including carotenoids, flavonoids, and anthocyanins, contain phenolic moieties that cooperate to inhibit the ROS-induced cascade of events.[26].Antioxidants are able to perform their function through altering DNA's binding mode[27]. Based on its DPPH and iron chelating scavenging activity, this study found that the crude chloroform extract possesses potent antioxidant properties. The DPPH quenching IC₅₀ value is recorded at 38.691g/mL, which is the lowest of all the extracts and is nearly equivalent to the standard. The iron reduction IC₅₀ for the crude chloroform extract was 59.81 g/mL. Our research indicates that the extract has a high total phenolic content, as well as high levels of flavonoids and antioxidant phytochemicals. We tested the extracts' anticancer effects on a human lung cancer cell line. Our research showed that the chloroform extract of the same source presented remarkable anti-mutagenic response against lung cancer cell line as well, even though methanol extract

of the areal part of *F. indica* was shown to inhibit proliferation of human colon cancer cell via inhibition of ROS generation [9]. Cancer cells were inhibited by 22.83% in a crude chloroform extract. The demise of cells was clearly seen under an inverted microscope. Changes in the cell's morphology, such as the fragmentation of the nucleus, the destruction of the mitochondria, or the clustering of the chromosomes, can all lead to cell death. The GCMS analysis was performed to identify the active chemicals in various biological processes. Although GCMS analysis of plant root extract has been previously reported[13], the examination of the plant's aerial component was new to this investigation. Phytol was the most abundant component in both the chloroform and methanol extracts, as determined by our research. Phytol, an essential oil extracted from plants, has been shown to have beneficial effects as an antioxidant, anti-inflammatory, anti-hyperlipidemic, and antibacterial [28]. Inducing apoptosis of human gastric cancer cells has been demonstrated for this compound[29], albeit the mechanism of action is poorly explained. Antioxidant, antibacterial, and antifungal properties have all been attributed to cyclopentapropionic acid, 2-undecyl, methyl ester, another significant component in crude chloroform extract [30]. There are also significant amounts of chemicals such as decane, benzyl alcohol, pentadecanoic acid, 14-methyl, undecane, and 9,12-octadecadienoic acid. Hepatoprotective, antihistaminic, and hypocholesterolemic effects of 9,12-octadecadienoic acid have been described [31].

Our investigation in computer-aided in-silico methods was supported by a molecular docking analysis. Methods like these also show how ligands interact with receptor proteins to boost the biological effects of phyto-constituents. The proteins topoisomerase II and calyculin were chosen because they are unique to human lung cancer cell lines, and porin was chosen because it has been shown to have greater expression in the A549 cell line[32]. The binding affinity was best expressed by topoisomerase II, which was chosen along with two other proteins. The highest binding affinities (-6.6kcal/mol and - 6.7kcal/mol, respectively) with topoisomerase II were demonstrated by phytol and pentadecanoic acid, 14-methyl. These substances in the extracts may be responsible for inhibiting the growth of human lung cancer cells because the protein is specific to this cell line. Binding affinities more than - 5kcal/mol were also observed for benzyl alcohol and neophytadiene. Therefore, with the necessary structural alterations, these phyto components can be considered lead compounds to create medicines for human lung cancer. The reaction can be transmitted via contact between the chemical and the target protein. Because of its ability to reduce cellular stress, aid in DNA damage repair, and limit oncogene expression, TP53 is considered a key tumor suppressor protein[36]. Breast cancer cells can be stimulated to express TP53 by a wide variety of phytochemicals, including curcumin, coumarin, and vanillin[33]. The expression of TP53 in cancer cells is one mechanism by which the active ingredients can confer anticancer effects. In our PASS study, we have carried out prediction study against numerous cell line of human lung cancer correlated to our in-vitro investigation. In that case, pentadecanoic acid, 14 methyl seemed to exhibit prominent anticancer activity with Pa value 0.543 against non-small cell lung cancer. Our molecular docking score of the phyto-constituents with TP53 protein reveals that phytol can be a promising lead compound to exhibit anticancer property. Phytol has been proved to interact with active sites of both topoisomerase and TP53 protein. In case of topoisomerase, hydrophobic interaction was carried out by pi alkyl and alkyl bond formation with Ile A: 217, Ile:125, Ile:121 and Phe A:142. Hydrogen bond was

formed with Asn A: 121 while topoisomerase was interacted with phytol. Phytol formed bonds by both hydrophobic-interaction with alkyl and pi-alkyl (Phe A:1553; Ile A:1587; Leu A:1547) interlinkage as well as polar interaction with conventional hydrogen bond (Asp A:1521). Pentadecanoic acid, 14 methyl was identified which showed maximum docking affinity (-6.7 kcal/mol) against topoisomerase contrast to the other compounds in the experiment. Moreover, the compound showed promising interaction to this particular protein rather than other proteins. As topoisomerasell is a specific protein involved in expression of lung cancer cell line, it can be hypothesised that pentadecanoic acid,14 methyl can be a lead compound to be anti-carcinogenic via a particular pathway to inhibit expression of that specific protein.

