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Research Article

In vitro Biological efficacy and in silico assessment of anti-lung cancer activity of Acalypha fruticosa Forssk.

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Abstract
Acalypha fruticosa Forssk. is an evergreen rigid shrub predominantly found in the Siriya Kalvarayan hill region of the Eastern Ghats of India. *A. fruticosa* is used by tribal communities for various purposes, including the treatment of skin diseases, wounds, stomachaches, and poisonous insect and snake bites. The present study reported promising pharmacological properties of *A. fruticosa*, which has antioxidant, anti-cancer, and anti-inflammatory properties. The plant extract showed substantial antioxidant activity by inhibiting DPPH and ABTS radicals by 80% and 75%, respectively, at a 100 µg/mL concentration. The plant leaf extract exhibited significant inhibition of lung cancer cells (A549 cells) with an IC50 of 57.0 µg/mL while maintaining 80% cell viability in normal Vero cells at 100 µg/mL. The ethanolic leaf extract of *A. fruticosa* possesses 48% anti-inflammatory efficacy. Gas Chromatography-Mass Spectrometry (GC-MS) analysis identified around 44 individual volatile compounds in the ethanolic leaf extract. Using the SwissTarget database, compounds from the extract were screened for their binding ability to specific receptors associated with lung cancer (ERβ, COX-2, HER-2, and mTOR). Specific anti-cancer compounds, such as 1,2-Cyclohexanedione and 1,5-Hexadien-3-ol, demonstrated significant binding energy for the chosen receptors.1,5-Hexadien-3-ol, 3-methyl-6(methylthio)-1-(2,6,6-trimethyl-1-cyclohexen-1-yl)-, (E,E)- showed particularly noteworthy binding energy for all selected lung cancer receptors compared to other phytocompounds. Further identification of specific bioactive compounds and their interaction with lung cancer receptors will provide valuable insights into the plant's potential for developing therapeutic agents.

Keywords: *Acalypha fruticosa*, antioxidant, phytochemicals, lung cancer, GC-MS and molecular docking.

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Abbreviations: ABTS - 2,2-azinobis (3-ethylbenzothiazoline)-6-sulfonic acid; ASP -Aspirin; CHD -1,2-Cyclohexanedione; COX-2 - cyclooxygenase2; DLB - dactolisib; DPPH - (2,2'diphenyl 1-picryl hydrazyl); ERβ - Estrogen receptor β;HER-2 - Human epidermal growth factor receptor2; HMC - 1,5-Hexadien-3-ol, 3-methyl-6-(methylthio)-1-(2,6,6-trimethyl-1-cyclohexen1-yl)-, (E,E)-; LTB - Lapatinib; mTOR - mammalian target of rapamycin; MTT - [3-(4,5dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide]; NTB - Neratinib; PCM - Paracetamol; PGL - Pyrogallol; RFL - ridaforolimus; RMN - rapamycin; TLB - taselisib; TMN - toremifene.

Introduction

Acalypha fruticosa Forssk. is a shrub which belongs to the Euphorbiaceae and is widely prevalent in the Eastern Ghats and southern Western Ghats of Tamil Nadu. It is used in folk medicine by Malayali tribals native to the region to treat skin disease, wounds, stomachaches and poisonous bites (Govindu, S. and Adikay, S., 2014). The leaves possess immense medicinal value in curing digestive problems like colic, diarrhoea and dyspepsia; additionally, they treat burns and occasionally remedy bee stings (Seebaluck R. *et al*., 2015). The entire plant is prescribed for cough, cold and headache. The leaf extracts possess wound-healing properties and improve vision (Akshayaa, L. and Malaippan, S., 2021).

Furthermore, the plant extract has been healing problems like jaundice and fever (Thambiraj, J. *et al*., 2012). Hence, the bioactive compounds present in this species may have significant anti-inflammatory properties; since inflammation is one of the key players in cancer, cardiovascular disease and skin diseases, the phytochemicals present in this plant could inhibit inflammation-related diseases since phytochemicals could simultaneously possess a range of activities such as antioxidant, anti-inflammatory, anti-proliferative, etc. Depending on the different range of concentrations of each compound in crude extracts, phytochemical compounds could have multiple actions and exert synergistic effects (Serrano A., et al., 2018). Several ethnomedicinal herbs have been documented for their curative potential against various human ailments. Anti-microbial resistance and chemoresistance of anti-cancer drugs in clinical practice have recently increased unprecedentedly. As per an estimate, nearly 4.95 million people died due to anti-microbial resistance in 2019 alone, mainly because several clinically significant pathogens have become resistant to the currently available antibiotics repertoire (Alara, J.A. and Alara, O.R., 2024). The main phytochemical classes that possess nutritional and medicinal value are alkaloids, tannins, terpenoids, flavonoids, phenols, essential oils, and polysaccharides. These compounds have been isolated and investigated for their biological/therapeutical actions (Saxena, M. et al., 2013).

While ROS (reactive oxygen species) generated through routine aerobic oxygen metabolism may have beneficial properties, oxidative stress is characterized by abnormal/nonphysiological levels of ROS involved in the pathophysiology of several human disorders. One theory links oxidative stress to carcinogenesis as well as higher cancer progression rates (Kryston, T. B., *et al*., 2011). Cellular antioxidant enzymes are expressed to combat harmful free radicals (as part of routine aerobic respiration); non-physiologically high ROS levels overwhelm natural cellular antioxidant levels, disrupting cellular redox homeostasis. Hence, supplementation with antioxidants has been shown to quell free radical chain reactions which damage cellular membrane lipids and macromolecules such as RNA and DNA (Lu J. M. et al., 2010). Disruption of normal redox homeostasis is seen in inflammation, asthma, diabetes, gastrointestinal inflammatory disease, liver disease, ageing, cardiovascular disease, muscular degeneration and neoplasm/tumour formation (Cina, S. J. et al., 1996). Globally, lung cancer is the leading cancer type, which accounts for the highest mortality and morbidity rates in men (Schabath, M.B. and Cote, M.L., 2019). Dysregulation of cell signalling in cancer by pro-inflammatory cytokines and chemokines leads to higher activity of several lung cancer proliferative signal transduction pathways. COX 1 and 2 enzymes are the key proteins which convert arachidonic acid into pro-inflammatory eicosanoid molecules such as prostaglandins and thromboxanes. HER-2 receptors are overexpressed in both Small Cell Lung Cancer (SCLC) and Non-small-cell lung cancer (NSCLC). The present study shows that *A. fruticosa* ethanolic leaf extract possesses multiple biological activities *in vitro*. *In silico* corroboration of lung cancer receptor targets, including ERβ, COX-2, HER-2 and mTOR, supports the plant extract's prospective anti-inflammatory and anti-cancer properties.

Materials and methods

2.1. Sample collection and identification

Leaves of *A. fruticosa* were obtained from the Siriya Kalvarayan Hills in Kallakurichi district and used. *A. fruticosa* is an important source of Folk medicines followed by the ethnic community, and the herb is extensively found growing in Siriya Kalvarayan Hills. Rapinat Herbarium, St. Joseph's College, Trichy properly verified and authenticated the plant specimen.

2.2. Phytochemical analysis

Qualitative screening of *A. fruticosa* extract was done using the Brindha *et al*. method. Quantification of total carotenoid and total chlorophyll content was estimated using the Arnon method. The total sugar substance was calculated using the Dubois method. The determination of total lipid content was done using the gravimetric method. The amount of protein in the leaf extract was quantified by Lowry method. Total amino acid content was quantified by following the Troll and Cannan method. Total tannins, phenolics and flavonoids were estimated using the Folin-Ciocalteau method. The extraction procedures were performed using a soxhlet apparatus.

2.3. Antibacterial study

The well diffusion method (Annadurai P. et al., 2021) was used with six bacterial strains.

The bacterial species utilized in this study comprised *Staphylococcus aureus* - MTCC 9542, *Pseudomonas aeruginosa* - MTCC 1034, *Shigella flexneri* - MTCC 9543, *Klebsiella pneumoniae* - MTCC 8911, *Salmonella typhimurium* - MTCC 3224 and *Escherichia coli* MTCC 584. The nutrient broth was used for the sub-culture of selected bacterial strains and incubated at 37°C for 18 to 24 hours to revive the organisms. Subsequently, fresh overnight cultures were employed for the experimental procedures. Wells were cut using sterile, autoclaved steel borers and filled with plant extracts (at various concentrations) prepared using different solvents (50 µL). Each extract was tested in duplicate. Nutrient agar dispersion plates were inoculated with bacterial cultures after wells were cut. Sterile antibiotic discs (positive controls) were employed to obtain the inhibition zones. Standard antibiotics such as Chloramphenicol (30 µg), Amikacin (30 µg), Ampicillin (10 µg), Penicillin G (10 µg) and Methicillin (30 µg) (HIMEDIA) were used as reference or positive control Control wells contained equal amounts (50 µL) of the respective solvents. Agar plates containing bacteria were incubated at 37 °C for 24 hours. The zone of inhibitions were mesuared as the diameter of growth-free zones by treatment of both plant extract and antibiotic disc in mm, at the end of the incubation time.

2.4. DPPH assay

The antioxidant potential of *A. fruticosa* extract was done by following the protocol of Brand-Williams W. et al., (1995). 0.1 mM of 1 mL DPPH (2,2-DiPhenyl-1-PicrylHydrazyl) solution (violet in colour) was mixed with an equal amount of the *A. fruticosa* ethanolic extract at 10-100 µg/mL range. The mixed solutions were kept in the dark condition for 20 minutes at 37°C. After that, the loss of violet colour was measured at 520 nm by a UV-visible spectrophotometer. L-ascorbic acid was used as the positive control. The inhibition of DPPH free radicals was calculated by the formula given below:

Inhibition % = (Ac-As/Ac) × 100

Ac - absorbance of the control; As - absorbance of the sample.

