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Bioactive compounds extracted from leaves of *G. cyanocarpa* using various solvents in chromatographic separation showed anti-cancer and anti-microbial potentiality in in silico approach

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ABSTRACT

The study was carried out to determine the possible bioactive compounds from Ethanol, Methanol, Petroleum ether, and Dichloromethane fractions of *Glycosmic cyanocarpa* (*G. cyanocarpa*). Analysis of these extracts was performed using a mass spectrometer detector installed with gas chromatography (GC–MS/MS) utilizing a method named electron impact ionization (EI). The mass spectrum of each extract was compared against the information incorporated in the library (NIST and Wiley) which provides the chemical structure with the name and molecular masses of the identified compounds. A total of 73 compounds (25 from ethanol, 19 from methanol and 5 from dichloromethane (DCM), and 24 from petroleum ether fractions) were identified from various fractions of the plants. Fourier Transform infrared spectroscopy (FTIR) analysis of the crude extracts was done to identify the functional groups of the plant derived compounds. The isolated compounds were subjected to comparison with the standard drugs towards the active binding sites of epidermal growth factor receptor (EGFR) and dihydrofolate reductase (DHFR) proteins for further evaluation of their cytotoxic and antimicrobial activity, respectively. Stigmasterol, beta-Sitosterol, Pyrazol-5-amine, 3-methyl-1,4-diphenyl-, 1,2-Benzenediol,4-(2-aminopropyl)-18,19-Secoyohimban-19-oic acid, and Phenol, 2,4-bis(1,1-dimethylethyl)-, phosphite (3:1) showed maximum binding affinity towards EGFR and 16,17,20,21-tetradehydro-16-(hydroxymethyl)-, methyl ester, (15 beta.,16*E*)-, Stigmasterol, beta-Sitosterol, Pyrazol-5-amine, 3-methyl-1,4-diphenyl- revealed highest binding affinity towards DHFR receptor. According to the current research, *G. cyanocarpa* may be a useful natural source for controlling antibacterial and anticancer activity. For thorough phytochemical screening and determining precise mechanisms of action, additional research is required.

1. Introduction

Plants have served as the primary source of many incredible medicines throughout history and are ready to contribute to a wide range of novel treatments [1–4]. 80% of people globally receive primary health care from traditional healthcare, as reported by the World Health Organization (WHO) [5]. Naturally occurring compounds have been thoroughly investigated for potential novel medication developments [6,7]. In fact, for over 5000 years, plants have been applied as remedies [8], as a source of antibiotics, antineoplastic, analgesics, cardioprotective [9] and many more.

Plant secondary metabolites are a significant source of active medicinal compounds [10] having a great potential to act as drugs [11,12] to treat various diseases [13] including antimicrobial [9,14] and antiviral [15] activity. Over fifty percent of the medicinal products approved by the Food and Drug Administration (FDA) contain natural ingredients and their derivatives [9].

Glycosmis cyanocarpa (Blume) Spreng. Belongs to the family Rutaceae. The plant looks like a shrub or a tree usually. Flowers consist of four ovules in every locule as well as four-merous and eight stamens. Compared to wide, oblong, elliptical, or ovoid, fruits are longer. It is found in south east Asian region, especially Nepal, India, Bangladesh, Burma, Thailand, Sri Lanka, Malaysia, the Philippines, and western Indonesia [16]. *Glycosmis* species have produced numerous phytochemicals including Flavonoids [17], Alkaloids, Triterpenoids, Sterol [18], Essential Oils [19], Hydroquinone Diglycosides

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[20], Phenolic Glycosides [21], Isoflavones [22], and sulfur containing amides [23].

The species cyanocarpa has the ability to prevent rats from developing carrageenan-induced paw swelling [24]. Scientists have reported its antifungal and anti-insecticidal properties [23]. Numerous investigations revealed that *Glycosmis pentaphylla* can stop liver necrosis and hepatic damage [25]. Researchers have noted the extracts' efficacy in preventing the growth of life-threatening microbes including gram negative Klebisella, *E. coli* and Staphylococcus aureus as well as gram positive Streptococci [26]. Other studies confirm that, the ethyl acetate fraction of *Glycosmis parva* caused apoptosis, prevented cell growth, and arrested the cell cycle in HT29 cells [27,28]. The ethanolic extract of *Glycosmis pentaphylla's* antioxidant activity was determined utilizing the ABTS test method, Nitric oxide, and H_2O_2 scavenging protocols [29]. Another study observed that *Glycosmis pentaphylla* reveals its antidiabetic properties are comparable to Glybenclamide [30] as well as analgesic properties [31].

Over the past thirty years, the field of molecular docking has risen in prominence due to its relevance in structural molecular biology and the pursuit of structure-based drug discovery. This advancement has been notably accelerated by the significant expansion in computer accessibility and capabilities, alongside the increasing convenience of reaching small molecule and protein databases [32]. Automated molecular docking software aims to predict molecular interactions by determining favorable binding orientations and strengths, typically involving a small ligand and a larger protein target. While often referred to as ligand-protein docking, this process has expanded to include protein-protein interactions. Its applications in drug discovery are extensive, ranging from lead optimization and virtual screening to aiding mutagenesis studies and guiding X-ray crystallography [33].

Previously scholars have performed analytical approach like ^{1}H and ^{13}C NMR or 2D NMR to isolate compounds from the plant and the extracts were used to perform pharmacological activity of the plant in animal mice model [34]. They investigate cytotoxic, antioxidant and antimicrobial properties in mice model. However, we approached in different angle where we used the extraction and isolation of bioactive compounds from solvents extraction method and characterized with GC–MS/MS and FTIR analytical technique. The isolated compounds and their mass spectra have been compared with literature and that determined their chemical structure and IUPAC name. In addition, we studied molecular docking of the identified compounds to see their antimicrobial and anticancer properties.

2. Method

2.1. Sample collection

The *G. Cyanocarpa* leaves were obtained in November 2018 from South Eastern part of Bangladesh, named as Remakri in Thanchi Upazila of Bandarban District. A voucher specimen of the plant was stored for future use at the Bangladesh National Herbarium in Mirpur, Dhaka, where it was taxonomically recognized.

2.2. Preparation of plant extract

The leaves were washed with water and allowed to dry in the sun for a few days. The dried leaves were then processed at the BRiCM Lab using a high-capacity grinding equipment to form a coarse powder. The leaf was ground into a powder and placed in a protective reagent container (3.5 L) soaked with 3.0 L of methanol (distilled). To enable thorough mixing, accompanying occasional shaking and stirring, the bottles with its contents was locked with its cap and stored for 10 days.

Whatman No. 1 filter paper and a brand-new cotton plug was used to filter the mixture. A vacuum rotary evaporator was used to evaporate the filtrate below 40 °C until a gummy mass (73.5 g) was produced. A portion (15 g) of the methanol extract (concentrated) was fractionated by using the method initiated by [35] and modified version of [36]. In a nutshell, 7.5 g of the raw extract was titrated in water and 90% methanol. Afterward, the produced yield solution was well segregated with the use of increasing polarity solvents such petroleum ether, chloroform, and ethyl acetate. In order to dry out all of the organic soluble fractions for subsequent examination, a rotary evaporator was used at a low temperature.

