Full Length Research Article

© 2023, Centre for Advanced Research in Agricultural Sciences
Research Journal of Agricultural Sciences **14**(6): 1670–1676 (Nov-Dec 2023)
DOI: http://rjas.org/Article/Article/5665

ISSN: 0976-1675 (P)
ISSN: 2249-4538 (E)

GC–MS and Molecular Docking Analyses of Phytocompounds from *Dichrostachys cinerea* Stem Bark and Their Effect on Lung Cancer Target Protein EGFR

SWADHINI SUBRAMANIAM*[1] and DHANALAKSHMI JAGANATHAN[2]

[1-2] P.G. and Research Department of Biochemistry, Bharathidasan College of Arts and Science, Ellispettai, Erode - 638 116, Tamil Nadu, India
[1] Department of Biochemistry, Navarasam Arts and Science College for Women, Arachalur, Erode-638101, Tamil Nadu, India

Received: 13 Aug 2023; Revised accepted: 20 Oct 2023; Published online: 03 Nov 2023

Abstract

The objective of the current work is to employ GC-MS analysis to screen phytocompounds present in the ethanolic extract of *Dichrostachys cinerea* stem bark, with a focus on their potential anti-lung cancer properties, *in silico*. In the current work, the major phytocompounds that were identified using GC-MS analysis were utilized to conduct molecular docking studies in order to determine their impact on the target protein associated with lung cancer. The molecular docking studies were conducted using PyMoL and AutoDock docking software. The GC-MS analysis yielded a selection of two compounds that demonstrated the most promising potential against the target protein, EGFR, associated with lung cancer. The two major compounds, namely octadecanoic acid and 9-octadecenoic acid (Z)-(CAS)-oleic acid, demonstrated a notable level of binding affinity with the target protein associated with lung cancer. The interaction and inhibition of octadecanoic acid and 9-octadecenoic acid (Z)-(CAS)-oleic acid with the EGFR were confirmed with distinct amino acid residues such as asparagine (ASN-65), histidine (HIS-63 and HIS-80), glutamine (GLN-195), and arginine (ARG-203), respectively. Therefore, the findings of this investigation suggest that the phytocompounds derived from the ethanol extract of *Dichrostachys cinerea* stem bark possess numerous bioactive phytocompounds and therapeutic potential in the management of lung cancer.

***Key words*:** *Dichrostachys cinerea*, EGFR receptor, Molecular docking, Phytocompounds, Lung cancer

Medicinal plants serve as the primary sources of various phytochemicals, constituting the largest biological reservoirs of these compounds. The therapeutic effects of plants can be due to the existence of secondary metabolites. Plants not only serve as a source of vital nutrients for human beings, but they also offer biologically active substances that possess several therapeutic properties for human health and the management of many diseases [1]. Plants are known to have numerous major secondary metabolites, which demonstrate extensive therapeutic activities like antioxidant, antibacterial, anti-inflammatory, antimicrobial, and anticancer activities [2]. The utilization of phytomedicines has been predominantly attributed to their favorable characteristics, such as safety, accessibility, affordability, and social acceptance, in comparison to synthetic pharmaceuticals [3]. Furthermore, these phytochemicals are extensively used in various industries, such as food, pharmaceuticals, cosmetics, etc. In developing countries such as India, traditional medicines serve as the predominant form of healthcare, with the use of plants in traditional medicinal practices due to the existence of phytochemical substances [4].

Despite the fact that breast cancer has surpassed lung cancer as the most prevalent form of cancer globally, lung cancer ranks as a major cause of cancer-associated deaths. According to Sung *et al*. [5], it was reported that there was a total of 2,206,771 newly diagnosed cases of lung cancer globally in the year 2020, resulting in 1,796,144 deaths due to lung cancer [6]. Epidermal growth factor receptors (EGFRs) are a major group of receptor tyrosine kinases (TK) that are activated in many cancers. The EGFR and its associated family members play a significant role in a multifaceted signaling cascade that regulates several cellular mechanisms such as growth, transduction, migration, and survival in tumor cells. The involvement of EGFR and its family members in various aspects of cancer development has positioned them as promising targets for anti-cancer treatment [7]. The abnormal expression of EGFR has been demonstrated to have a significant impact on the formation and proliferation of cancerous cells, as it is implicated in various cellular mechanisms like growth and programmed cell death [8].

Medicinal formulations developed from plants often involve the use of crude extracts, which comprise a mixture of phytocompounds and are employed for the treatment of several diseases [9]. Numerous plants have several secondary metabolites, although only a limited subset of these have undergone extensive studies and demonstrated their significant potential as a rich reservoir of bioactive compounds. The establishment of effective screening protocols is of utmost

***Correspondence to:** Swadhini Subramaniam, E-mail: swathini93@gmail.com; Tel: +91 9965722233

Citation: Subramaniam S, Jaganathan D. 2023. GC–MS and molecular docking analyses of phytocompounds from *Dichrostachys cinerea* stem bark and their effect on lung cancer target protein EGFR. *Res. Jr. Agril. Sci*. 14(6): 1670-1676.

importance in the exploration of new chemical compounds and in ensuring quality control [10]. Gas chromatography-mass spectrometry (GC-MS) has been extensively utilized in the field of plant research for the purpose of functional group analysis and the identification of diverse bioactive chemicals [11]. GC-MS is a widely accepted and reliable analytical technique employed for the purpose of identifying several chemicals, like alkaloids, flavonoids, organic acids, and amino acids, within plant extracts. Furthermore, there has been a significant advancement in computer-based tools that serve as reliable techniques for drug discovery. These tools are capable of screening potential medications derived from bioactive chemicals found in herbal plants [12]. Computational prediction techniques are employed for *in silico* forecasting of pharmacological, pharmacokinetic, and toxicological outcomes, and they play a pivotal role in the determination of strategies that contribute to the progress of pharmaceutical and technological advancements [13]. Molecular docking represents a cost-effective and highly efficient strategy employed in the development and evaluation of pharmaceutical compounds [14].