2.5. ABTS assay

The ABTS (2,2-azino-bis-3-ethylbenzothiazoline-6-sulphonic acid) solution (Re, R., et al., 1999) was prepared in 7 mM conc. by mixing with double distilled water. The ABTS solution was dissolved with 2.45 mM conc for free radical production of potassium persulfate (1:1). The mixure of samples was kept in dark at 35 °C for 12-16 hrs. After incubation, it generated more free radical cations ($ABTS^{\bullet+}$), then dissolved (decolourized) with double distilled water to change the OD value to 0.7 at 734 nm. Furthermore, the different range (10-100 µg/mL) of *A. fruticosa* leaf extract was combined with the newly prepared $ABTS^{\bullet+}$ solution. Ascorbic acid was used as a positive control. The absorbance was measured at 734nm by UV-visible spectrophotometer.

$ABTS^{\bullet+}$ scavenging of presentations was calculated as per the DPPH assay formula.

2.6. Anti-cancer activity

Mossman B.T.(1983) method was followed for the cytotoxic activity of *A. fruticosa* extract with A549 and Vero cell line, using MTT (3-(4, 5-dimethylthiazolyl-2)-2, 5diphenyltetrazolium bromide) assay (*in vitro*). The lung cancer cell lines were obtained from NCCS at Pune, maintained in DMEM (Dulbecco's modified Eagle's medium) medium with 10% of Foetal bovine serum, 100 µg/mL of streptomycin and 100 µg/mL of penicillin and incubated in CO_2 (50 µg/mL) at 37 °C. After these cells came together, the various concentrations of *A. fruticosa* leaf extracts were combined and incubated for 24 hrs. The remaining plant extracts were removed from the 24-well plate by cleaning with PBS (pH 7.4) or DMEM (lacking serum). Additionally, 100 µL/well of MTT (0.5%) was combined, and the cells were incubated for four hrs. Thereafter, 1 mL of DMSO was added to all the wells. The absorbance was calculated at 570 nm with the help of a UV-visible spectrophotometer, and DMSO was considered blank. The following formula calculated cell activity (%):

Cell activity (%) = Treated cells / Control cells × 100

2.7. Anti-inflammatory activity

HRBC (human red blood cell) membrane stabilization mehod of Annadurai P. et al., 2022 was followed. The healthy human fresh blood sample was taken from volunteers and mixed with same volume of clean (disinfected) Alsever solution [citric acid (0.9%), sodium chloride (0.72%), dextrose (2%) and sodium citrate (0.8%)] as an anticoagulant. Then, the mixture was allowed to spin at 10,000 rpm for 15 min at 37 °C. The supernatant was removed, and RBC (red blood cell) was washed with standard saline buffer. The anti-inflammatory property was checked with RBC suspension culture.

2.8. GC-MS analysis

The ethanolic leaf extract of *A. fruticosa* was analyzed using GC-MS (Gas Chromatography Mass Spectrophotometer - Perkin Elmer Clarus 500, Connecticut, USA) equipped with flame ionization detector, capillary column (30 m length × 0.25 mm ID coated with 5% phenyl 95% dimethylpolysiloxane) with a film thickness of 0.25 µm for quantification purposes (Gomathi D., et, al., 2015).

2.9. Molecular docking

Molecular docking was done (Vijesh A. et al., 2013) to recognize the molecular connections between the 3D model of PDB ID: ER-β, COX-2, HER-2 and mTOR and *A. fruticosa* compounds using AutoDock 4.2. The receptor and ligands were changed into PDBQT files from PDB format through the standard AutoDock procedure. AutoGrid was modified, and the magnitude of XYZ was set at 90 x 90 x 90 $Å^3$ correspondingly.

3. Results and discussion

3.1. Phytochemical analysis

Plant communities are reservoirs of natural compounds that effectively treat many human diseases owing to their diverse therapeutic properties such as antiviral, anti-diabetic, antimicrobial, anti-ulcer, antioxidant, anti-inflammatory, anti-cancer properties, etc. diet containing phytochemicals can prevent cancer and help manage disease. Plant metabolites like tannins, flavonoids, catechins, terpenoids, steroids, saponins, alkaloids and phenolic compounds serve as natural defence against plant pathogens. Flavonoids are capable of controlling the risk of cardiovascular diseases, inflammatory diseases and cancer (Ballard C.R. et al. 2019). Phytochemical classes such as flavonoids, alkaloids and tannins possess anti-cancer effects (Chowdhury S. et al., 2017). Plants containing many flavonoids and alkaloids could provide substantial antibacterial activity (Jubair N. et al., 2021). We have identified and quantified plant compounds in the present investigation using qualitative and quantitative assays and GC-MS analysis. Qualitative screening of *A. fruticosa* leaf extracts obtained using six different solvents *viz.* aqueous (H_2O), methanol (CH_3OH), ethanol (C_2H_6O), acetone (C_3H_6O), chloroform ($CHCl_3$) and hexane (C_6H_{14}) for their phytochemical classes showed that ethanolic extract possesses the maximum number of phytochemical types (Table 1). Quantitative tests using *A. fruticosa* leaf extracts shown in Table 2 revealed the presence of 74.26 2.3 mg/g of phenols (gallic acid as standard), 48.03 1.4 mg/g of tannins(tannic acid standard) and 38.26 0.8 mg/g of flavonoids (rutin as standard). Around 44 different essential volatile compounds were identified/quantified through GC-MS techniques.

Table 1. Qualitative analysis of *A. fruticosa* leaf extract

Test	Solvents					
	Aqueous	**Methanol**	**Ethanol**	**Acetone**	**Chloroform**	**Hexane**
Triterpenoids	-	+	+	+	-	+
Sugars	-	+	+	+	+	-
Catechins	-	-	-	-	-	-
Flavonoids	-	-	+	-	-	-
Saponins	+	-	-	-	+	+
Tannins	+	+	+	+	+	+
Anthraquinones	-	-	-	-	-	-
Aminoacids	+	-	+	-	-	-
Sterols	+	-	-	+	+	+
Carbohydrates	+	-	-	-	+	-

(+) – present, (-) - absent

Table 2. Quantitative analysis of *A. fruticosa* leaf extract

Test	Percentageof compounds (mg/g)
Chlorophyll *a*	7.22±1.7
Chlorophyll *b*	18.2± 0.5
Total Chlorophyll	19.64± 2.4
Total carotenoids	2.48± 1.2
Total sugar	51± 1.1
Total protein	1.1± 1.8
Total lipids	12± 2.6
Total free amino acid	18.15± 1.5
Total phenol	74.26± 2.3
Total tannin	48.03± 1.4
Total flavonoids	38.26± 0.8

3.2. Inhibition of Bacterial growth

Herbal extracts possess bio-active phytochemicals which are used to heal pathological infections. Several researchers have focused on exploring plants and other natural secondary metabolites as anti-microbial agents because of their synergistic actions and ability to combat several receptors simultaneously (Vaou, N. et al., 2021). Pathogenic bacteria are responsible for many infectious diseases like pneumonia, leptospirosis, diphtheria, cholera and tuberculosis. Phytochemicals possess antibacterial activities *in vitro*, as shown by numerous herbs and essential oils from aromatic plant species (Barbieri R. et al., 2017). Plant extracts in agar culture plates have controlled the growth and proliferation of several microbial strains as per previous studies.

Nevertheless, the antibacterial activity of a given plant can vary based on the geographical location from which the plant was obtained, the parts of the plant used, the types of solvents used for extraction and the experimental methodology for bioactivity assessment (Farahmandfar R. et al., 2019). Siriya Kalvarayan hills possess rich plant diversity, as evidenced by numerous floristic species with promising ethnopharmacological

values. Some of these species have no known literature reports and are used to heal human diseases by the local tribals who live in the villages of the Siriya Kalvarayan hills. Hence, the ethnopharmacological attributes of some relatively poorly studied plant species have been a prominent goal of our research group. In this study, we have used six different bacterial strains to assess the antibacterial efficacy of A. *fruticosa* ethanolic leaf extract. In the concentration range of 6-10 mg/mL, the crude ethanolic leaf extract yielded a significant zone of inhibition against *Escherichia coli* (20-28 mm), *Klebsiella pneumonia* (21-28 mm) and *Shigella flexneri* (12-28 mm) (Figure 1). Positive control antibiotic drugs yielded better zones of inhibition: Chloramphenicol (30 µg) and Amikacin (30 µg). Crude plant extracts are not as efficacious as purified antibiotics; however, in ayurvedic and traditional medicine, many plant-based formulations have significant antibacterial activity, and therefore, the anti-microbial actions of plant compounds cannot be completely ignored. The zones of inhibition (mm) exhibited by plant extracts and positive control drugs are shown in Table 3.