2.3. Gas chromatography-mass spectroscopy (GC–MS) analysis

The beneficial compounds that were separated from the leaves of *G. cyanocarpa* using the electron impact ionization (EI) technique were examined using a gas chromatograph (GC–MS/MS, Shimadzu, Japan) linked to a mass spectrometer (GC–MS/MS TQ 8040, Shimadzu, Kyoto, Japan). A fused silica capillary column with the following specifications was used: Rxi-5 ms; 30 m; 0.25 mm ID; and 0.25 m. The temperature of the column was set at 50 °C. All of the specimens were delivered in split mode, with the injection temperature maintained at 250 °C. The oven was preheated to 500 °C for one minute, 200 °C for 2 min, and then 300 °C for 7 min. The pressure of the gas chromatography was 53.5 kPa with total flow of 11.0 mL/min having 1.0 mL/min column flow. By comparing the mass spectra of each extract with the information incorporated in the NIST and Wiley libraries, the chemical names, structures, and molecular masses of the bioactive components in each extract were identified [37,38]. Total 39 min. of run time was required to perform GC–MS/MS analysis. The voltage (relative mode) of the detector was 0.6 kV. The ion source temperature was 230 °C while the interface temperature was 250 °C. Solvent cut time was made 3.5 min. Data acquisition mode was Q3 scan mode and m/z value was set for 50 to 600.

2.4. Compound characterization by FTIR

Perhaps one of the most effective instruments for determining various types of chemical bonds (functional groups) that are present in compounds is the FTIR. For FTIR analysis, dried powders containing various solvent extracts of each plant material were utilized. To create the transparent sample disc, 5 mg of the dried extract powder was encapsulated in 100 mg of KBr pellet. Each plant specimen's powdered sample was placed into an FTIR Spectroscope (Shimadzu, IR Affinity1, Japan) with a resolution of 4 cm^{-1} and a scan range of 400 to 4000 cm^{-1}.

2.5. Molecular docking study

Several software including PyRx, PyMoL 2.3, DiscoveryStudio 4.5, and Swiss PDB viewer have been used to evaluate computer-based docking of identified compounds from different extracts of *G. cyanocarpa* leaves.

2.6. Preparation of ligand

All the stated compounds in the Table 1–3 were searched and downloaded their 3D structures in SDF format from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/ (accessed on 30 June 2023)). Additionally, the 3D SDF structures off two standards Lapatinib (PubChem CID_208908) and Ciprofloxacin (PubChem CID_2764) were also downloaded from the website [39,40]. All of these compounds, along with two standards, were serially loaded into the discovery studio 4.5 with their PubChem CIDs to align them into a ligand library. It needs to be stated that all the compounds were optimized using the Pm6 semiempirical method to increase docking precision [41].

2.7. Selection of target protein

A computational docking was conducted to reveal the potential cytotoxic and antimicrobial activity of identified 5, 19, 24 and 25 compounds from dichloromethane (DCM), Methanol, Petroleum ether and

Table 1
Phytochemical compounds of *G. cyanocarpa* extracted with ethanol solvent.

Serial No.	Retention Time (min)	Area (%)	Compound name
1	3.526	0.69	1,6-Dideoxy-l-mannitol
2	3.785	1.2	Butanoic acid, ethyl ester
3	5.45	7.92	Glycerin
4	9.028	1.04	2-Methoxy-4-vinylphenol
5	10.564	0.76	cis-3-Hexenyllactate
6	10.613	1.59	2-Oxabicyclo[2.2.2]octan-6-ol, 1,3,3-trimethyl-, acetate
7	10.771	0.59	.beta.-d-Glucopyranose, 1,6-anhydro-
8	11.2	1.3	Ethanol, 2-[2-(2-methoxyethoxy)ethoxy]-
9	11.329	1.15	3′,5′-Dimethoxyacetophenone
10	11.426	1.9	3-Hydroxy-7,8-dihydro-.beta.-ionol
11	12.095	0.88	6-Methyl-5-octen-2-one
12	12.247	5.68	4-(6,6-Dimethyl-2-methylenecyclohex-3-enylidene) pentan-2-ol
13	13.291	5.9	4-((1*E*)-3-Hydroxy-1-propenyl)-2-methoxyphenol
14	13.732	2.82	6-Hydroxy-4,4,7a-trimethyl-5,6,7,7a-tetrahydrobenzofuran-2(4*H*)-one
15	14.412	1.54	Hexaethylene glycol dimethyl ether
16	16.086	1.65	Phthalic acid, butyl undecyl ester
17	16.735	1.37	trans-Sinapyl alcohol
18	17.179	2.79	Nonadecanoic acid
19	17.536	1.71	1,4-Cyclohexanediol, (Z)-
20	20.815	0.93	Benzyl .beta.-d-glucoside
21	20.976	0.59	2-Hexanone, 6-phenyl-
22	22.556	24.78	9-Octadecenamide, (Z)-
23	30.924	1.81	1,2-Benzenediol,4-(2-aminopropyl)-
24	31.148	26.31	Pyrazol-5-amine, 3-methyl-1,4-diphenyl-
25	31.809	3.1	Arborinine

Table 2
Phytochemical compounds of *G. cyanocarpa* extracted with Methanol solvent.

Serial No.	Retention Time (min)	Area (%)	Compound name
1	3.733	6.76	3,3-Dimethoxy-2-butanone
2	3.793	2.16	1,3-Dioxolane-4-methanol, 2-ethyl-
3	14.32	2.15	Neophytadiene
4	18.439	15.96	Phytol (terpenes)
5	18.814	2.09	2,8,9-Trioxa-5-aza-1-silabicyclo(3.3.3)undecane, 1-methoxy-
6	22.627	21.14	9-Octadecenamide, (Z)-
7	22.755	5.4	18,19-Secoyohimban-19-oic acid, 16,17,20,21-tetradehydro-16-(hydroxymethyl)-, methyl ester, (15.beta.,16*E*)-
8	22.805	2.47	(7*E*,11*E*)-1-(2-Hydroxyethyl)-2,3-epoxy-1,7,11-trimethylcyclotetradecadiene
9	22.83	4.22	2-(3-Hydroxy-propyl)-cyclohexanol
10	22.963	2.54	Geranyl ethyl ether 1
11	23.01	1.97	Chloromethyl nonanoate
12	23.146	3.55	2-Hexen-1-ol, 2-ethyl-
13	24.641	2.58	Chloroacetic acid, 4-hexadecyl ester
14	24.831	2.49	Tetrapentacontane, 1,54-dibromo-
15	29.477	5.07	Squalene (Triterpenes)
16	32.191	3.28	(1-Cyanocyclohexyl) carbamate
17	33.911	4.91	Vitamin E
18	35.995	3.73	Stigmasterol
19	37.109	4.86	beta.-Sitosterol

Table 3
Phytochemical compounds of *G. cyanocarpa* extracted with DCM solvent.