The use of herbal plants for the purpose of treating or preventing various ailments has a longstanding historical foundation and persists as a prevalent practice in numerous regions across the world. *Dichrostachys cinerea* (L.) is one such plant, which is also known as *Cailliea dichrostachys* or *Dichrostachys glomerata*. This particular plant belongs to the Mimosaceae family and is characterized as a terrestrial herbaceous species. The distribution of this species is extensive over India and Africa, while it is regarded as an invasive species within the Caribbean region. The utilization of various parts of *Dichrostachys cinerea*, including its bark, roots, and fruit pods, has been seen in traditional medicinal practices to treat prevalent diseases like fever, pain, laxatives, and diarrhea [15]. According to several previous studies, *Dichrostachys cinerea* has been found to demonstrate antioxidant, antibacterial, anticancer, and anti-inflammatory characteristics [16-18]. In the current study, efforts have been made to screen the phytochemicals in the ethanolic extract of the *Dichrostachys cinerea* stem bark and subject it to *in silico* analysis of identified major bioactive compounds of lung cancer-associated EGFR protein.

MATERIALS AND METHODS

Description and collection of plant material

Dichrostachys cinerea (L.) is a shrub that can reach a maximum height of eight feet. Its branches terminate in thorns. The foliage exhibits a bipinnate arrangement, with each pinna adorned with a glandular structure. The hanging flowers, measuring 2.5 cm in length, consist of an upper portion and a yellow hermaphroditic lower portion that is sterile and ranges in color from purple to pink. The fruit pods have a unique ornamental look due to their twisted and indehiscent nature. Additionally, it is worth noting that this particular plant species thrives in dense soil compositions, exhibiting a high degree of prevalence within local regions.

The distribution of this species encompasses regions in the southern, central, and tropical regions of the Indian subcontinent. Fresh barks of *Dichrostachys cinerea* were collected in April 2023 in Erode district, Tamil Nadu, India. The botanical specimens were prepared, authenticated, and deposited in the Institutional Herbarium Center.

Preparation of the plant extract

The shade-dried barks were sliced into pieces and powdered using a micro-crusher. The resultant fine powder was initially subjected to maceration, where 100 g of the powder was soaked in 500 ml of ethanol for a duration of 24 hours. This process was facilitated by a magnetic stirrer, with the aim of extraction. The solvent was extracted and the resulting residue was subsequently undergoing filtration using filter paper and subsequent evaporation through the use of a Rotavapor. The ethanolic extract of the plant was obtained and stored at a temperature of 4°C for further experimentation.

GC-MS analysis of ethanolic extract of Dichrostachys cinerea

The identification of bioactive compounds involved the use of GC-MS analysis. This analysis was conducted using a Shimadzu-2010+ machine, which consisted of an AOC-20i auto sampler and a GC connected to a MS. The analysis was performed under certain conditions, as outlined below: The experiment utilized a column with dimensions of 0.32mm in diameter, 30m in length, and a thickness of 0.50µm. The column was operated in electron impact mode at an energy level of 70 eV. Helium gas with a purity of 99.999% was employed as the carrier gas, flowing at a constant rate of 1.73 ml/min. A volume of 0.5 µl was used with a split ratio of 10:1. The injector temperature was 270 ºC, while the ion-source temperature was set at 200 ºC. The oven temperature was set at 40 ºC and isothermally for 2 min. Then, the temperature was increased at a rate of 8 ºC per minute until it reached 150 ºC. Following this, the temperature was further increased at the same rate until it reached 250 ºC. Finally, the oven temperature was held isothermally at 280 ºC for a period of 20 min. The mass spectra were acquired using an electron energy of 70 electron volts (eV), with a scanning interval of 0.5 seconds, and including fragments ranging from 40 to 450 Daltons (Da). The overall duration of the GC process is 51.25 mins. The software utilized for the processing of mass spectra and chromatograms was Turbo Mass Ver 5.2.0. The interpretation of the GC-MS analysis was performed utilizing the National Institute of Standards and Technology (NIST) database, which encompasses a comprehensive collection of over 62,000 patterns. A comparison was conducted between the spectrum of the unidentified component and the spectra of the known components archived in the NIST library.

In silico analysis

Ligand preparation

The ligand structures were obtained from the PubChem database by retrieving the canonical SMILES representations. The conversion of the ligands into a PDB file was performed using Open Babel 2.4. The optimization of the structure was carried out by Argus Lab. The Kollman-united partial atomic charges were incorporated.

Protein preparation

The 3D crystallographic structure of the EGFR 696-1022 T790M/V948R variant in combination with SKLB (5X2F) was acquired from the RCSB Protein Data Bank (http://www.rcsb.org/). The process of eliminating the water molecules was conducted with the Discovery Studio 4.1 Visualiser software. Both polar and nonpolar hydrogens were included. The Gasteiger charges were calculated, and the resulting data was stored in the pdbqt format using Autodock tools.

Molecular docking

The molecular docking study was conducted with AutoDock Tools 4.0. The process of docking the small molecule with the macromolecules, specifically the EGFR, is utilized. The program is designed to forecast the manner in which a substrate or drug is bound to a receptor. The receptor and small-molecule ligands were transformed into PDBQT format from PDB file format using the established AutoDock procedure. The AutoGrid software was calibrated, and the XYZ dimensions were established as 60 x 60 x 60 Å3, correspondingly. The spacing between atoms was established at a value of 0.375 Å, while Gasteiger charges and polar hydrogen atoms were included in the EGFR macromolecule. Before initiating the docking process, the active torsions and torsional degree of freedom of tiny molecules were tuned.