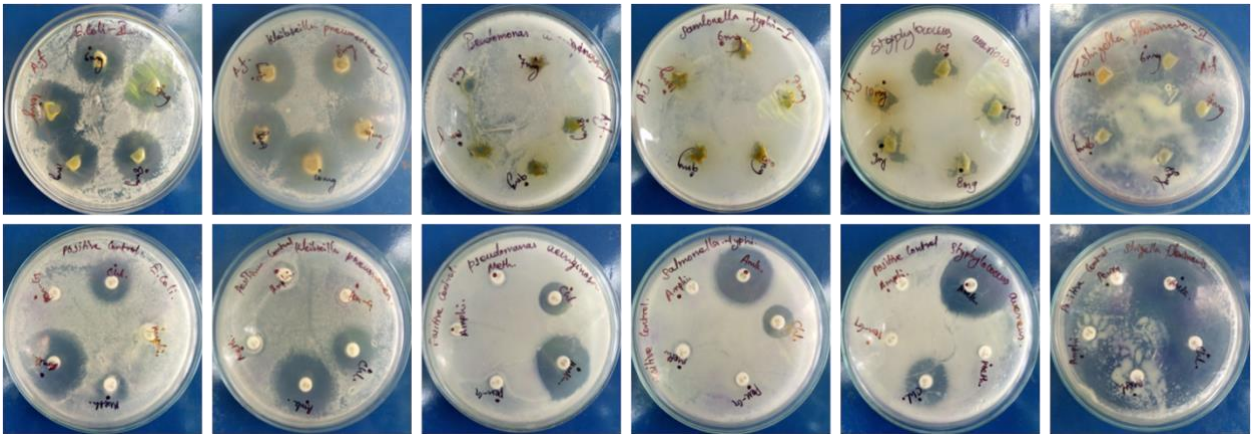


Figure 1. The ethanolic leaf extract of *A. fruticosa* was performed for *in vitro* antibacterial assay against various bacterial strains, which are *Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Salmonella typhimurium, Staphylococcus aureus* and *Shigella flexneri*.

Table 3. Antibacterial activity of *A. fruticosa* ethanolic leaf extract

Antibiotic (µg/disc)	**Inhibition zone in diameter (mm)**						
	Concentration of *A. fruticosa* leaf extract (mg/mL)	***Escherichia Coli***	***Klebsiella pneumoniae***	***Pseudomonas aeruginosa***	***Salmonella typhimurium***	***Staphylococcus aureus***	***Shigella flexneri***
-	6	20 ± 1.8	21 ± 2.0	NE	NE	NE	12 ± 0.8
-	7	23 ± 2.0	21 ± 1.3	NE	NE	NE	16 ± 1.1
-	8	23 ± 1.1	23 ± 1.0	NE	NE	NE	19 ± 1.3
-	9	25 ± 0.8	26 ± 1.7	NE	NE	NE	24 ±1.9
-	10	28 ± 1.9	28 ± 2.2	NE	NE	NE	28 ± 2.0
Chloramphenicol (30)	-	14 ± 1.5	16 ± 1.0	13 ± 1.1	10 ± 1.7	14 ± 1.1	28 ± 0.9
Amikacin (30)	-	30 ± 1.7	29 ± 1.4	27 ± 2.2	28 ± 0.9	34 ± 0.8	36 ± 0.4
Ampicillin (10)	-	NE	NE	NE	NE	NE	NE
Penicillin G (10)	-	NE	NE	NE	NE	NE	NE
Methicillin (30)	-	7 ± 1.1	NE	NE	NE	NE	NE

NE – no effect detected

3.3. Determination of free radical scavenging activity

ROS and free radicals are correlated with increased ageing, higher incidence of diseases and disease pathophysiology (Valko M. et al. 2007). Oxidative stress is counter-balanced by antioxidant enzymes and non-enzymatic antioxidants such as glutathione, ascorbic acid and Vitamin E. Some compounds have significantly higher antioxidant power and can slow ageing and disease progression rates (E Obrenovich M. et al. 2011). Medicinal plants, fruits and vegetables are richly endowed with antioxidant plant compounds such as terpenoids, polyphenols, phytosterols, phytoestrogens and Vitamin C and E (Jideani, A. I. et al. 2021). In the DPPH and $ABTS^{\bullet+}$ radical scavenging assays carried out in this work, the ethanolic leaf extract possessed the best radical scavenging against DPPH and ABTS radicals. At 100 µg/mL, *A. fruticosa* extract yielded 78% free radical scavenging activity; it was relatively lower than the corresponding positive control drug L-ascorbic acid, which scavenged DPPH radicals by 90% at the same concentration, as shown in Figure 2a. In the $ABTS^{\bullet+}$radical scavenging activity

results, *A. fruticosa* leaf extract yielded 75% free radical inhibition at 100 µg/mL concentration (Figure 2b). L-ascorbic acid used as a positive control drug possessed 89% inhibition at the same concentration range.

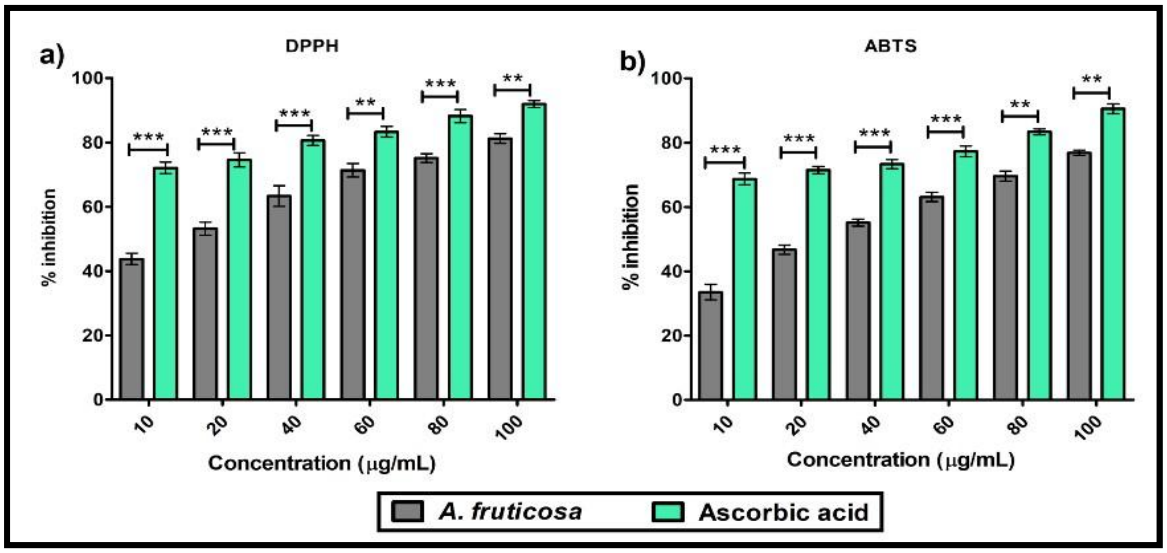


Figure 2. Antioxidant efficacy of *A. fruticosa* leaf extract: The values specify means ± standard deviation (n=3). The mean variation is significant at the range of *p <0.05, **p <0.01 and ***p <0.001 against the standard: a) DPPH and b) $ABTS^{\bullet+}$.

3.4. Anti-cancer effect of *A. fruticosa* on lung cancer

The fight against lung cancer involves a multi-faceted approach, integrating conventional treatments, targeted therapies, natural compounds, and advanced research techniques. Continued innovation and research are critical to improving outcomes for lung cancer patients (Block, K.I. et al., 2015). While certain plant compounds, such as the *Catharanthus* alkaloids, are highly cytotoxic to cancer cells, most plant phytochemicals are known to lack side effects/harmful actions (Efferth T. et al., 2019). Combining treatments like chemotherapy with targeted therapies or natural compounds can enhance anti-cancer effects and overcome resistance (Rejhová, A. et al., 2018). The MTT experiment showed that *A. fruticosa* extract was more cytotoxic to the A549 lung cancer cell line than normal Vero cells. MTT assay assessed the cytotoxic activity using the A549 human lung cancer cell line. At a concentration range of 10-100 µg/mL, the plant extract proffered inhibition of cancer cell viability by close to 20-75%, while it inhibited normal Vero cells by 0-20% at the same concentration range (Figure 3). At a comparable concentration range of 100 µg/mL, the plant extract inhibited cancer cells by more than 55% with respect to control Vero cells, demonstrating higher *in vitro* cytotoxicity against lung cancer cells. Higher cell death can be seen in lung cancer cells (Figure 4).

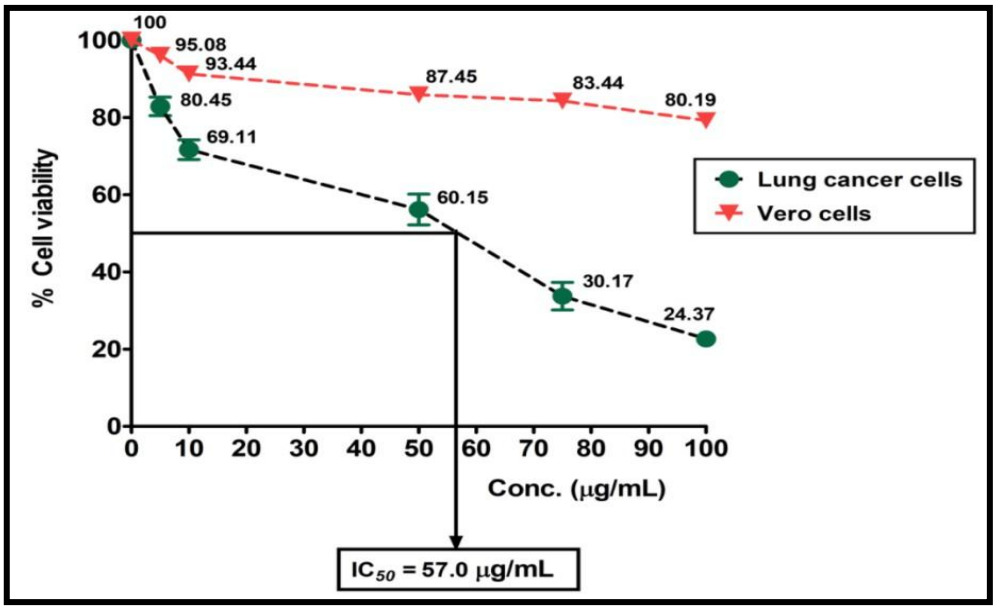


Figure 3. Anti-cancer effect of *A. fruticosa* on lung cancer.