Serial No.	Retention time (min)	Area (%)	Compound name
1	22.563	1.85	9-Octadecenamide, (Z)-
2	24.841	0.62	Pentatriacontane
3	31.162	34.38	Dodecanoic acid, 1,2,3-propanetriyl ester
4	31.23	42.26	Pyrazol-5-amine, 3-methyl-1,4-diphenyl-
5	31.914	20.89	Arborinine

Ethanol fraction of *G. cyanocarpa* leaves extract respectively. To assess the cytotoxic potential of the 3D crystal structure of epidermal growth factor receptor (EGFR) [PDB ID: 1XKK] [40] as well as to evaluate the antimicrobial effect of the dihydrofolate reductase (DHFR) [PDB ID: 4M6J] [39]. 3D crystal structure was downloaded from the protein data bank (https://www.rcsb.org/ (accessed on 30 June 2023))

2.8. Ligand-protein binding

The current computer-aided ligand-protein interaction has been drawn to estimate probable binding profiles of phytocompounds with their affinities to the target molecules. This molecular drug-protein linking technique was carried out using a highly sophisticated PyRx-Autodock Vina, and the molecular docking was done using semi-flexible modeling. To ensure that the ligands exclusively bind to the required macromolecule, the literature-based amino acids with their ID have been selected, and the protein has first been loaded and formatted to the desired macromolecule. For the target EGFR, Leu 718, Val 726, Ala 743, Lys 745, Met 766, Lys 775, Arg 776, Leu 777, Leu 788, Thr 790, Gln 791, Leu 792, Met 793, Gly 796, Cys 797, Leu 799, Asp 800, Arg 803, Leu 844, Thr 854, Asp 855, and Phe 856 were used as target site(El Azab et al. [40]). Also, Ala9, Ile16, Leu93, Ser92, Arg91, Arg77, Glu78,

Ser76, Leu75, Lys54, Val120, Ser119, Lys55, Thr56, Ser118, Gly117, and for 1R4U, Arg 176, Val 227, Gln 228, Asn254, and His 256 were selected as target amino acid during DHFR docking (Khatun et al. [31]). Consequently, all of the SD files of the ligands were imported and thereafter reduced into pdbqt format with the Open Bable tool in the PyRx-AutoDock Vina software in order to match the best optimal hit during docking against these chosen macromolecules. However, these active amino sites were kept under a grid box through grid mapping. The center_x = 15.855, center_y = 34.570, center_z = 35.827, dimension_x = 24.902, dimemnsion_y = 19.335, and dimension_z = 31.936 were maintained for EGFR. While the grid mapping for DHFR were fixed to center_x = 3.655, center_y = −3.613, center_z = −18.763, dimesnsion_x = 21.0232, dimension_y = 27.490, dimension_z = 27.503. In the meantime, supportive functions were kept as default. Then the final docking was conducted through AutoDock Vina (version 1.1.2) to find out the ligands' affinity towards the macromolecule. Last, the result was conceptualized, and best-fitting 2D and 3D models were predicted using BIOVIA Discovery Studio version 4.5.

2.9. ADME/T analysis

In modern drug design, there is an increasing trend towards utilizing computer-based methods to evaluate various pharmacokinetic aspects of potential drugs. These aspects include absorption, distribution, metabolism, excretion, and toxicology, as well as assessing the drug's likeliness to be effective. These combined analyses, known as ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicology), play a crucial role in the process of discovering new drugs [42].

A commonly used strategy involves studying the pharmacological properties of compounds, as demonstrated by the research carried out at pkcsm (http://biosig.unimelb.edu.au/pkcsm/prediction). Furthermore, online tools like SwissADME (http://www.sib.swiss) have gained popularity for their capacity to predict drug likeliness, often evaluated using the Lipinski rules. These rules, originally proposed by Lipinski, establish criteria that indicate the potential oral suitability of a compound. Specifically, a compound is considered suitable for oral administration if it satisfies certain conditions: its molecular weight is under 500 atomic mass units (amu), it possesses fewer than 5 hydrogen bond donor sites, fewer than 10 hydrogen bond acceptor sites, and a lipophilicity value (LogP) not exceeding 5 [43].

3. Result and discussion

3.1. Phytochemicals from GC–MS/MS analysis of ethanol extract of G. cyanocarpa

25 chemicals have been found in an ethanol-based extract of *Glycosmic cyanocarpa*, according to Table 1 and Fig. 1. We measured the peak area % to represent each compound's relative concentration. Highly abundant compounds were Pyrazol-5-amine (26.31%), 9-Octadecenamide, (Z)- (24.78%), 4-((1*E*)-3-Hydroxy-1-propenyl)-2-methoxyphenol (5.9%), 4-(6,6-dimethyl-2 methylenecyclohex-3-enylidene) pentan-2-ol (5.68%), Arborinine (3.1%) and 6-Hydroxy-4,4,7a-trimethyl-5,6,7,7a-tetrahydrobenzofuran-2(4*H*)-one (2.82%). All these compounds have various pharmacological properties. Cancer is one of the deadliest diseases in medical field. Various reports have demonstrated the efficacy of arborinine against cancer cell line. A new prospective therapeutic candidate for ovarian cancer is arborinine, an alkaloid that greatly inhibits the cell proliferation and tumor growth of SKOV3 in a dose-dependent way. This resulted in decreased LSD1 expression and increased H3K4m1 expression [44]. Additionally, it has demonstrated antagonistic action against renal cell carcinoma [45]. It is reported to be effective against human breast cancer cell line (T-47D) as well as human (COLO-205) colon cancer cell [46]. Pyrazol-5-amine, a heterocyclic compound, potentially has its synthetic versatility [47] and most pyrazol has biological (anti-neoplastic, antimicrobial, anti-inflammatory, anti-diabetic, antidepressant, antitumor, insecticide properties [48–51]. The modifications carried out in the pyrazol moiety reveals the antiproliferative activity in different cancer cells [52]. 9-Octadecenamide, (Z)- has shown as a potent antibacterial and antiviral potentiality [53]. The plant reveals a lot of phenolic compounds in much abundant form. 2-Methoxy-4-vinylphenol has reported to produce aroma in various food products [54]. The compound 4-(6,6-dimethyl-2-methylenecyclohex-3-enylidene) pentan-2-ol showed melamine and dyes effects [55]. Phenolic compound 4-((1*E*)-3-Hydroxy-1-propenyl)-2-methoxyphenol showed Antimicrobial, Antioxidant, Anti-inflammatory properties [56]. A derivative of 3′,5′-dimethoxyacetophenone inhibits aldose reductase, causes collagenase, and has anticancer effects on human leukemic cells [57]. Beer aroma is impacted by the norisoprenoids 3-hydroxy-7,8-dihydro-beta-ionol during fermentation [58]. There have been reports of nonadecanoic acid (19-carbon fatty acid) having lethal effects on a variety of human cancer cell lines, including the HCT-15, SK-OV-3, A549, and SK-MEL-2 [59]. Moreover, high fatty acid and ester groups demonstrate their cytotoxic activity which scholars have previously detected [60].