The study employed the Genetic Algorithm (GA) using the Lamarckian principle, and the computational program was configured to execute a total of ten docking runs. The efficacy of the inhibitors was assessed by evaluating their binding energy, specifically the Gibbs free energy (ΔG) values, which were measured in units of kilocalories per mole (kcal/mol). The utilization of PyMoL, a software program designed for molecular visualization, facilitated the examination of the interactions occurring between the target molecule and small molecules. Additionally, PyMoL was employed to quantify the distance, specifically the bond length, inside the docked complex. The optimal output postures were examined to assess the interactions occurring between the receptor and ligand. The 2D conformations of the most promising compounds were created using Accelrys Discovery Studio Visualizer 2.5.

RESULTS AND DISCUSSION

The medicinal plants are pivotal players in the treatment and prevention of numerous ailments, while also mitigating the potential for unwanted side effects in comparison to conventional therapies. Plants have been utilized as medicinal resources throughout human history [19]. The crucial aspect of scientific study involves the identification of biologically active chemicals within plants, since this serves as a foundation for subsequent pharmacological developments [20]. Naturally found compounds derived from herbal reservoirs that possess therapeutic properties have significantly contributed to the development of numerous chemotherapeutic agents. These compounds are expected to continue playing a crucial role in the field of drug discovery, offering promising aspects for future advancements [21].

Recently, there has been significant research conducted on medicinal plants to discover the primary agent that can effectively manage chronic diseases and enhance therapeutic efficacy due to the occurrence of numerous phytocompounds [22]. There has been a surge in scientific inquiry regarding the study of therapeutic plants. Therapeutic utilization of natural substances for the treatment of various diseases has been documented since ancient civilizations. Based on a report by the WHO, it has been observed that 80% of individuals globally rely on plant-based remedies to address their fundamental healthcare requirements [23].

The assessment of the chemical profile of plants helps determine the several pharmacological properties exhibited by them. Nevertheless, it is crucial to note that there is currently a lack of literature documenting the use of GC-MS for the purpose of plant metabolic characterization to identify the diverse range of bioactive chemicals found in ethanolic extracts of *Dichrostachys cinerea*. Hence, the GC-MS study was conducted to screen the phytocompounds of the *Dichrostachys cinerea* bark extract. In the study, the comprehensive examination of the bark extract of *Dichrostachys cinerea* resulted in the identification of 30 distinct peaks. GC–MS chromatogram of the ethanolic extract of *Dichrostachys cinerea* bark is shown in (Fig 1).

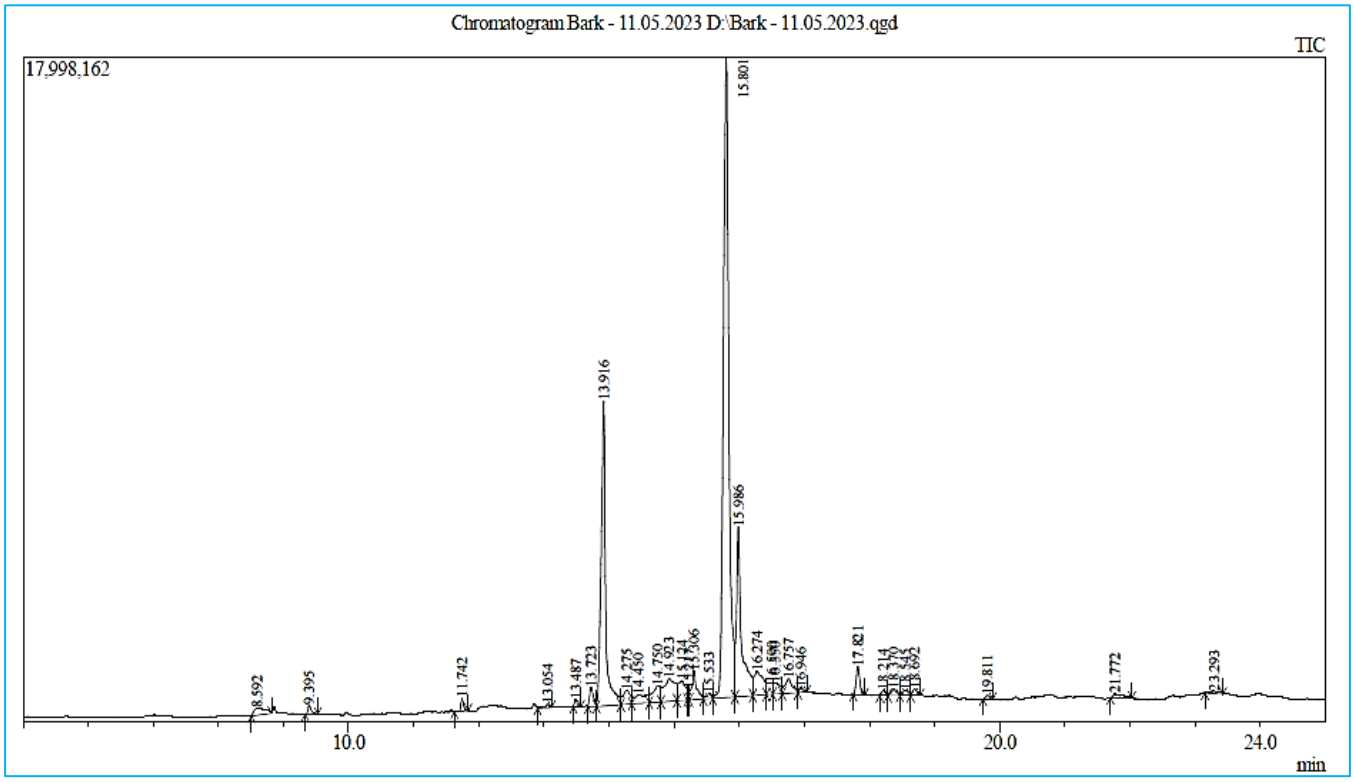

Fig 1 GC–MS chromatogram of the ethanolic extract of *Dichrostachys cinerea* bark

These peaks were assigned to specific bioactive compounds, and the identification process involved comparing the properties of the observed peaks to those already known in the NIST library (Table 1).