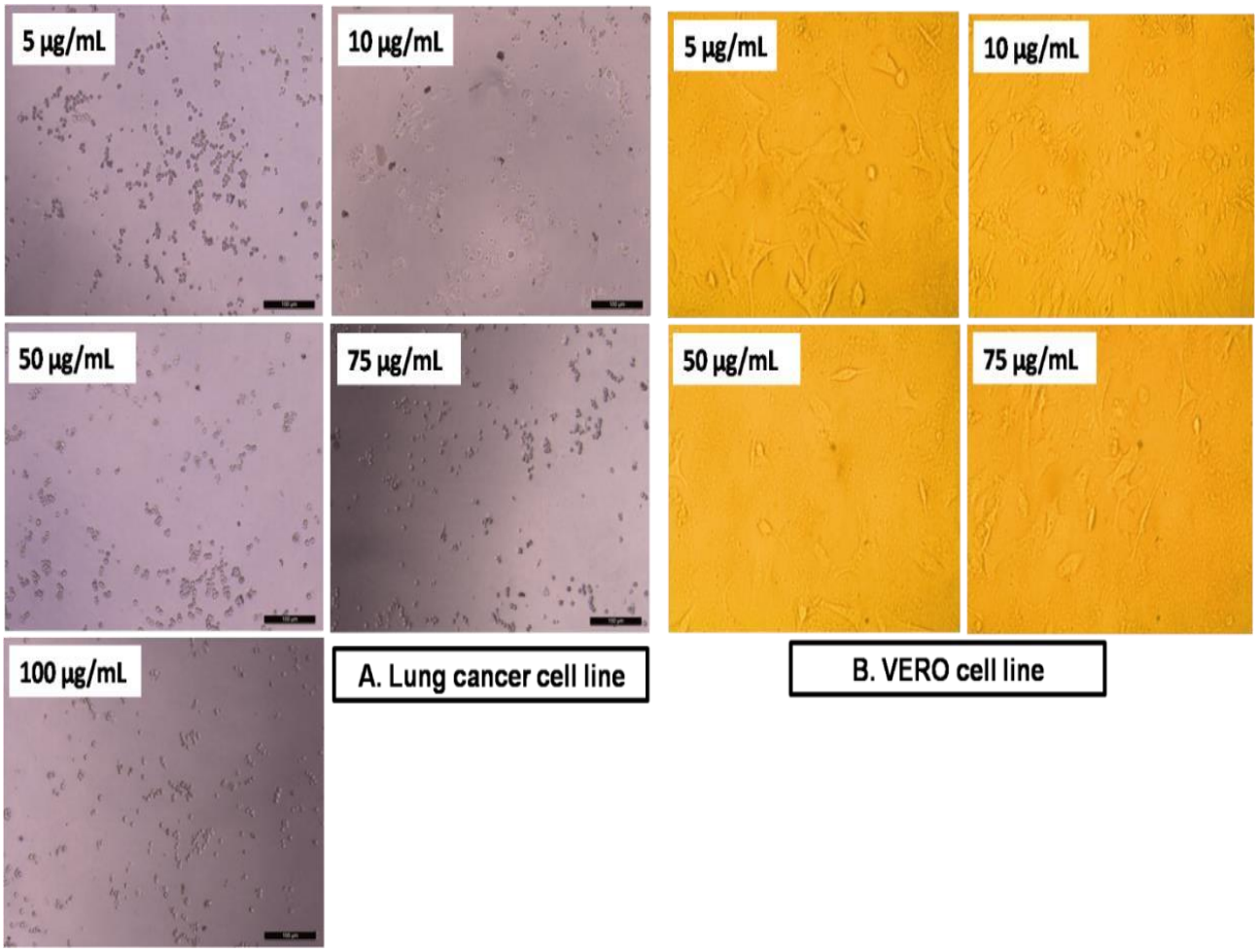


Figure 4. Cytotoxic effect of *A. fruticosa* extract towards lung cancer and VERO cell lines.

3.5. Anti-inflammatory efficacy of *A. fruticosa* leaf extract

Anti-inflammatory efficacy refers to the effectiveness of a substance or treatment in reducing inflammation and biological changes of body cells to harmful stimuli such as pathogens, damaged cells, or irritants (Placha, D. and Jampilek, J., 2021). The efficacy of anti-inflammatory agents is crucial in managing different inflammatory conditions such as asthma, arthritis and inflammatory bowel disease (Laveti D. et al., 2013). The immediate response to injury or infection is redness, heat, swelling, and pain. It involves activating immune cells, releasing cytokines, and increasing blood flow to the affected area (Ji J. et al., 2023). A prolonged inflammatory response can lead to tissue damage and is associated with various diseases, such as rheumatoid arthritis, cardiovascular diseases, and certain cancers (Chen, L. et al., 2018). Many leaf extracts inhibit the activity of enzymes like cyclooxygenase (COX) and lipoxygenase (LOX), reducing the synthesis of pro-inflammatory prostaglandins and leukotrienes (Nworu, C.S. and Akah, P.A., 2015).

The anti-inflammatory effects of *A. fruticosa* leaf extracts of various concentration ranges (100-800 µg/mL), the crude ethanolic leaf extract gained 48 % cell viability at 800 µg/mL compared with aspirin as a positive control drug.Considerable attention given to their potential as a natural efficacy anti-inflammatory (Figure 5). These extracts often contain bioactive compounds that can modulate inflammatory pathways.

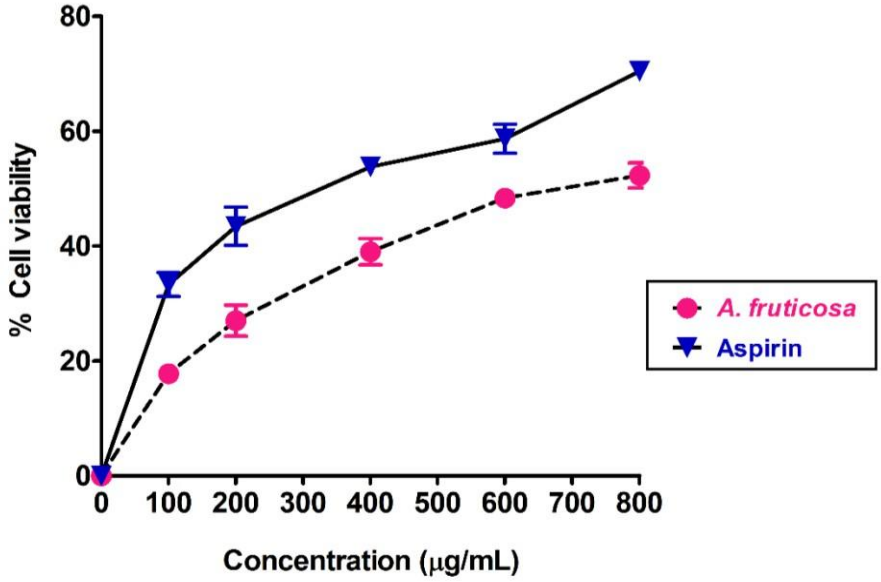


Figure 5: Anti-inflammatory efficacy of *A. fruticosa* leaf extract

3.6. GC-MS analysis of ethanolic leaf extract of *A. fruticosa*

The mass spectrometer examines the molecules eluted at various periods to determine the compounds' composition and structure (Cuyckens, F. and Claeys, M., 2004). Various peaks with different m/z ratios are produced as large molecules are fragmented into smaller ones. The peaks were compared to a recognized component database in the GC-MS library (Prasad A. et al., 2019). Ten different compounds were identified using GC-MS. Figure 6 displays the mass spectrum of the bioactive chemicals with their retention time (RT). The names, molecular weights and chemical formulae of the identified compounds are shown in Table 4.