3.2. Phytochemicals from GC–MS/MS analysis of methanol extract of G. cyanocarpa

From Table 2, it is shown that 19 compounds are available in methanol extract of the plant *Glycosmic Cyanocarpa*. Among them, the most abundant constitutes are 9-octadecenamide, (Z)- (21.14%), Phytol (15.96%), Squalene (5.07%), Vitamin E (4.91%), beta-Sitosterol (4.86%) and neophytadiene. (2.15%). Phytol has proved to be a good biological entity in many research. A decrease in enzymatic antioxidants (glutathione peroxidase) caused by the high quantities of intracellular reactive oxygen species (ROS) produced by phytol ultimately results in cell death by causing cell cycle arrest and severe DNA damage [61]. Researchers have also shown that phytol decreases biofilm formation, lessens flagella movement, and suppresses the generation of pyocyanin, adopting *Pseudomonas aeruginosa* as an experimental model [62]. Squalene is a fundamental metabolite in the sterol synthesis pathway [63] and results from squalene-enriched diets are encouraging, highlighting both quenching activity and augmentation of antioxidant systems brought on against ROS and RNS burst [64]. Neophytadiene, a diterpene, has anticonvulsant and anxiolytic-like implications, and the GABAergic system is likely involved [65]. The plant exposes phytosterols which have numerous activity. In the brine shrimp lethality testing, plants possessing beta sitesterol exhibit antinociceptive [66], anxiolytic, antibacterial and antifungal activity with no revealing toxicity [67,68]. In rodents, it has anxiolytic and sedative effects [69], although there are no reports of the same effects in humans. Additionally, Stigmasterol prevents skin cancer by raising lipid peroxide levels and causing DNA damage [70] and suppresses the growth of cholangiocarcinoma by downregulating VEGFR-2 and TNF-alpha [71] and researchers discovered that it can successfully prevent the aggregation of ovarian cancer cells as tumor-forming ovarian cancer cells failed to develop and dispersed [72]. Besides antioxidant properties [73], in leukemia, vitamin E or alpha-tocopherol has been shown to reduce the suppression of cytotoxic T-cell activation by myeloid-derived suppressor cells and to stimulate dendritic cells' antigen-specific immunological responses [74,75]. All the evidence found in the literature supports that the plant will be a good fit for various pharmacological potentiality (Fig. 2).

3.3. Phytochemicals from GC–MS/MS analysis of DCM extract of G. cyanocarpa

5 compounds were found in the DCM fraction of the plant extract which is shown in Table 3 and Fig. 3. Among them most abundant phytochemicals are Pyrazol-5-amine, 3-methyl-1,4-diphenyl- (42.26%), Arborinine (20.89%) and Dodecanoic acid, 1,2,3-propanetriyl ester

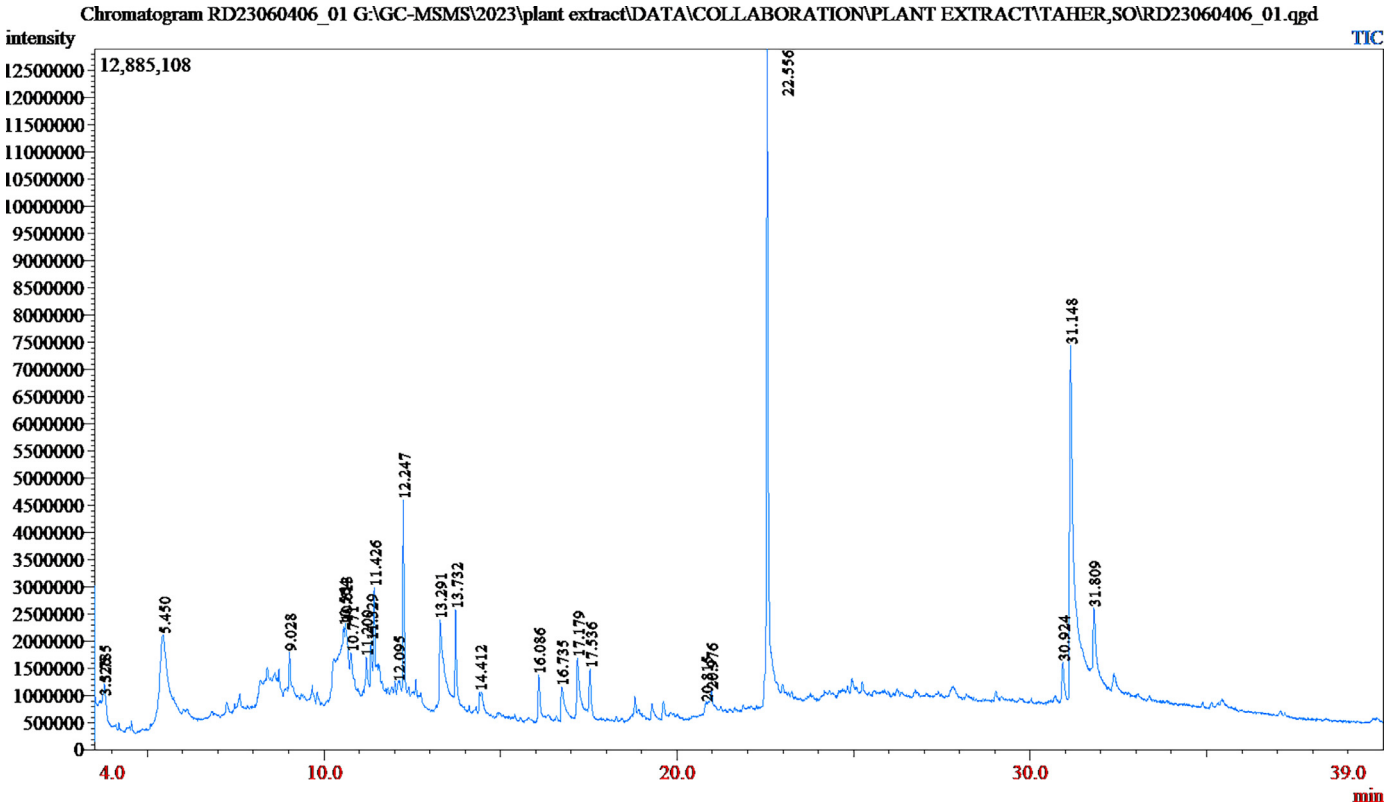

Fig. 1. GC–MS chromatograms of *G. cyanocarpa* extracted with ethanol solvent.