Table 1 Phytocompounds detected by GC–MS analysis of the ethanolic extract of *Dichrostachys cinerea* bark

Peak#	R. Time	Area	Area (%)	Height	Height (%)	Name of the compound
1	8.592	2338314	1.06	178295	0.46	Xanthosine (CAS) Xanthine riboside
2	9.395	856568	0.39	248184	0.64	Dodecanoic acid
3	11.742	979518	0.44	347033	0.90	Tetradecanoic acid
4	13.054	488763	0.22	90279	0.23	1,2-Benzenedicarboxylic acid, diundecyl ester
5	13.487	541253	0.24	195057	0.50	Hexadecanoic acid, methyl ester
6	13.723	2258852	1.02	531209	1.37	9-Octadecenoic acid (Z)- (CAS) Oleic acid
7	13.916	39090486	17.67	8264314	21.31	n-Hexadecanoic acid
8	14.275	2897320	1.31	389051	1.00	E-11-Hexadecenal
9	14.450	3191049	1.44	232185	0.60	Hexane, 2,2,3,3-tetramethyl-
10	14.750	3761166	1.70	452919	1.17	OLEIC ACID, PROPYL ESTER
11	14.923	7187135	3.25	614613	1.59	7-Methyl-Z-tetradecen-1-ol acetate
12	15.124	4232399	1.91	538890	1.39	1-Tetracosanol
13	15.217	568259	0.26	295009	0.76	Magnesium, bis(4-N,n-butylmethylaminobutyl
14	15.306	4010963	1.81	795071	2.05	METHYL 9,9-DIDEUTERO-OCTADECANO
15	15.533	760867	0.34	147842	0.38	Heptadecanoic acid, 16-methyl-, methyl ester
16	15.801	101014030	45.66	17353313	44.75	9-Octadecenoic acid (Z)- (CAS) Oleic acid
17	15.986	24653344	11.14	4623503	11.92	Octadecanoic acid
18	16.274	6335847	2.86	649368	1.67	9,12-Octadecadienoic acid (Z,Z)- (CAS) Linol
19	16.500	1919511	0.87	320485	0.83	4-Diazodamantanone
20	16.550	2193940	0.99	311720	0.80	Oxirane, hexyl-
21	16.757	3124214	1.41	390388	1.01	9,12-Octadecadienoic acid (Z,Z)- (CAS) Linol
22	16.946	555384	0.25	127677	0.33	2-Ethyl-2-methylsuccinic acid
23	17.821	2790895	1.26	755252	1.95	Isoaromadendrene epoxide
24	18.214	636251	0.29	148020	0.38	(E)-13-Docosenoic acid
25	18.370	995111	0.45	177603	0.46	9-Hexadecenoic acid (CAS)
26	18.545	856977	0.39	150666	0.39	1-Eicosanol
27	18.692	750003	0.34	175275	0.45	Eicosanoic acid
28	19.811	437025	0.20	103229	0.27	Hexadecanal (CAS) PALMITIC ALDEHYDE
29	21.772	1077501	0.49	101742	0.26	Hexadecanoic acid, 2-hydroxy-1-(hydroxymet
30	23.293	739188	0.33	65861	0.17	3-HYDROXYMETHYL-A-NORCHOLESTA
		221242133	100.00	38774053	100.00	

From the outcomes listed in (Table 1), the major compounds identified in the ethanolic extract of *Dichrostachys cinerea* bark are n-Hexadecanoic acid, 9-Octadecenoic acid (Z)-(CAS)-Oleic acid, and Octadecanoic acid, which were noted in the RT of 13.916, 15.801, and 15.986, respectively. The phytochemical analysis conducted on extracts of *Dichrostachys cinerea* has identified the presence of many compounds, including tannins, sterols, triterpenes, reductionist chemicals, polyphenols, flavonoids, and cardiotonic heterosides [24]. The chromatogram of the best detected phytochemicals from GC-MS examination of *Dichrostachys cinerea* bark extract is shown in (Fig 2-4).