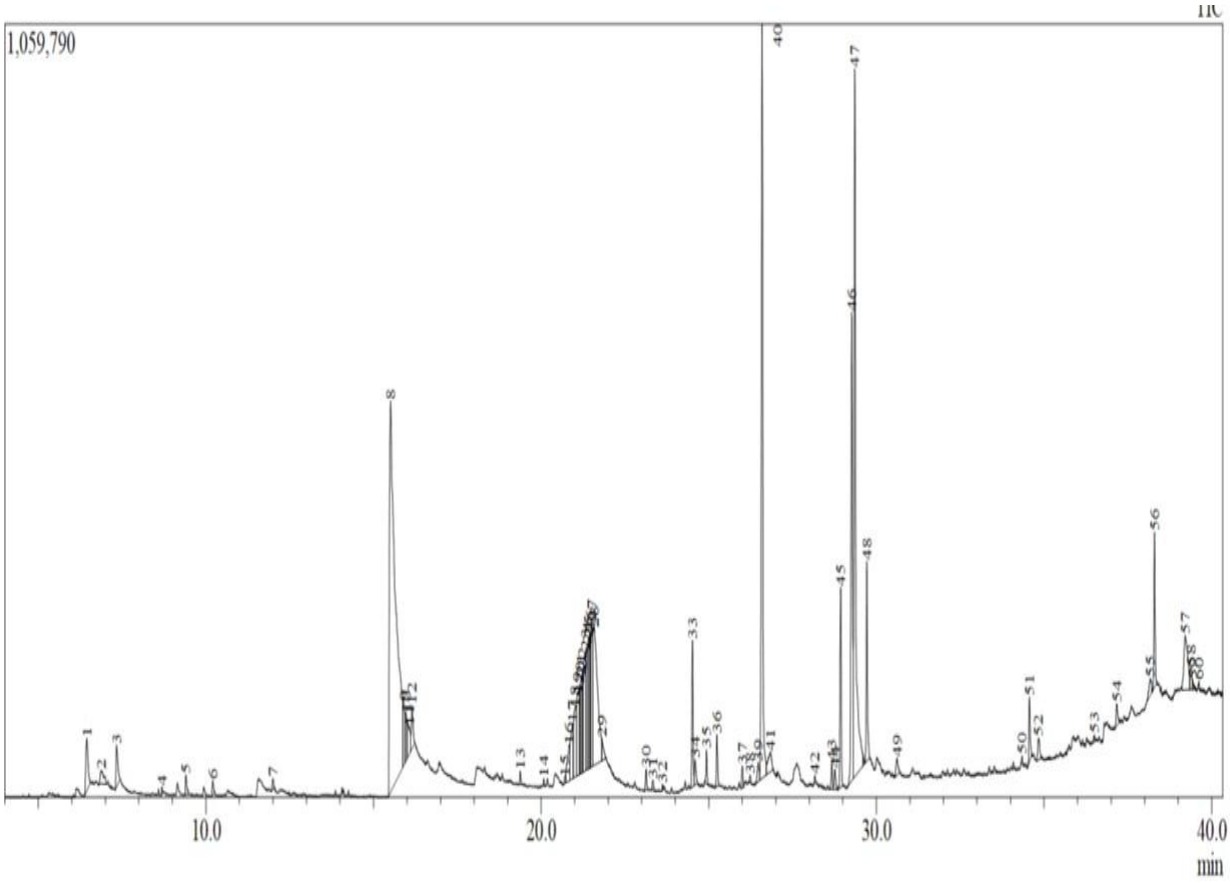


Figure 6. GC-MS spectrum of *A. fruticosa* leaf extract

Table 4. The list of volatile compounds obtained from the *A. fruticosa* leaf extract by GC-MS analysis

Peak No.	Retention time	Area (%)	Height (%)	Compound name	Molecular Weight g/mol	Molecular Formula	Uses
1	6.452	1.13	1	3-Dimethylsilyloxypentadecane	285.6	$C_{17}H_{37}OSi$	No significant report
2	6.877	0.45	0.24	1,2-Cyclohexanedione	112.13	C_6H8O_2	No significant report
3	7.338	0.73	0.79	Itaconic anhydride	112.08	$C_5H_4O_3$	No significant report
4	8.692	0.05	0.13	1-Hexadecanol	242.44	$C_{16}H_{34}O$	No significant report
5	9.404	0.28	0.37	Levoglucosenone	126.11	$C_6H_6O_3$	Cytotoxic effect, autoimmune diseases and heart diseases
6	10.206	0.18	0.27	4H-Pyran-4-one, 2,3-dihydro-3,5dihydroxy-6-methyl-	144.12	$C_6H_8O_4$	No significant report
7	12.001	0.13	0.2	4-Vinylphenol	120.15	C_8H_8O	Anti angiogenic activities, antimicrobial, antioxidant activity and anticancer activity
8	15.506	22.31	7.48	1,2,3-Benzenetriol	126.11	$C_6H_3(OH)_3$	Antioxidant activity
9	16.075	0.82	0.5	Methyl (3s*,4r*)-2,3,4,5-tetrahydro-4methyl-1,5-dioxo-1h-benz[c]azepine3-carboxylate	-	-	No significant report
10	16.146	0.72	0.82	3,5-Dimethyl-1-dimethylphenylsilyloxybenzene	256.41	$C_{16}H_{20}OSi$	No significant report
11	19.374	0.11	0.24	Hexadecanoic acid	256.42	$C_{16}H_{32}O_2$	Antibacterial, antiinflammatory and anti-cancer potential
12	20.076	0.05	0.13	Pivalic acid, 2-isopropoxyphenyl ester	236.31	$C_{14}H_{20}O_3$	No significant report
13	20.7	0.07	0.1	Pentane, 3-(bromomethyl)-	165.07	$C_6H_{13}Br$	No significant report
14	20.83	0.91	0.66	Mome inositol	-	-	No significant report
15	21.41	1.99	2.49	Methyl-beta-d-mannofuranoside	194.18	$C_7H_{14}O_6$	No significant report
16	21.82	0.32	0.42	Goitrin	129.18	C_5H_7NOS	No significant report
17	23.13	0.24	0.4	Hexadecanoic acid	256.42	$C_{16}H_{32}O_2$	Anti-cancer activity, antiinflammatory effect and antioxidants
18	23.333	0.11	0.19	2(5H)-Oxepinone, 6,7-dihydro-4methyl-	-	-	-
19	23.617	0.05	0.13	2-Methyl-5-oxohexanethioic acid, S-tbutyl ester	216.34	$C_{11}H_{20}O_2S$	No significant report
20	24.512	1.65	2.8	Neophytadiene	278.5	$C_{20}H_{38}$	Antiinflammatory activity
21	24.59	0.34	0.48	2-Pentadecanone, 6,10,14-trimethyl-	268.5	$C_{18}H_{36}O$	No significant report
22	24.932	0.36	0.65	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	296.5	$C_{20}H_{40}O$	No significant report

23	26.001	0.23	0.42	Hexadecanoic acid, methyl ester	270.5	$C_{17}H_{34}O_2$	Anti-microbial activity
24	26.232	0.07	0.14	1,9-Nonanediol, dimethanesulfonate	316.4	$C_{11}H_{24}O_6S_2$	No significant report
25	26.473	0.33	0.36	1,4-Dimethyladamantane #	164.29	$C_{12}H_20$	No significant report
26	26.593	10.31	14.4	l-(+)-Ascorbic acid 2,6dihexadecanoate	652.9	$C_{38}H_{68}O_8$	Anti-cancer, anti-inflammatory and hepatoprotective
27	26.843	0.72	0.37	.Beta.-Sitosterol	456.7	$C_{31}H_{52}O_2$	Anti-cancer, antiinflammatory and antioxidants
28	28.166	0.07	0.15	Undecanoic acid	186.29	$C_{11}H_{22}O_2$	Antioxidants and anti-microbial properties
29	28.668	0.29	0.51	9-Decen-1-yl acetate	198.30	$C_{12}H_{22}O_2$	No significant report
30	28.765	0.28	0.37	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	278.4	$C_{18}H_{30}O_2$	No significant report
31	28.933	2.15	3.81	Phytol	296.5	$C_{20}H_{40}O$	Anti-cancer, antiinflammatory activity and antioxidants
32	29.266	7.1	8.95	9,12-Octadecadienoic acid (Z,Z)-	562.9	$C_{36}H_{66}O_4$	No significant report
33	29.714	2.33	3.81	Octadecanoic acid	284.5	$CH_3(CH_2)_{16}COOH$	Antibacterial activity
34	34.341	0.07	0.15	1-Dodecanol	186.33	$CH_3(CH_2)_{10}CH_2OH$	No significant report
35	34.562	0.8	1.23	Hexadecanoic acid, 2-hydroxy-1(hydroxymethyl)ethyl ester	330.5	$C_{19}H_{38}O_4$	No significant report
36	34.83	0.4	0.42	1,2-Benzenedicarboxylic acid, diisooctyl ester	390.6	$(C_8H_{17}COO)_2C_6H_4$	Antibacterial activity
37	36.495	0.07	0.14	9,12-Octadecadienoic acid (z,z)-, trimethylsilyl ester	352.6	$C_{21}H_{40}O_2Si$	No significant report
38	37.169	0.35	0.42	Octadecanoic acid, 2,3dihydroxypropyl ester	358.6	$C_{21}H_{42}O_4$	No significant report
39	38.166	0.39	0.32	1-Heptatriacotanol	537.0	$C_{37}H_{76}O$	No significant report
40	38.294	1.75	2.96	Squalene	410.7	$C_{30}H_{50}$	Antioxidants and anti-cancer activity
41	39.213	2.04	1.04	(1R,4aR,4bS,7R,10aR)-1,4a,7-Trimethyl-7-vinyl-1,2,3,4,4a,4b,5,6,7,9,10,10adodecahydrophenanthrene-1carbaldehyde	286.5	$C_{20}H_{30}O$	No significant report
42	39.385	0.38	0.41	Dodecyl nonyl ether	312.6	$C_{21}H_{44}O$	No significant report
43	39.435	0.06	0.16	1,5-Hexadien-3-ol, 3-methyl-6(methylthio)-1-(2,6,6-trimethyl-1cyclohexen-1-yl)-, (E,E)-	280.5	$C_{17}H_{28}OS$	No significant report
44	39.615	0.06	0.14	(9Z,12Z,15Z)-3,7-Dimethyloct-6-en-1-yloctadeca-9,12,15-trienoate	416.7	$C_{28}H_{48}O_2$	No significant report

3.7. Molecular docking studies

The receptor proteins – human ERβ, COX2, HER-2 and mTOR, were docked with ten different phytochemical compounds using Autodock 4.2.6 that were identified using GC-MS analysis. The best binding energy (ΔG_B) results of receptor-ligand combinations alone are displayed in Figure 7. ER protein possesses widespread distribution and diverse biological functions, ERβ has emerged as a potential therapeutic target for various conditions, including menopausal symptoms, osteoporosis, neurodegenerative diseases, and certain cancers (Nilsson, S. and Gustafsson, J.Å., 2011). Selective agonists and antagonists targeting ERβ are being developed and evaluated for their therapeutic efficacy and safety profiles (Ellis, A. J., et al., 2015). The cyclooxygenase enzymes are pivotal players in synthesizing prostaglandins, which regulate numerous physiological processes in the body (Sales, K. J. and Jabbour, H.N., 2003). Understanding the regulation and function of COX enzymes has significant implications for developing therapeutic strategies targeting inflammation, pain, and other conditions influenced by prostaglandin signalling (Harizi, H., 2015.). The discovery of HER2 overexpression in certain cancers has led to the development of targeted therapies to inhibit HER2 signalling. These HER2targeted therapies have significantly improved patients' prognosis and survival rates with HER2positive breast cancer and other HER2-driven malignancies (Nahta R. et al., 2006). mTOR activity is tightly regulated by various cellular signals, including growth factors, nutrients, energy levels, and stress stimuli (Aramburu, J. et al., 2014). Growth factor signalling through the PI3K-Akt pathway leads to the activation of mTORC1, primarily via inhibition of the TSC1-TSC2 (tuberous sclerosis complex 1 and 2), a negative regulator of mTORC1. Amino acids, particularly leucine, also activate mTORC1 through a distinct mechanism involving the Rag GTPases and Ragulator complex (Dodd, K.M. and Tee, A. R., 2012). Lung cancer is a complex disease involving numerous molecular interactions, including those occurring at the surface of cancer cells. These interactions include proteins expressed on the cell surface, which play critical roles in cell signalling, adhesion, migration, and invasion. Some key protein surface interactions implicated in lung cancer include ERβ, COX-2, HER-2 and mTOR. Taken together, the docking results indicate that *A. fruticosa* compounds could be effective at inhibiting lung cancer cell lines, and a few of these receptor-ligand interactions could be responsible for the cell death and cytotoxic effects of the plant extract against lung cancer cells observed in the *in vitro* anti-cancer activity.