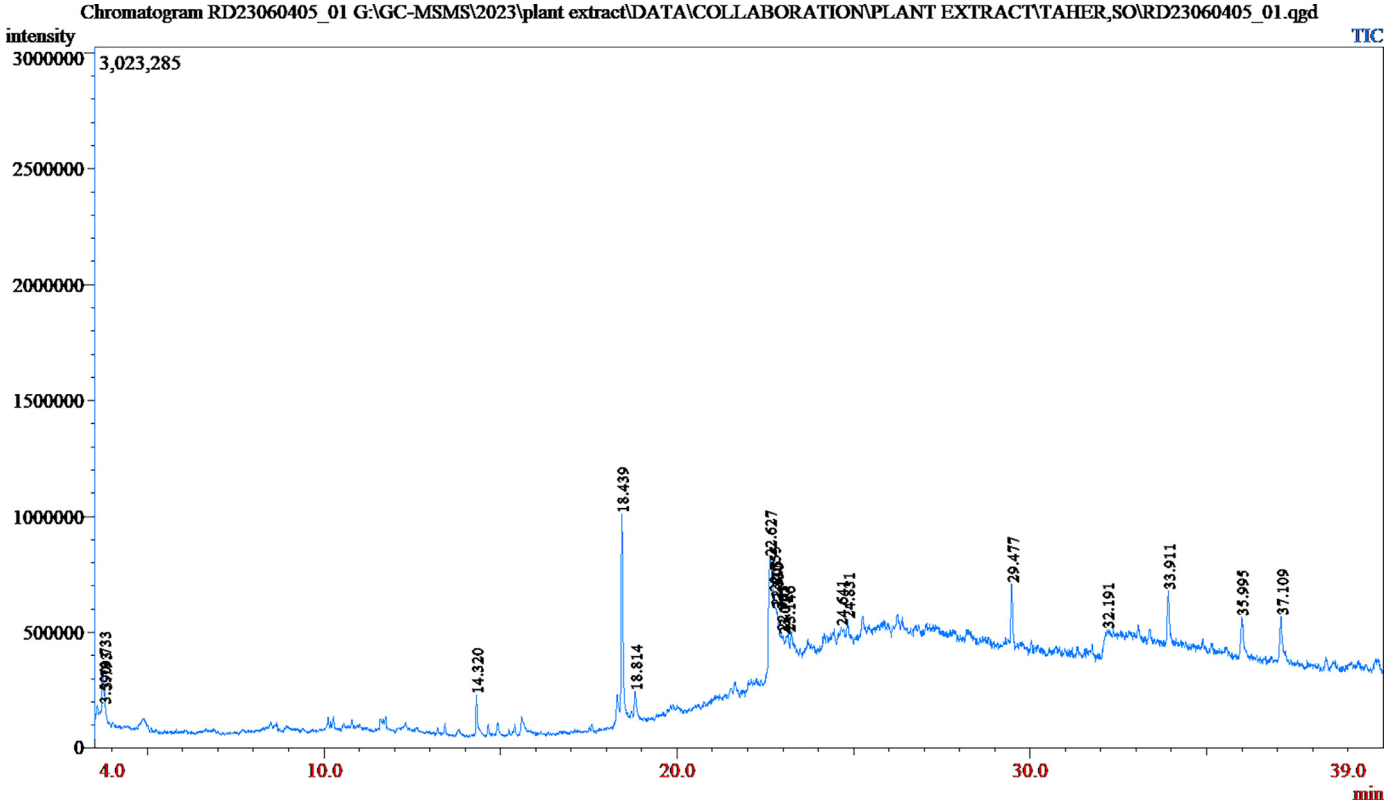

Fig. 2. GC–MS chromatograms of *G. cyanocarpa* extracted with methanol solvent.

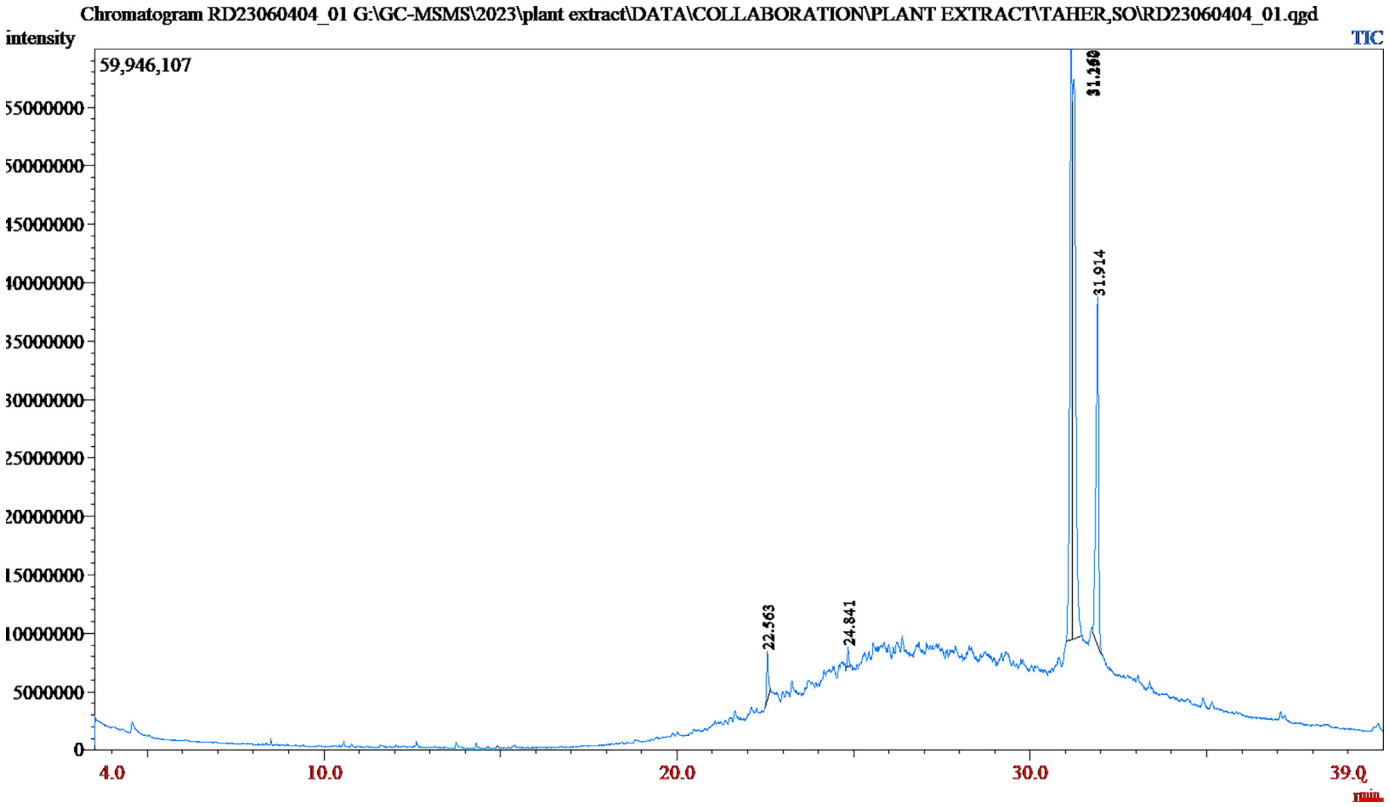

Fig. 3. GC–MS chromatograms of *G. cyanocarpa* extracted with DCM solvent.