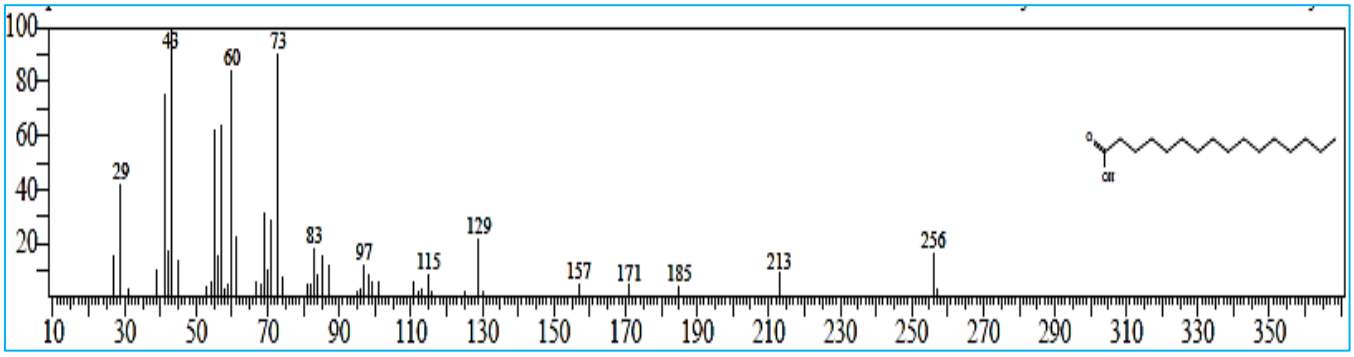

Fig 2 GC–MS chromatogram of the n-Hexadecanoic acid

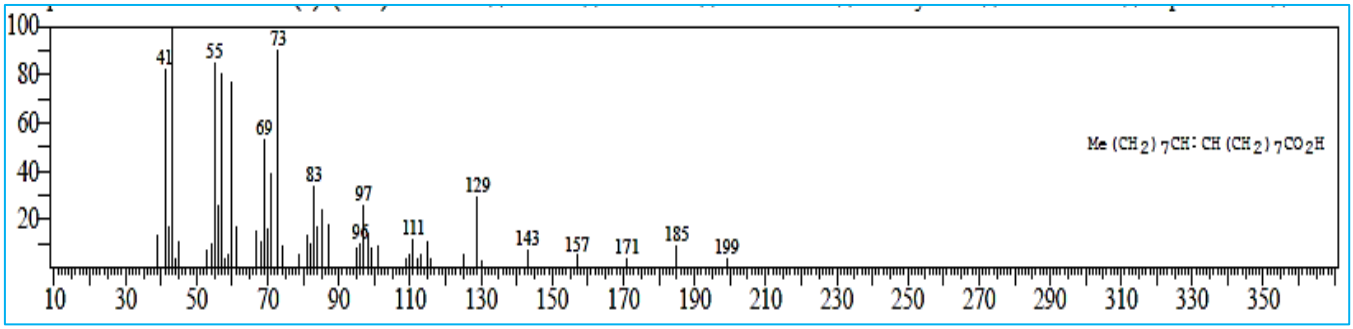

Fig 3 GC–MS chromatogram of the 9-Octadecenoic acid (Z)- (CAS) Oleic acid

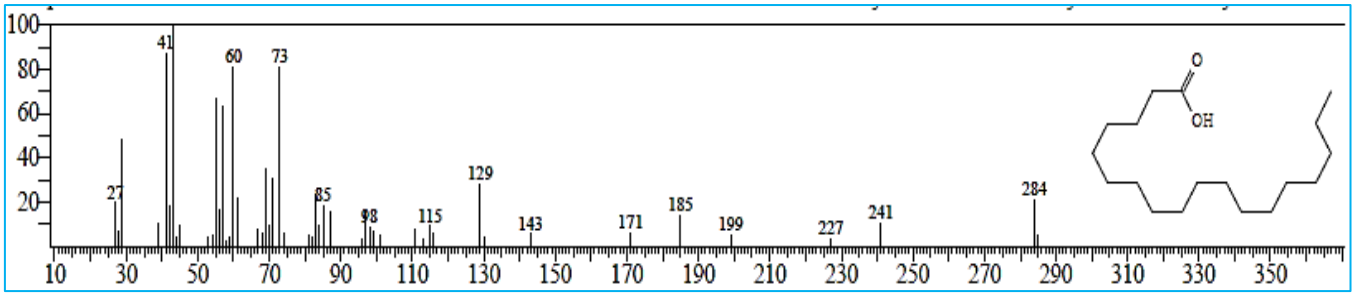

Fig 4 GC–MS chromatogram of the Octadecanoic acid

The activation of EGFR pathway is initiated by the dimerization of the receptor induced by ligand binding [25-26]. Various strategies have been established to disrupt and target the effects mediated by EGFR, owing to its functional involvement in a wide range of cellular activities. EGFR is a noteworthy therapeutic target in the treatment of NSCLCs [27-28]. The efficacy of gefitinib and erlotinib, which are reversible EGFR tyrosine kinase inhibitors (TKIs) based on 4-anilinoquinazoline, has been proven in the treatment of advanced NSCLCs with EGFR mutations [29]. Regrettably, it is commonly observed that NSCLCs harboring EGFR-activating mutations tend to acquire resistance to targeted therapies. Notably, in at least 50% of the cases where relapse occurs, resistance is attributed to a specific secondary mutation in the EGFR gene [30].

Moreover, numerous studies have provided evidence and demonstrated considerable applications of anti-EGFR candidates in various solid tumors, including NSCLC [31-33]. The effectiveness of TKIs employed in targeted therapy for cancer treatment is frequently restricted by the onset of resistance, generally attributed to the occurrence of novel mutations in targeted kinases. The development of small-molecule inhibitors targeting proteins participating in survival and apoptosis has emerged in recent times [34]. Several studies have identified small-molecule inhibitors that exhibit the ability to enhance the susceptibility of tumor cells to apoptosis upon inhibition of these kinases [35-37].

The use of molecular docking studies is essential in current drug design as a means to comprehend the interaction between drugs and receptors [38]. Computational methodologies provide robust support and facilitate the development of innovative and highly effective inhibitors by elucidating the intricate mechanisms behind drug-receptor interactions. The objective of ligand-protein docking is to anticipate the prevailing binding configuration between a ligand and a protein that possesses a well-established three-dimensional structure [39].