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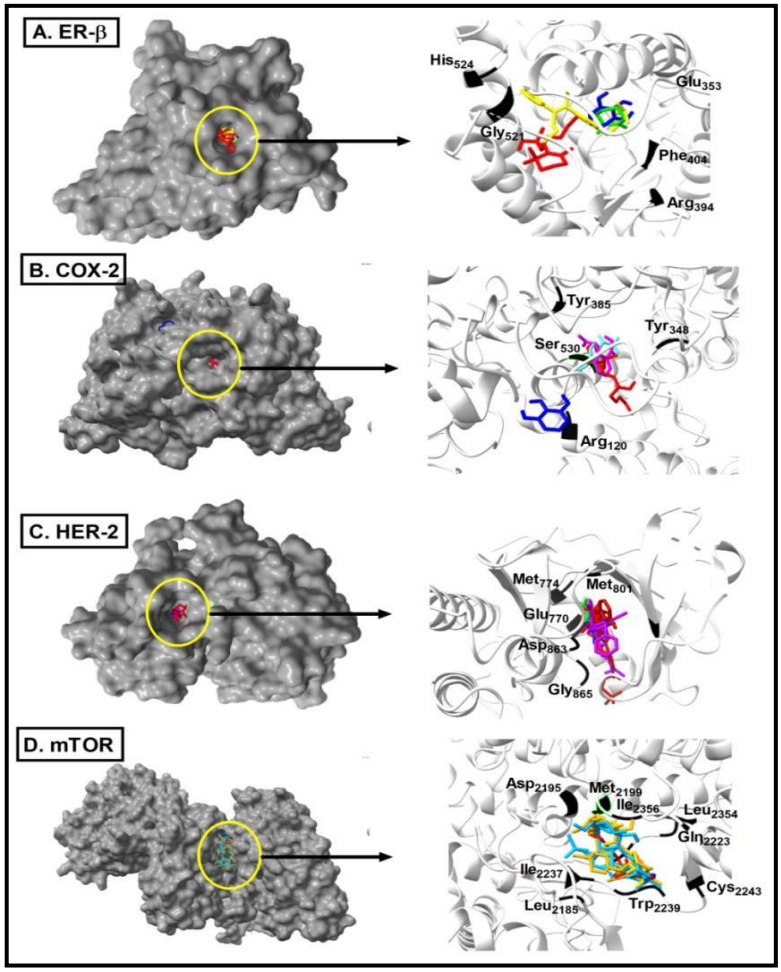


Figure 7. The surface, binding location of Macromolecule-ligand interaction of lung cancer proteins. The PDB IDs are ERβ (3OS8), COX-2 (5F1A), HER-2 (3PP0) and mTOR (4JT6).

Table 5. Molecular docking binding scores of *A. fruticosa* and commercial drugs with lung cancer receptors.

Protein Targets	Ligands	Binding energy	Ligand efficiency	inhib_constant (□M)	IE	VDE	EE
3OS8 ChainA (ER-β)	1,2-Cyclohexanedione(CHD)	-4.78	-0.6	315.46	-4.78	-4.67	-0.11
	1,5-Hexadien-3-ol, 3methyl-6-(methylthio)-1-(2,6,6-trimethyl-1cyclohexen-1-yl)-, (E,E)- (HMC)	-8.48	-0.45	0.616	-10.26	-10.22	-0.04
	Pyrogallol (PGL)	-4.87	-0.54	268.32	-5.77	-5.2	-0.57
	Toremifene (TMF)	-10.13	-0.35	0.037	-12.81	-12.22	-0.59
5F1A ChainA (COX-2)	1,2-Cyclohexanedione (CHD)	-	-	-	-	-	-
	1,5-Hexadien-3-ol, 3methyl-6-(methylthio)-1-(2,6,6-trimethyl-1cyclohexen-1-yl)-, (E,E)- (HMC)	-8.05	-0.42	1.26	-9.84	-9.81	-0.03
	Pyrogallol (PGL)	-4.26	-0.47	758.84	-5.15	-4.98	-0.17
	Aspirin (ASP)	-5.09	-0.39	185.99	-6.28	-6.06	-0.22
	Paracetamol (PCM)	-5.67	-0.52	69.92	-6.27	-6.19	-0.07
3PP0 ChainA (HER-2)	1,2-Cyclohexanedione (CHD)	-4.45	-0.56	547.3	-4.45	-4.45	0.0
	1,5-Hexadien-3-ol, 3methyl-6-(methylthio)-1-(2,6,6-trimethyl-1cyclohexen-1-yl)-, (E,E)- (HMC)	-7.97	-0.42	1.45	-9.76	-9.6	-0.15
	Pyrogallol (PGL)	-4.52	-0.5	482.8	-5.42	-5.02	-0.39
	Neratinib (NTB)	-10.85	-0.27	0.011	-14.13	-13.50	-0.63
	Lapatinib (LTB)	-9.13	-0.23	0.203	-12.41	-12.27	-0.14
4JT6 ChainA (mTOR)	1,2-Cyclohexanedione (CHD)	-5.14	-0.64	171.63	-5.14	-5.14	0.0
	1,5-Hexadien-3-ol, 3methyl-6-(methylthio)-1-(2,6,6-trimethyl-1cyclohexen-1-yl)-, (E,E)- (HMC)	-8.32	-0.44	800.44	-10.11	-10.02	-0.09
	Pyrogallol (PGL)	-5.14	-0.57	171.47	-6.03	-5.87	-0.16
	Rapamycin (RMN)	-10.12	-0.16	0.038	-12.81	-12.57	-0.24
	Ridaforolimus (RFL)	-9.83	-0.14	0.062	-12.81	-12.57	-0.24

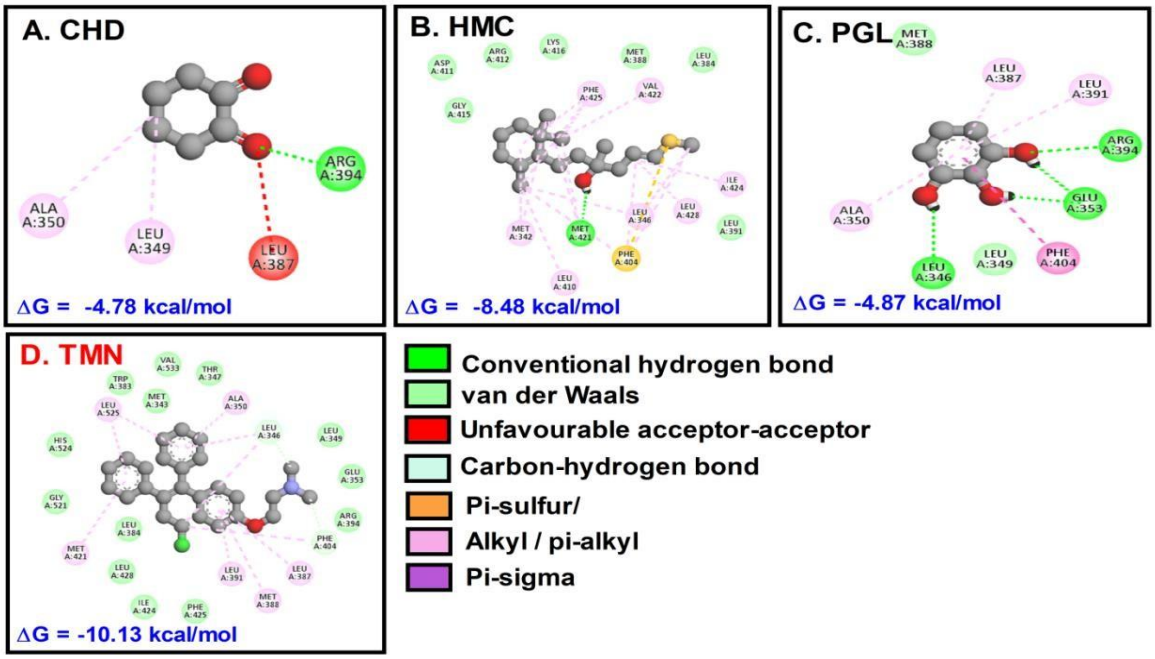


Figure 8. The different types of interactions between *A. fruticosa* compounds and 3OS8 (ERβ).