(34.38%). All of these compounds are availabe in other fractions of the plant in high concentration, which indicates that the plant possesses these compounds at a significant amount. Arborinine, an alkaloid, has been found in the plant's ethanol extract. Prior research has demonstrated that arborinine is lethal to the cancer cell lines HeLa, MCF-7, and A431 with IC50 values of 1.84, 11.74, and 12.95 M, respectively [76]. Scholars have identified Arborinine from the same genus *Glycosmic parva* and found it's functionality in upregulated caspase-3, −7, and downregulated Bcl2-L1 of HeLa cell, which suggests it's action against cervical cancer [77]. Another study suggests that can act as a powerful electron-transport inhibitor [78] which may be a useful tool in the ongoing development of a new class of herbicide. Trilaurin, also known as dodecanoic acid, 1,2,3-propanetriyl ester, has demonstrated its application in occlusive or viscosity-increasing agents as well as skin conditioning agents [79]. Pentatriacontane (C-35) an alkane, was also found in minute amounts. Surprisingly our investigation revealed the existence of (Z)-9-Octadecenamide, also known as oleamide, in the fractions. This substance has been associated with neurological activity; indeed, it is a lipid-like component that aids in sleep [80] and helps in neurological development and pain attenuation activities [81]. There is evidence that claims it is a potential algaecidal and fungicidal agent [82].

3.4. Phytochemicals from GC–MS/MS analysis of petroleum ether extract of G. cyanocarpa

Pertoleum ether fraction showed 24 compounds in GC–MS/MS analysis. Many of the compounds found in other fractions also. Dodecane, tetradecane, hexadecane, eicosane and heneicosane are all *n*-alkanes, which are straight-chain hydrocarbons and cyclotetradecane is a cycloalkane, which is a cyclic hydrocarbon. In recombinant *Escherichia coli*, dodecane has been employed as an oxygen vector to boost fumarase activity by raising ATP levels and altering the energy metabolic route [83]. Tetradecane has been identified as a bioactive component of *Abelmoschas moschatus* flower extract, which has antioxidant, antimicrobial, flavor, hypocholesterolemic and larvicidal activities [84]. Hexadecane, also known as cetane, is a major component of diesel fuel and has been used as a model compound for studying the combustion and pyrolysis of alkanes [85]. *Candida albicans*, a fungus that can cause mucosal or systemic infections, has been shown to have antibiofilm capability by eicosane. 60% of the *C. albicans* biofilm may be inhibited by eicosane at a concentration of 100 g/mL without affecting growth. Additionally, it decreased the adherence and attachment of yeast cells to the medium and lowered the genes necessary for hyphal development and the production of biofilms [86]. Eicosane has been reported to have antitumor activity against human breast cancer cells by inducing apoptosis and inhibiting angiogenesis. Cyclotetradecane has been used as a building block for the synthesis of macrocyclic compounds with potential biological applications, such as antibacterial, antifungal, antiviral and anticancer agents. Phenolic compound phenol, 2,4-bis(1,1-dimethylethyl)-, phosphite (3:1) might have the anti-enterococcal and antioxidant activity [87].

3.5. FTIR analysis of crude extracts

In the present study, the FTIR spectroscopy was used to identify the functional groups based on the peak values in the IR present in the crude extracts. Plant powders were subjected to FTIR analysis and the functional groups of the components were separated based on its peaks. The results obtained indicated the presence of following functional groups viz., free alcohol; inter- and intra-molecular bonded alcohol, alkane, aromatic compounds, imine or oxime or ketone or alkene, phenol and amine stretching (Table 4).

Based on the peak values in the IR radiation band, the FTIR spectrum was utilized to determine the functional groups of the active components present in the extract. Based on the extract's peak ratio, the constituents' functional groups have been separated after it was run through the FTIR. The existence of N–H, O–H, C=C, C–H, C–O, C–N, C–F, S–O functional groups and benzene derivatives was verified by the FTIR analysis re-

Table 4
Phytochemical compounds of G. cyanocarpa extracted with Petroleum ether solvent.

Serial No.	Retention time (min)	Area (%)	Compound name
1	3.53	0.76	Cyclohexane, 1,2,3-trimethyl-, (1.alpha.,2.beta.,3.alpha.)-
2	3.57	0.47	1,1,4-Trimethylcyclohexane
3	3.635	0.23	*trans*-2,4-Dimethylthiane, *S,S*-dioxide
4	3.708	0.34	Cyclohexane, 1,2,4-trimethyl-
5	3.765	1.43	Ethylbenzene
6	3.865	10.08	3,5-Octadiyne
7	4.139	1.69	*o*-Xylene
8	4.185	1.01	Nonane
9	4.98	0.22	Benzene, 1-ethyl-3-methyl-
10	5.4	0.27	Benzene, 1,2,4-trimethyl-
11	7.7	0.4	Dodecane
12	9.738	1.7	Tetradecane
13	11.58	1.82	Hexadecane
14	11.671	0.32	Cyclotetradecane
15	12.608	0.46	Eicosane
16	13.82	1.22	Heneicosane
17	14.013	0.29	Cyclotetradecane
18	16.968	0.24	Decane, 4-cyclohexyl-
19	22.552	0.37	9-Octadecenamide, (Z)-
20	24.824	0.25	2-Methylhexacosane
21	29.285	0.62	Tetratetracontane
22	29.482	0.21	Squalene
23	33.903	0.28	Vitamin E
24	38.367	0.46	Phenol, 2,4-bis(1,1-dimethylethyl)-, phosphite (3:1)

Table 5
FTIR peak assignment table of plant extracts compared with standard chart.

Absorption (cm^{-1})	Appearance	Functional group	Compound class	Comments
3724.70		Unknown	Unknown	
3505.77	Strong, broad	O–H stretching	Alcohol	
2928.07	Strong, broad	O–H stretching	Carboxylic acid	Usually centered on 3000 cm^{-1}
	Weak, broad	O–H stretching	Alcohol	Intra-molecular bonded
	Strong, broad	N–H stretching	Amine salt	
	Medium	C–H stretching	Alkane	
2378.33	Strong	O=C=O stretching	Carbon dioxide	
1634.74	Strong	C=O stretching	Conjugated acid	Dimer
	Strong	C=O stretching	Conjugated aldehyde	
	Medium	C=C stretching	Alkene	Disubstituted (*cis*)
	Strong	C=C stretching	Alkene	Monosubstituted
1531.55	Strong	N–O stretching	Nitro compound	
1457.28		Unknown		
1384.95	Medium	O–H bending	Alcohol	
	Strong	S=O stretching	Sulfate	
	Strong	S=O stretching	Sulfonyl chloride	
	Strong	C–F stretching	Fluoro compound	
	Medium	O–H bending	Phenol	
1269.22	Strong	C–N stretching	Aromatic amine	
	Strong	C–O stretching	Aromatic ester	
	Strong	C–O stretching	Alkyl aryl ether	
1080.18	Strong	C–O stretching	Primary alcohol	
693.44	Strong	C=C bending	Alkene	Disubstituted (*cis*)
			Benzene derivative	

sults in the Table 5. It has been demonstrated that FTIR spectroscopy is a sensitive and dependable technique for determining the biomolecule composition.