Conclusion

This comprehensive study provides valuable insights into the anti-cancer potential of *F. indica* extracts, particularly against lung cancer cell lines. By integrating various methodologies including phytochemical analysis, antioxidant activity assessment, GCMS analysis, and in-silico molecular docking, we have elucidated the bioactive compounds and their mechanisms of action. Our findings not only support the traditional medicinal uses of *F. indica* but also highlight its promising anti-cancer properties. Further research is warranted to explore its therapeutic potential and pave the way for the development of novel anti-cancer agents derived from natural sources.

Declarations

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Contributions

MAB: conducted all experiments, data curation, statistical analysis and writing original manuscript; MSP: provided valuable input to manuscript development and enhanced the manuscript through proofreading; MBI: Methodology and resources. G. M. MR: GC-MS data analysis and data curation; SRR: Help in

running the GC-MS instrument and data analysis; MEI: conceptualised the comprehensive research plan, supervision, and leading manuscript writing and editing.

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Ethics declarations

Ethics approval and consent to participate

All methods were conducted following the appropriate guidelines and regulations.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

Data availability

All data are available by reasonable request from the corresponding author.

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Competing interests

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Figures

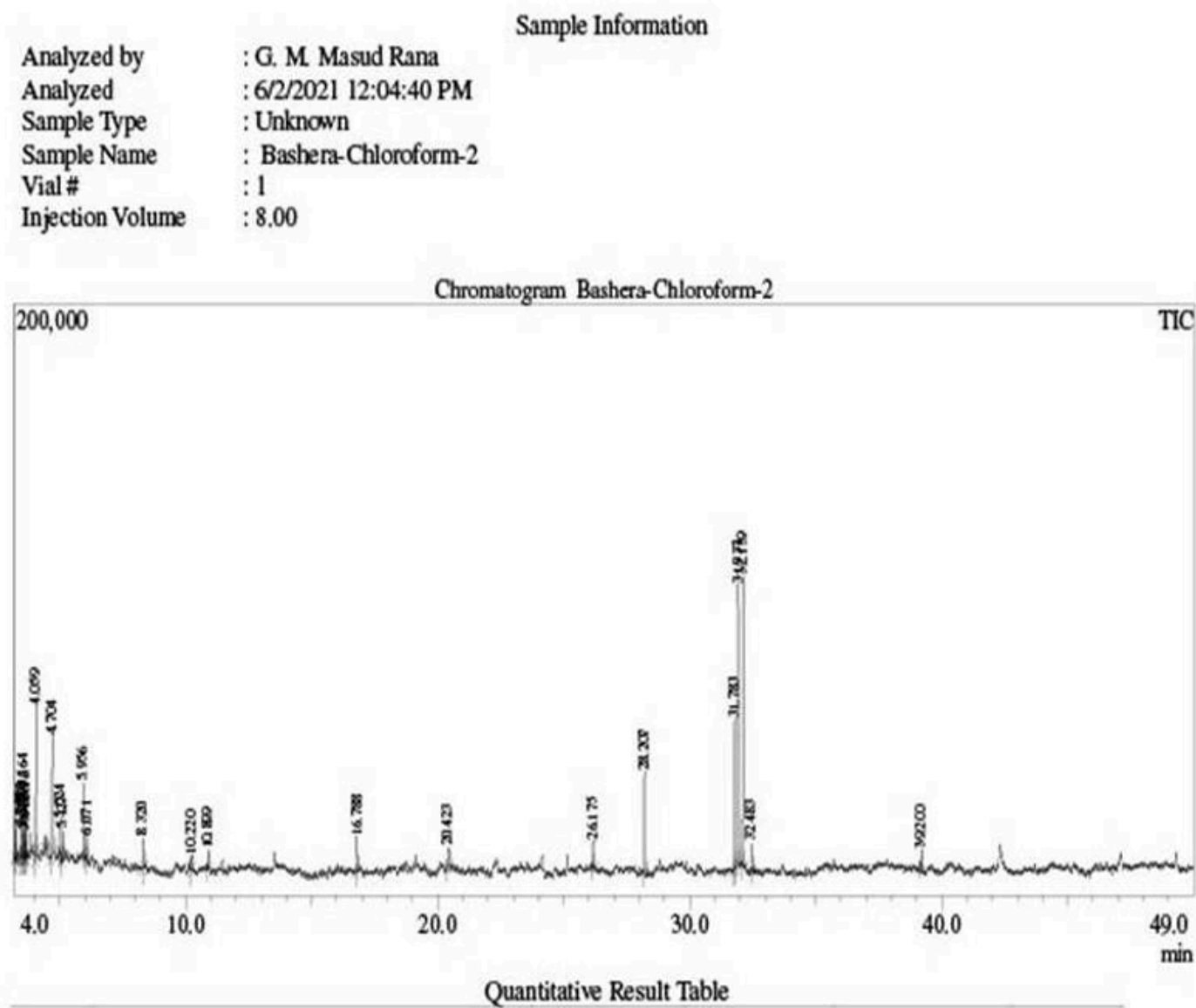


Figure 1

GC-MS spectra of crude chloroform extract

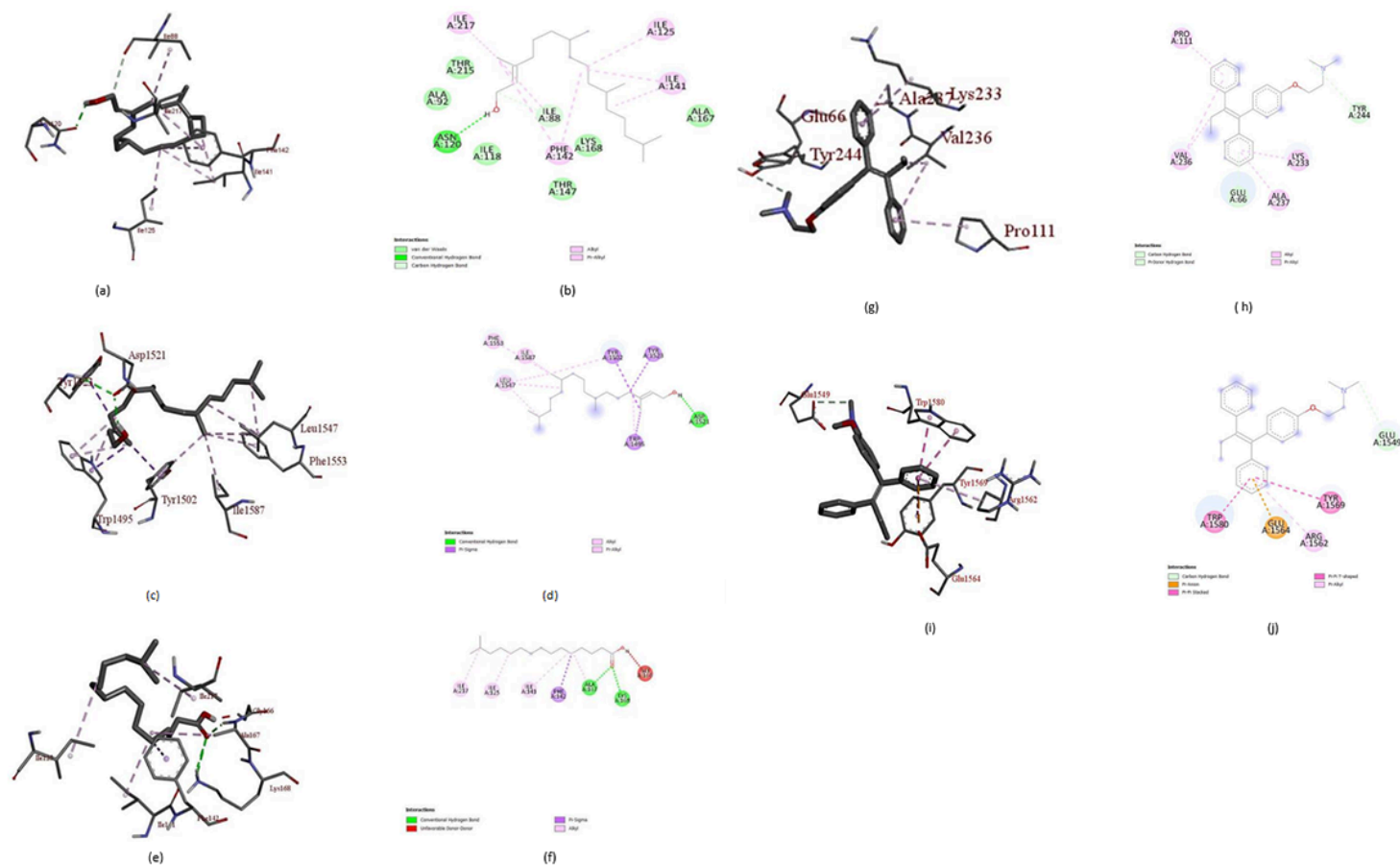


Figure 2

3D and 2D interaction diagram for (a-b) phytol-topoisomerase II, (c-d) phytol-Tp53, (e-f) Pentadecanoic acid,14-methyl and topoisomerase II (g-h) Tamoxifen-topoisomerase II, and (i-j) Tamoxifen-Tp53, respectively.