ERβ, like other nuclear receptors, consists of several functional domains, including a DNA-binding domain (DBD), a ligand-binding domain (LBD), and two activation function domains (AF-1 and AF-2). The

DBD allows ERβ to bind to specific DNA sequences known as estrogen response elements (EREs), while the LBD facilitates ligand binding and receptor dimerization. The AF-1 and AF-2 domains recruit co-regulatory proteins that modulate gene transcription. In human Nonsmall cell lung cancer (NSCLC), ERβ is overexpressed in NSCLC tissues as well as individual cells and an astounding 60-80% of lung cancer tissues were shown to possess overexpression of this receptor (Rodriguez-Lara, V. et al., 2018) in different cellular locations like cytoplasm, nucleus, mitochondria and plasma membrane. Estrogen ligand binding (eg. estradiol) to ER leads to its homodimerization and transactivation and activation of several pathways such as a) PI3K/IKK/NFkB, b) RAS/RAF/MEK/ERK and c) PI3K/AKT/Bcl-XL, which can affect cell proliferation, mitochondrial biogenesis, enhanced cell survival, invasion and metastasis (Maggi, A. 2011). In conjunction with the PI3K/AKT pathway, activation of mTOR is also known to occur, enhancing cancer cell survival and resistance to apoptosis. The binding of compounds CHD, HMC and PGLto ERβ ligand binding domain (PDB ID: 3OS8) can be seen with energies of -4 to -8 Kcal/mol, with HMC possessing < -8 kcal/mol; in contrast, the control drug TMN binding to the same site with greater favourability than the plant compounds (ΔG = -10.13) shown in Table 5 and Figure 8.

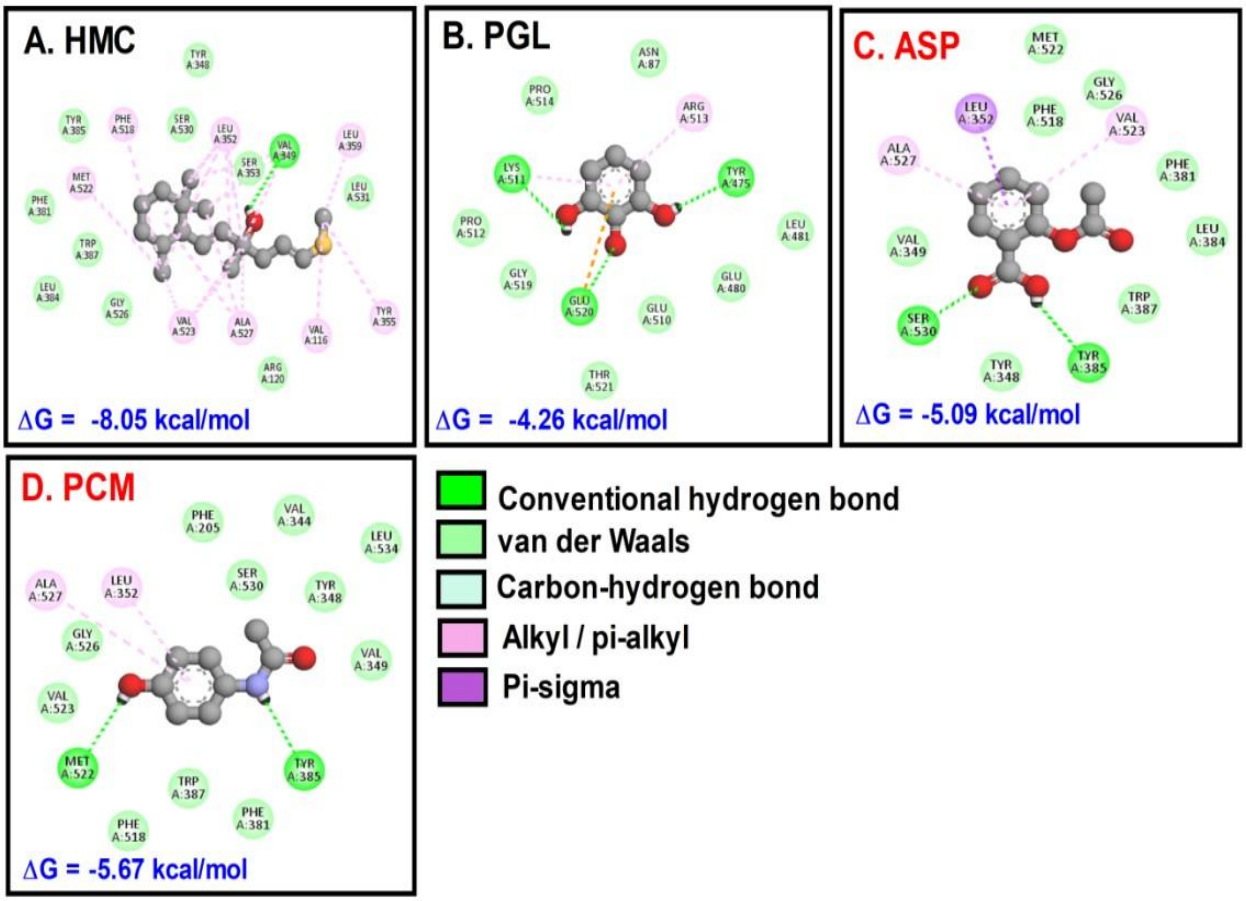


Figure 9.The different types of interactions between *A. fruticosa* compounds and 5F1A (COX-2).

The higher suppression of COX activity could occur because of plant compound binding. Inhibition of the inducible prostaglandin H-synthase/PGHS2 (COX2) enzyme is beneficial in preventing heart attacks as well as inflammatory signals, which could lead to the generation of tissue-specific downstream pro-inflammatory products of prostaglandins and thromboxanes, which are known to bind to their receptors and trigger inflammation, pain, mitogenesis and other diverse effects which could enhance cancer proliferation (Wang, D., & DuBois, R. N., 2006). COX2 is a key pharmacological target in lung cancer as it is implicated in the etiology as well as the progression of lung cancer; patients with higher COX2 expression and activity in several lung cancer types have poorer survival rates (Brown, J. R. and DuBois, R.N., 2004). Binding of the plant compounds HMC and PGL to COX2 (PDB ID: 5F1A) occurred with favourable energies of -8.05 and -4.26, respectively; in contrast, control drugs PCM and ASP showed poorer binding with energies of -4 to -5 kcal/mol (See Table 5 and Figure 9).

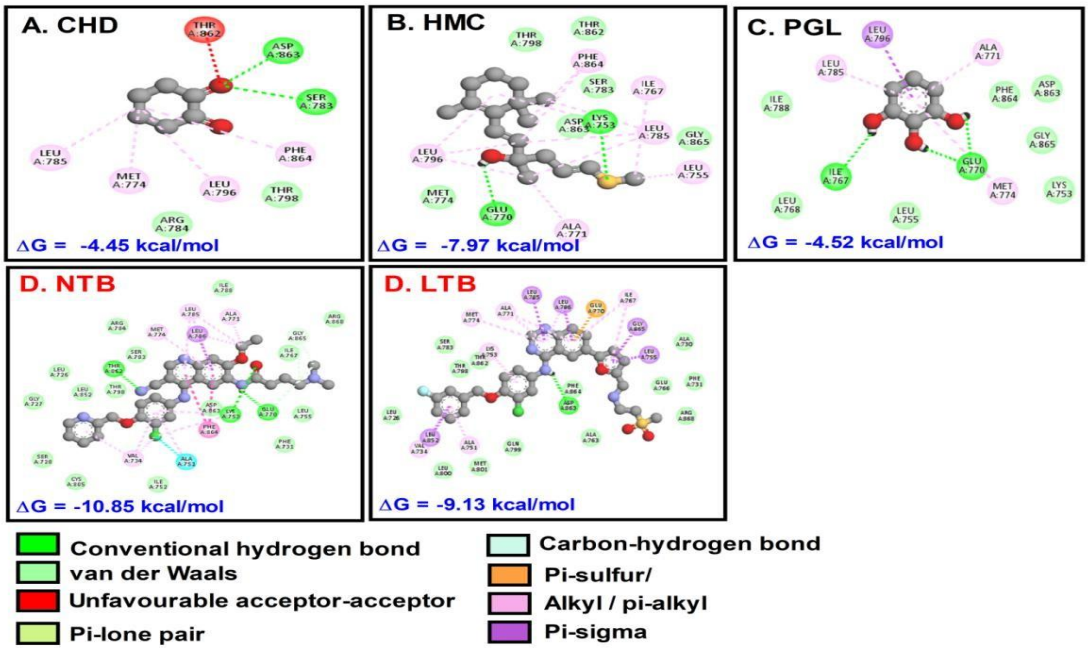


Figure 10. The different types of interactions between *A. fruticosa* compounds and 3PP0 (HER-2).

HER2 or ERBB2 is a cell surface receptor of the EGFR family that signals gene expression changes in response to the binding of diverse hormones to the HER2 receptor. Pathways such as PI3K/AKT/mTOR and RAS/RAF/MEK/ERK pathway are activated, leading to blockage of apoptosis and enhanced cell survival; hence, HER2 is one of the most important oncotargets (Joshi, S. K. et al., 2020). Amplification or overexpression of HER2 is associated with various cancers, most notably lung cancer. HER2-positive breast cancer accounts for approximately 20-30% of all breast cancer cases and is known to be more aggressive and less responsive to conventional therapies. HER2 overexpression leads to dysregulated cell growth and survival, promoting tumour progression and metastasis. One of the most important ways to prevent cancer proliferation is through blockage of HER2 receptor; compounds that bind to HER2 and prevent natural hormone ligand binding can effectively block the proliferative signalling of HER2. In this context, HMC was superior to PGL and CHD with ΔG of -7.97 (Table 5 and Figure 10), while the control compounds LTB and NTB docked with much lower binding energies and could be much more effective *in vivo*.