3.6. Molecular docking analysis

Table 6 represents that compound **18** exhibited the strongest affinity against EGFR with a binding affinity score of −9.7 kcal/mol followed by Compound 19 (−9.2 kcal/mol). Also, Compound 67 manifested a promising binding score of −8.3 kcal/mol while compounds **22** and **45** exhibited prominent binding affinities against the EGFR with the values of −8.2 kcal/mol, where compounds **8** and **46** scored −8 kcal/mol. In comparison, the standard Lapatinib manifested a binding affinity of −9.8 kcal/mol. Besides, compounds **7**, **14**, **15**, **17**, **23**, **34**, and **42** conveyed binding affinity against this receptor between a range of −7.1 to −7.9 kcal/mol (Figs. 4 and 5).

Fig. 6(a) shows compound **18** bound with 9 amino acids of EGFR including LEU 718, VAL 726, ALA 743, LYS 745, MET 766, LEU 777, LEU 788, CYS 797, and LEU 844, where compound **19** bonded with LEU 718, VAL 726, ALA 743, LYS 745, MET 766, LEU 777, LEU 785, CYS 797, and LEU 844. However, the standard Lapatinib exhibited interaction with the 14 amino acids of EGFR including LEU 718, VAL 726, ALA 743, LYS 745, MET 766, ARG 776, LEU 777, THR 790, GLN 791, MET 793, ASP 800, LEU 844, ASP 755, and PHE 856.

Shown in Fig. 6(b), for DHFR, the highest binding affinity (−8.5 kcal/mol) was observed for compounds **7** and **18**. Also, compounds **9** and **14** conveyed notable affinity against DHFR with scores of −8.1 and −7.9 kcal/mol compared to the standard Ciprofloxacin score of −8.2 kcal/mol. Also, the binding affinities lower than −7.1 against DHFR was observed for **8**, **17**, **22**, **23**, and **45** (Fig. 7).

However, an interaction between compound **7** with 7 amino acids of DHFR including PHE 88, LYS 98, LEU 99, GLN 102, PRO 103,

Table 6
The in silico docking scores for the identified chemicals from *G. cyanocarpa* based on molecular interactions measured with regard to binding affinity (kcal/mol) and the standard drugs Cirprofloxacin and Lapatinib, during the interaction with EGFR (1XKK) and DHFR (4M6J) for assessing the cytotoxic and antimicrobial properties respectively.

Fraction	Compound num.	Compound name	Pubchem CID	Binding affinities	
				EGFR (1XKK)	DHFR (4M6J)
MeOH	1	3,3-Dimethoxy-2-butanone	140,871	−4.7	−4
MeOH	2	1,3-Dioxolane-4-methanol, 2-ethyl-	546,241	−5.1	−4.5
MeOH	3	Neophytadiene	10,446	−6.8	−5.4
MeOH	4	Phytol	5,280,435	−6.5	−5.6
MeOH	5	2,8,9-Trioxa-5-aza-1-silabicyclo(3.3.3)undecane, 1-methoxy-	199,318	−2	−1.8
	6	9-Octadecenamide, (*Z*)-	5,283,387	−6.4	−5.5
Me/EtOH/PEther					
MeOH	7	18,19-Secoyohimban-19-oic acid, 16,17,20,21-tetradehydro-16-(hydroxymethyl)-, methyl ester, (15.beta.,16*E*)-	5,365,002	−7.5	−8.5
MeOH	8	(7*E*,11*E*)-1-(2-Hydroxyethyl)-2,3-epoxy-1,7,11-trimethylcyclotetradecadiene	101,618,282	−8	−7.4
MeOH	9	2-(3-Hydroxy-propyl)-cyclohexanol	566,185	−5.4	−5.1
MeOH	10	Geranyl ethyl ether 1	5,365,847	−6.1	−5.2
MeOH	11	Chloromethyl nonanoate	525,230	−5.5	−4.9
MeOH	12	2-Hexen-1-ol, 2-ethyl-	5,362,614	−4.9	−4.5
MeOH	13	Chloroacetic acid, 4-hexadecyl ester	543,598	−6	−5.3
MeOH	14	Tetrapentacontane, 1,54-dibromo-	545,963	−7.1	−7.9
MeOH	15	Squalene	638,072	−7.8	−7.3
MeOH	16	(1-Cyanocyclohexyl) carbamate	565,518	−5.9	−5.7
MeOH/P.ether	17	Vitamin E	14,985	−7.9	−7.8
MeOH	18	Stigmasterol	5,280,794	−9.7	−8.5
MeOH	19	.beta.-Sitosterol	222,284	−9.2	−8.1
DCM	20	Pentatriacontane	12,413	−5.8	−5.2
DCM	21	Dodecanoic acid, 1,2,3-propanetriyl ester	10,851	−6	−6.1
DCM	22	Pyrazol-5-amine, 3-methyl-1,4-diphenyl-	619,285	−8.2	−7.3
DCM/EOH	23	Arborinine	5,281,832	−7.9	−7.8
EtOH	24	1,6-Dideoxy-l-mannitol	542,304	−5.4	−4.6
EtOH	25	Butanoic acid, ethyl ester	7762	−4.2	−3.6
EtOH	26	Glycerin	753	−5.6	−5.2
EtOH	27	2-Methoxy-4-vinylphenol	332	−6.2	−5.4
EtOH	28	cis-3-Hexenyllactate	5,364,231	−5.8	−5.9
EtOH	29	2-Oxabicyclo[2.2.2]octan-6-ol, 1,3,3-trimethyl-, acetate	175,002	−5.4	−5.1
EtOH	30	.beta.-d-Glucopyranose, 1,6-anhydro-	2,724,705	−4.4	−3.9
EtOH	31	Ethanol, 2-[2-(2-methoxyethoxy)ethoxy]-	8178	−5.7	−5.5
EtOH	32	3′,5′-Dimethoxyacetophenone	95,997	−6.4	−6.2
EtOH	33	3-Hydroxy-7,8-dihydro-.beta.-ionol	519,379	−5.3	−4.9
EtOH	34	6-Methyl-5-octen-2-one	5,363,290	−7.2	−6.6
EtOH	35	4-(6,6-Dimethyl-2-methylenecyclohex-3-enylidene) pentan-2-ol	5,370,028	−6	−5.5
EtOH	36	4-((1*E*)-3-Hydroxy-1-propenyl)-2-methoxyphenol	1,549,095	−6.4	−5.8
EtOH	37	6-Hydroxy-4,4,7a-trimethyl-5,6,7,7a-tetrahydrobenzofuran-2(4*H*)-one	14,334	−4.9	−4.5
EtOH	38	Hexaethylene glycol dimethyl ether	70,621	−6.9	−6.2
EtOH	39	Phthalic acid, butyl undecyl ester	6,423,450	−6.2	−5.6
EtOH	40	trans-Sinapyl alcohol	5,280,507	−6.7	−5.1
EtOH	41	Nonadecanoic acid	12,591	−1.1	−0.9
EtOH	42	1,4-Cyclohexanediol, (Z)-, TMS derivative	554,465	−7.2	−6.8
EtOH	43	Benzyl .beta.-d-glucoside	188,977	−6.5	−5.5
EtOH	44	2-Hexanone, 6-phenyl-	26,530	−6.4	−5.3
EtOH	45	1,2-Benzenediol,4-(2-aminopropyl)-	521,660	−8.2	−7.3
EtOH	46	Pyrazol-5-amine, 3-methyl-1,4-diphenyl-	619,285	−8	−8.2
P. Ether	47	Cyclohexane, 1,2,3-trimethyl-, (1.alpha.,2.beta.,3.alpha.)-	6,432,164	−5.6	−5.1
P. Ether	48	1,1,4-Trimethylcyclohexane	35,365	−5.3	−4.9
P. Ether	49	*trans*-2,4-Dimethylthiane, *S,S*-dioxide(cis)	543,891	−5	−5
P. Ether	50	Cyclohexane, 1,2,4-trimethyl-	91,517	−5.5	−4.9
P. Ether	51	Ethylbenzene	7500	−5.1	−4.7
P. Ether	52	3,5-Octadiyne	140,066	−4.5	−4.1
P. Ether	53	*o*-Xylene	7237	−5.2	−4.8
P. Ether	54	Nonane	8141	−4.7	−4.3
P. Ether	55	Benzene, 1-ethyl-3-methyl-	12,100	−5.7	−5.1
P. Ether	56	Benzene, 1,2,4-trimethyl-	7247	−5.8	−5.3
P. Ether	57	Dodecane	8182	−5.3	−4.4
P. Ether	58	Tetradecane	12,389	−5.7	−4.3
P. Ether	59	Hexadecane	11,006	−6	−4.4
P. Ether	60	Cyclotetradecane	67,524	−6.7	−6.7
P. Ether	61	Eicosane	8222	−5.9	−5
P. Ether	62	Heneicosane	12,403	−5.8	−4.8
P. Ether	63	Decane, 4-cyclohexyl-	524,439	−6.4	−5.7
P. Ether	64	Tetratetracontane	23,494	−5.9	−5.5
P. Ether	65	2-Methylhexacosane	150,931	−6.3	−5.4