The (Fig 5-6) illustrate the three-dimensional conformation of the target protein, EGFR. A comprehensive examination of the ethanolic extract of *Dichrostachys cinerea* bark using GC-MS demonstrated the occurrence of a total of 30 phytocompounds. Among these, two compounds were successfully subjected to docking studies with a target protein, EGFR, associated with lung cancer. Both octadecanoic acid and 9-octadecenoic acid (Z)-(CAS)-oleic acid compounds exhibited favorable binding affinity towards the target protein EGFR.

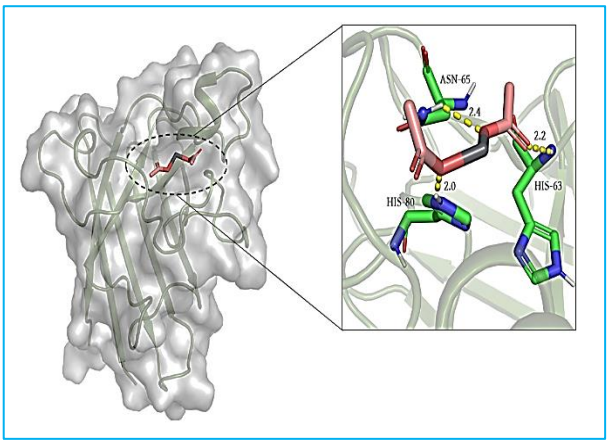

Fig 5 2D and 3D interaction of phytocompound octadecanoic acid with the target protein EGFR

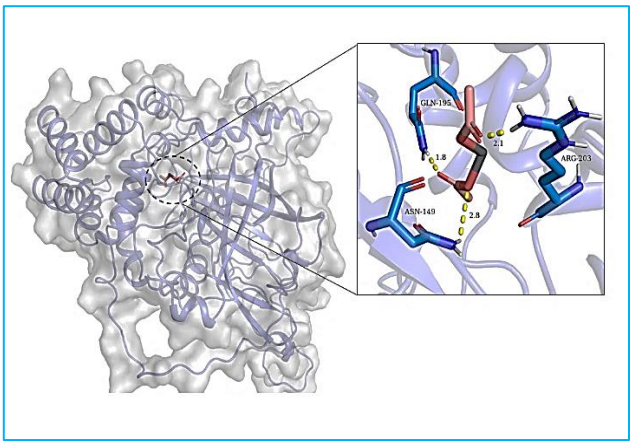

Fig 6 2D and 3D interaction of phytocompound 9-Octadecenoic acid (Z)- (CAS) Oleic acid with the target protein EGFR

Table 2 Interaction of phytocompounds from the ethanolic extract of the of *Dichrostachys cinerea* bark with the target protein EGFR

Compound name	Binding affinity (kcal/mol)	No. of bonds	Interacting residues
Octadecanoic acid	-8.38	3	His 80 His 63 ASN-65
9-Octadecenoic acid (Z)- (CAS) Oleic acid	-4.6	3	ASN-149 GLN-195 ARG-203

The molecular interactions between the EGFR with octadecanoic acid and 9-octadecenoic acid (Z)-(CAS)-oleic acid are depicted in (Fig 5-6), respectively. Various categories of non-covalent interactions have been recognized in the context of receptor-ligand interactions. The compounds derived from *Dichrostachys cinerea*, specifically octadecanoic acid and

9-octadecenoic acid (Z)-(CAS)-oleic acid, were used for the molecular docking study with EGFR. The octadecanoic acid exhibited interaction with distinct amino acid residues such as asparagine (ASN-65) and histidine (HIS-63 and HIS-80), as depicted in Figures 5 and 6. The interaction of 9-Octadecenoic acid (Z)- (CAS) Oleic acid with the EGFR was confirmed with distinct amino acid residues such as glutamine (GLN-195), asparagine (ASN-149), and arginine (ARG-203), respectively (Table 2).

The significance of natural products in the identification of anticancer compounds to treat cancer has increased [40]. The documentation of computational algorithm approaches in medicinal synthetic chemistry is extensive, but their utilization in the study of natural phytocompounds is limited and has not been well investigated. The docking mechanism performs a virtual identification process on a library collection of chemicals. The outcomes are then organized based on their scores, and hypothetical hypotheses are developed regarding the structural mechanisms via which the ligands inhibit the target receptor. This process is crucial for the improvement of drug discovery [41-42].

Natural phytochemicals have the potential to act as small-molecule inhibitors, offering potential benefits to cancer patients in terms of cost-effectiveness and well-being. Plants possess not only nutritional sources but also phytocompounds that work in the prevention of diseases and the enhancement of overall health. Plants synthesize phytochemicals, sometimes referred to as secondary metabolites, through many chemical pathways. Previous studies have demonstrated the therapeutic activity of numerous phytochemicals on human cellular functions [43]. A few of these phytochemicals are biologically active compounds that occur naturally and possess significant potential in the field of anticancer research.

Octadecanoic acid and 9-Octadecenoic Acid (Z)-(CAS)-Oleic Acid are such compounds, which are examined in this present study using molecular docking assays to assess their protein-ligand interaction. The interaction and inhibition of octadecanoic acid and 9-octadecenoic acid (Z)-(CAS)-oleic acid with the EGFR were confirmed with distinct amino acid residues.