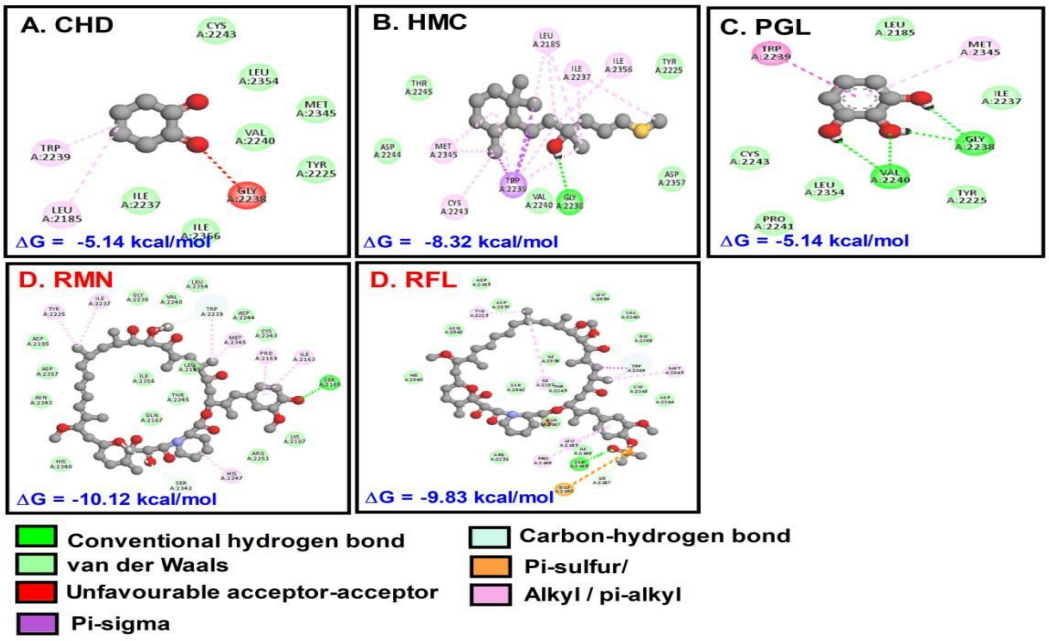


Figure 11.The different types of interactions*A. fruticosa* compounds and 4JT6 (mTOR).

The mTOR (mammalian target of rapamycin) is a crucial protein kinase that regulates cell growth, proliferation, metabolism, and survival in response to various environmental cues. It acts as a central component of two distinct protein complexes, mTOR Complex 1 (mTORC1) and mTOR

Complex 2 (mTORC2), each with unique downstream targets and functions (Weichhart, T., 2012). Triggering HER2 activity can also activate mTOR kinase, thereby sensing cellular oxygen, nutrient and energy levels. mTOR activation leads to enhanced uptake of amino acids and insulin action in cellular processes like anabolism and autophagy. mTOR activity is dysregulated in several cancers, and hence, we explored the docking of phyto compounds from the plant extract to mTOR at its kinase site (Anifowose, L.O. et al., 2023). HMC docked to this site with an energy of 8.32, while the other compounds, PGL and CHD, did not possess appreciable binding scores. Once again, the control compounds RFL and RMN had much lower ΔG values in the -9 to -10 kcal/mol range (Figure 11). Interaction residues refer to specific amino acids within proteins that directly participate in molecular interactions with other molecules, such as other proteins, nucleic acids, or small molecules. These residues are typically located at the interface of protein-protein or proteinligand complexes and play critical roles in mediating the specificity and affinity of interactions. All the chosen receptors for the molecular docking studies and the interaction residues are listed in Table 6.

Table 6. Interaction between *A. fruticosa* compounds and lung cancer targets.

Protein targets	Compounds	Van der Waals/Conventional hydrogen bond	Alkyl/pi-alkyl	Pisigma/Pi -Donor hydrogen bond/Un favorableDonar-Donar	Pi-Pi Tshaped/ Pianion/P i-Lone Pair/Pisulfur
ER-β (3OS8 chain A)	1,2-Cyclohexanedione (CHD)	Arg394	Leu349 and Ala350	Leu387	-
	1,5-Hexadien-3ol, 3-methyl-6(methylthio)-1(2,6,6-trimethyl-1-cyclohexen-1yl)-, (E,E)- (HMC)	Met421, Gly415, Asp411, Arg412, Lys416, Met388, Keu384 and Leu391	Phe425, Val422, Ile424, Leu428, Leu346, Leu410 and Mey342	-	Phe404
	Pyrogallol (PGL)	Arg394, Glu353, Leu346, Leu349 and Met388	Leu387, Leu391 and Ala350	Phe404	-
	Toremifene (TMF)	Gly521, His524, Trp383, Met343, Val533, Thr347, Leu346, Leu349, Glu353, Leu384, Leu428, Ile424, Phe425, Phe404 and Arg394	Leu525, Ala350, Met421, Leu391, Leu387 and Met388	-	-
5F1A chainA (COX-2)	1,5-Hexadien-3ol, 3-methyl-6(methylthio)-1(2,6,6-trimethyl-1-cyclohexen-1yl)-, (E,E)- (HMC)	Val349, Gly526, Leu384, Trp387, Phe381, Tyr385, Ser530, Tyr348, Ser353, Leu531 and Arg120	Met522, Phe518, Val523, Ala527, Val116, Tyr355, Leu359 and Leu352	-	-
	Pyrogallol (PGL)	Leu346, Glu353, Arg394, Leu349 and Met388	Leu387, Leu391 and Ala350	Phe404	-
	Aspirin (ASP)	Ser530, Tyr835, Val349, Tyr348, Trp387, Leu384, Phe381, Phe518, Gly526 and Met522	Ala527, Leu352 and Val523	-	-
	Paracetamol (PCM)	Met522, Tyr385, Trp387, Phe518, Phe381, Val523, Gly526, Ser530, Tyr348, Val349, Phe205, Val344 and Leu534	Ala527and Leu352	-	-
3PP0 ChainA (HER-2)	1,2-Cyclohexanedione (CHD)	Asp863, Ser783, Thr798 and Arg784	Leu785, Met774, Leu796 and Phe864	Thr862	-
	1,5-Hexadien-3ol, 3-methyl-6(methylthio)-1(2,6,6-trimethyl-1-cyclohexen-1yl)-, (E,E)- (HMC)	Lys753, Glu770, Gly865, Met774, Asp863, Ser783, Thr798 and Thr862	Phe864, Ile767, Leu785, Leu755, Ala771 and Leu796	-	-
	Pyrogallol (PGL)	Ile767, Glu770, Leu768, Leu755, Lys753, Gly865, Phe864, Asp863 and Ile788	Ala771, Met774 and Leu785	Leu796	-
	Neratinib (NTB)	Thr788, Arg784, Ser783, Ile767, Gly865, Arg368, Leu726, Thr862, Thr798, Leu852, Gly727, Ser728, Cys805, Ile752, Asp863, Lys753,	Leu785, Ala771, Met774, Leu786 and Val734	-	Phe864 and Ala751

		Glu770, Leu755 and Phe731			
	Lapatinib (LTB)	Leu726, Leu800, Met801, Gln799, Ala763, Asp363, Phe364, Ala763, Glu766, Phe731, Arg868, Thr862, Thr798,Ser783and Ala730	Met774, Lys753, Ala771, Ile 767, Ala751, Val734, Leu785, Leu796, Gly865, Leu755 and Leu852	-	Glu770
4JT6 ChainA (mTOR)	1,2-Cyclohexanedione (CHD)	Cys2243, Leu2354, Met2345, Val2240, Tyr2225, Ile2237 and Ile2356	Trp2239 and Leu2185	Gly2238	-
	1,5-Hexadien-3ol, 3-methyl-6(methylthio)-1(2,6,6-trimethyl-1-cyclohexen-1yl)-, (E,E)-(HMC)	Gly2238, Val2240, Asp2357, Asp2244, Thr2245 and Tyr2225	Leu2185, Ile2237, Ile2356, Met2345, Cys_{2243} and Trp2239	-	-
	Pyrogallol (PGL)	Cys2243, Leu2354, Pro2241, Val2240, Tyr2225, Gly2238, Ile2237 and Leu2185	Trp2239 and Met2345	-	-
	Rapamycin (RFL)	Asp2195, Gly2238, Val2240, Leu2354, Trp2239, Asp2244, Cys2243, Asp2195, Asp2357, Asn2343, Ile2356, Leu2185, Thr2245, Gln2167, Ser2165, Lys2187, Arg2251, Ser2342 and His2340	His2247, Ile2163, Pro2169, Met2345, Tyr2225 and Ile2237	-	-
	Ridaforolimus (RFL)	Asp2185, Asp2357, Asn2348, Leu2354, Val2240, Gly2238, Ile2356, Trp2239, His2340, Ser2342, Thr2243, Cys2248, Asp2244, Gln2167, Arg2251, Ile2163 and Ser2165	Tyr2225, Ile2234, Met2343, Leu2185 and Pro2169	-	Glu2190

CONCLUSION

According to the outcomes of the GC-MS analysis, the ethanolic extract of *A. fruticosa* leaves contains several bioactive substances with a range of therapeutic advantages that may be utilized to treat various ailments. The phytochemical studies showed the presence of diverse secondary metabolites, and the estimation of phenolics, tannins, and flavonoids showed substantial amounts of these compounds. The antibacterial, antifungal, anti-inflammatory and cytotoxic effects were studied *in vitro*, and the results demonstrate that the leaf extract of *A. fruticosa* effectively combats inflammation and suppresses lung cancer cell survival. Molecular docking of key proteins overexpressed in lung cancer- such as HER2, ERβ, mTOR and COX2 revealed that HMC had favourable binding to these targets. Hence, the results show that these compounds may be responsible for suppressing cancer cell proliferation. The pharmacodynamics and kinetic characteristics of these phytochemicals require more study and many more receptor-ligand docking studies and dynamics simulation studies. Purified compounds need to be studied *in vitro*, and the mechanism of lung cancer cell death could be explored in future investigations.

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