CONCLUSION

The major objective of the current work was to detect various phytocompounds found in the *Dichrostachys cinerea* bark extract using GC-MS analysis, and the major identified compounds were subjected to molecular docking studies with Epidermal growth factor receptors (EGFR). Octadecanoic acid and 9-Octadecenoic Acid (Z)-(CAS)-Oleic Acid are the major bioactive compounds detected by the GC-MS study. Octadecanoic acid and 9-Octadecenoic acid (Z)-(CAS)-Oleic acid exhibited notable binding affinity towards the target protein EGFR receptors, which is confirmed by molecular docking studies. Based on our research findings, it was proven that the plant *Dichrostachys cinerea* has the capacity to facilitate the development of potential and reliable pharmaceuticals for various diseases, particularly lung cancer. Furthermore, additional works are warranted to examine the bioactivity of the substance and conduct clinical trials in order to facilitate the development of novel drug candidates.

LITERATURE CITED

1. Liu RH. 2003. Health benefits of fruits and vegetables are from additive and synergistic combinations of phytochemicals. *Am. Jr. Clin. Nutrition* 78: S517-S520.
2. Ezeonu CS, Ejikeme CM. 2016. Qualitative and quantitative determination of phytochemical contents of indigenous Nigerian softwoods. *New Jr. Science* 2016: 5601327.
3. Chikezie P, Ibegbulem C, Mbagwu F. 2015. Medicinal potentials and toxicity concerns of bioactive principles. *Med. Aromat. Plant Sci. Biotechnology* 4: 1-15.
4. Ningombam DS, Singh PK. 2014. Ethnobotanical study of *Phologacanthus thyrsiformis* Nees: A conserved medicinal plant of Manipur, Northeast India. *Int. Jr. Her. Med*. 1: 10-14.
5. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A. 2021. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer Jr Clin*. 71: 209-249.
6. Herbst RS, Morgensztern D, Boshoff C. 2018. The biology and management of non-small cell lung cancer. *Nature* 553: 446-454.
7. Grandis JR, Sok JC. 2004. Signaling through the epidermal growth factor receptor during the development of malignancy. *Pharmacol. Ther*. 102: 37-46.
8. Biscardi JS, Ishizawar RC, Silva CM, Parsons SJ. 2000. Tyrosine kinase signalling in breast cancer: epidermal growth factor receptor and c-Src interactions in breast cancer. *Breast Cancer Research* 2(3): 203-210.
9. Migliaccio A, Di Domenico M, Castoria G, Nanayakkara M, Lombardi M, de Falco A, Bilancio A, Varricchio L, Ciociola A, Auricchio F. 2005. Steroid receptor regulation of epidermal growth factor signaling through Src in breast and prostate cancer cells: steroid antagonist action. *Cancer Research* 65(22): 10585-10593.
10. Yadav R, Khare RK, Singhal A. 2017. Qualitative phytochemical screening of some selected medicinal plants of Shivpuri District (MP). *Int. Jr. Life Sci. Research* 3: 844-847.
11. Satapute P, Murali KP, Kurjogi M, Jogaiah S. 2019. Physiological adaptation and spectral annotation of Arsenic and Cadmium heavy metal-resistant and susceptible strain *Pseudomonas taiwanensis*. *Environ. Pollution* 251: 555-563.
12. Sliwoski G, Kothiwale S, Meiler J, Lowe EW. 2014. Computational methods in drug discovery. *Pharmacol. Rev*. 66: 334-395.
13. Loza-Mejia MA, Salazar JR, Sanchez-Tejeda JR. 2018. In silico studies on compounds derived from Calceolaria:phenylethanoid glycosides as potential multi-target inhibitors for the development of pesticides. *Biomolecules* 8: 121.
14. Lee K, Kim D. 2019. In silico molecular binding prediction for human drug targets using deep neural multi-task learning. *Genes* 10: 906.
15. Subramaniam S, Jaganathan D. 2021. A comprehensive review on *Dichrostachys cinerea*. *Jr. Univ. Shanghai Sci. Technology* 23: 1298-1312.
16. Viol DI, Chagonda LS, Munodawafa T, Muchuweti M, Moyo SR, Mericli AH. 2013. Antioxidant activity and total phenolic contents of some traditional medicinal plants from Zimbabwe. *Jr. Biol. Act. Prod. Nat*. 3: 345-352.
17. Kambizi L, Afolayan AJ. 2001. An ethnobotanical study of plants used for the treatment of sexually transmitted diseases (njovhera) in Guruve District, Zimbabwe. *Jr. Ethnopharmacology* 77: 5-9.

18. Hassan HS, Sule IM, Musa MA, Musa YK, Abubakar SM, Hassan SA. 2012. Anti-inflammatory activity of crude saponin extracts from five Nigerian medicinal plants. *Afr. Jr. Tradit. Complement. Altern. Med*. 9: 250-255.
19. Atanasov AG, Waltenberger B, Pferschy-Wenzig EM, Linder T, Wawrosch C, Uhrin P, Temml V, Wang L, Schwaiger S, Heiss EH, Rollinger JM, Schuster D, Breuss JM, Bochkov V, Mihovilovic MD, Kopp B, Bauer R, Dirsch VM, Stuppner H. 2015. Discovery and resupply of pharmacologically active plant-derived natural products: A review. *Biotechnology Adv*. 33(8): 1582-1614.
20. Farid MM, Hussein SR, Ibrahim LF, El Desouky MA, Elsayed AM, El Oqlah AA, Saker MM. 2015. Cytotoxic activity and phytochemical analysis of *Arum palaestinum* Boiss. *Asian Pacific Jr. Tropical Biomed*. 5(11): 944-947.
21. Sulejman R. 2010. Wild medicinal plants and their usage in traditional human therapy. *Journal of Medicinal Plants Research* 4(11): 1003-1027.
22. Khursheed A, Ahmad S, Khan K-u-R, Tousif MI, Aati HY, Ovatlarnporn C, Rao H, Khurshid U, Ghalloo BA, Tabassum S. 2022. Efficacy of phytochemicals derived from roots of *Rondeletia odorata* as antioxidant, antiulcer, diuretic, skin brightening and hemolytic agents-a comprehensive biochemical and in silico study. *Molecules* 27: 4204.
23. Dias DA, Urban S, Roessner U. 2012. A historical overview of natural products in drug discovery. *Metabolites* 2: 303-336.
24. Bolleddu R, Venkatesh S, Rao MM, Shyamsunder R. 2019. Investigation of the pharmacognostical, phytochemical, and antioxidant studies of various fractions of *Dichrostachys cinerea* root. *Jr. Nat. Sci. Med*. 2: 141.
25. Siwak DR, Carey M, Hennessy BT. 2010. Targeting the epidermal growth factor receptor in epithelial ovarian cancer: Current knowledge and future challenges. *Jr. Oncology* 2010: 568938.
26. Tzahar E, Waterman H, Chen X. 1996. A hierarchical network of interreceptor interactions determines signal transduction by Neu differentiation factor/neuregulin and epidermal growth factor. *Mol. Cell Biology* 16: 5276-52787.
27. Lynch TJ, Bell DW, Sordella R, Gurubhagavatula S, Okimoto RA, Brannigan BW, Harris PL, Haserlat SM, Supko JG, Haluska FG, Louis DN, Christiani DC, Settleman J. 2004. Activating mutations in the epidermal growth factor receptor underlying responsiveness of nonsmall-cell lung cancer to gefitinib. *N. Engl. Jr. Med*. 350: 2129-2139.
28. Rosell R, Carcereny E, Gervais R, Vergnenegre A, Massuti B, Felip E, Palmero R, Garcia-Gomez R, Pallares C, Sanchez JM, Porta R, Cobo M, Garrido P. 2012. Erlotinib versus standard chemotherapy as first-line treatment for European patients with advanced EGFR mutation-positive non-small-cell lung cancer (EURTAC): A multicentre, openlabel, randomised phase 3 trial. *The Lancet Oncology* 13: 239-246.
29. Mitsudomi T, Morita S, Yatabe Y, Negoro S, Okamoto I, Tsurutani J, Seto T, Satouchi M, Tada H, Hirashima T, Asami K, Katakami N, Takada M. 2010. Gefitinib versus cisplatin plus docetaxel in patients with non-small-cell lung cancer harbouring mutations of the epidermal growth factor receptor (WJTOG3405): An open label, randomized phase 3 trial. *The Lancet Oncology* 11: 121-128.
30. Kobayashi S, Boggon TJ, Dayaram T, Janne PA, Kocher O, Meyerson M, Johnson BE, Eck MJ, Tenen DG, Halmos B. 2005. EGFR mutation and resistance of non-small-cell lung cancer to gefitinib. *N. Engl. Jr. Med*. 352: 786-792.
31. Petrelli F, Borgonovo K, Cabiddu M. 2011. Cetuximab and panitumumab in KRAS wild-type colorectal cancer: A meta-analysis. *Int. Jr. Colorectal Dis*. 26: 823-833.
32. Petrelli F, Barni S. 2011. Anti-EGFR-targeting agents in recurrent or metastatic head and neck carcinoma: A meta-analysis. *Head Neck* 2011: 10.
33. Rocha-Lima CM, Soares HP, Raez LE. 2007. EGFR targeting of solid tumors. *Cancer Control* 14: 295-304.
34.Martinelli E, Ciardiello D, Martini G, Troiani T, Cardone C, Vitiello PP. 2020. Implementing anti-epidermal growth factor receptor (EGFR) therapy in metastatic colorectal cancer: Challenges and future perspectives. *Ann. Oncology* 31: 30-40.
35. Harrison PT, Vyse S, Huang PH. 2020. Rare epidermal growth factor receptor (EGFR) mutations in non-small cell lung cancer. *Semin Cancer Biology* 61: 167-179.
36. Soo RA, Lim SM, Syn NL, Teng R, Soong R, Mok TS. 2018. Immune checkpoint inhibitors in epidermal growth factor receptor mutant non-small cell lung cancer: Current controversies and future directions. *Lung Cancer* 115: 12-20.
37. Yamaoka T, Kusumoto S, Ando K, Ohba M, Ohmori T. 2018. Receptor tyrosine kinase-targeted cancer therapy. *Int. Jr. Mol. Science* 19: 3491.
38. Mahto MK, Yellapu NK, Kilare RB, Chamarthi NR, Bhaskar M. 2014. Molecular designing and in silico evaluation of darunavir derivatives as anticancer agents. *Bioinformation* 10(4): 221-226.
39. Megana KSNM, Suneetha Y. 2019. In-silico molecular screening of natural plant products for the identification of novel potential chemotherapeutic agents against breast cancer. *International Journal of Pharmaceutical Sciences and Research* 10(10): 4546-4651.
40. Shapira N. 2017. The potential contribution of dietary factors to breast cancer prevention. *European Journal of Cancer Prevention* 26(5): 385-395.
41. Feinstein WP, Brylinski M. 2015. Calculating an optimal box size for ligand docking and virtual screening against experimental and predicted binding pockets. *Journal of Cheminformatics* 7: 18-25.
42. Duran-Iturbide NA, Diaz-Eufracio BI, Jose LF. 2020. In silico ADME/Tox profiling of natural products: A focus on Biofacquimacs. *ACS Omega* 5: 16076-16084.
43. Liu X, Zhang H, Yan J, Li X, Li J, Hu J, Shang X, Yang H. 2022. Deciphering the efficacy and mechanism of *Astragalus membranaceus* on high altitude polycythemia by integrating network pharmacology and in vivo experiments. *Nutrients* 14(23): 4968.