(*continued on next page*)

Table 6 (*continued*)

Fraction	Compound num.	Compound name	Pubchem CID	Binding affinities	
				EGFR (1XKK)	DHFR (4M6J)
P. Ether	66	Squalene	638,072	−7.8	−6.4
P. Ether	67	Phenol, 2,4-bis(1,1-dimethylethyl)-, phosphite (3:1)	91,601	−8.3	−5.8
Standards		Cirprofloxacin	2764	–	−8.2
		Lapatinib	208,908	−9.8	–

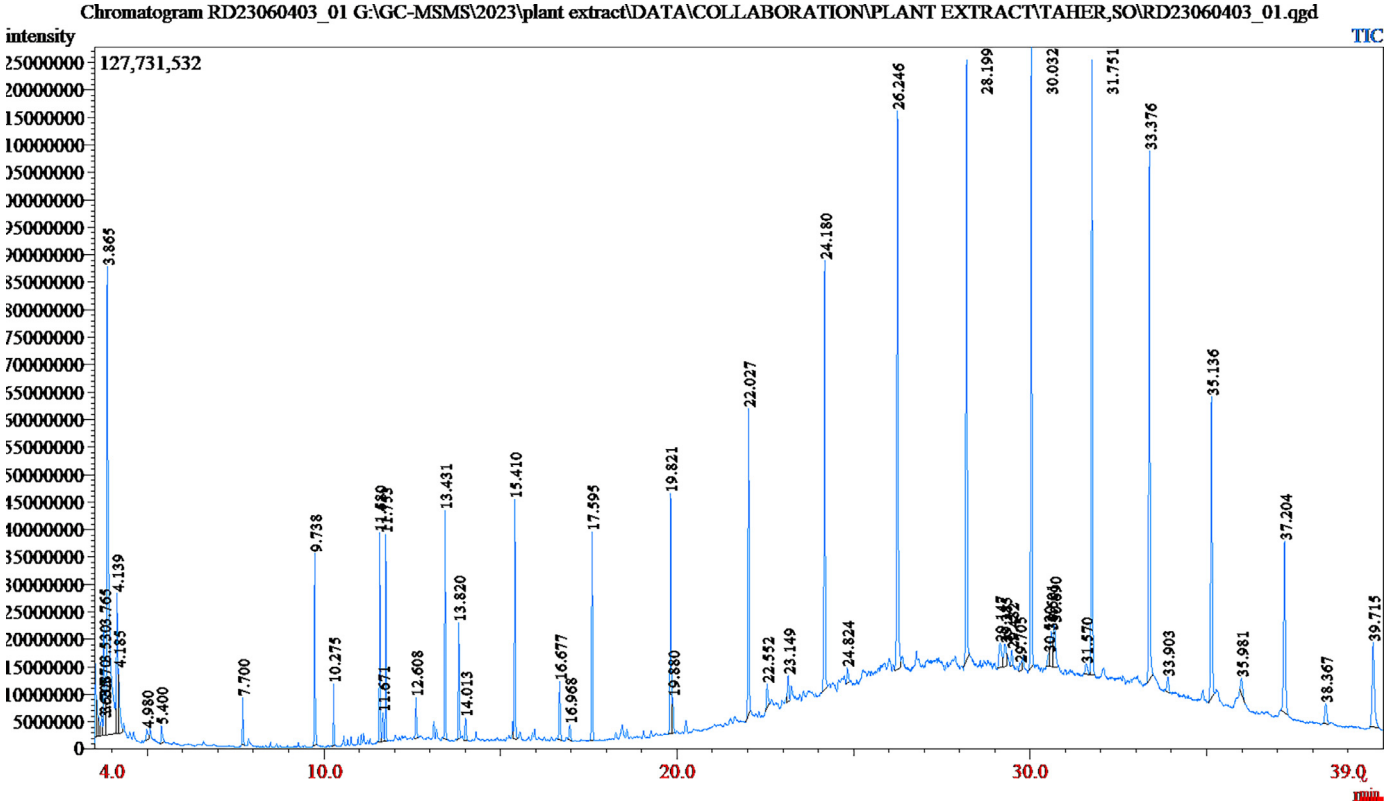

Fig. 4. GC–MS/MS chromatograms of *G. cyanocarpa* extracted with petroleum ether solvent.

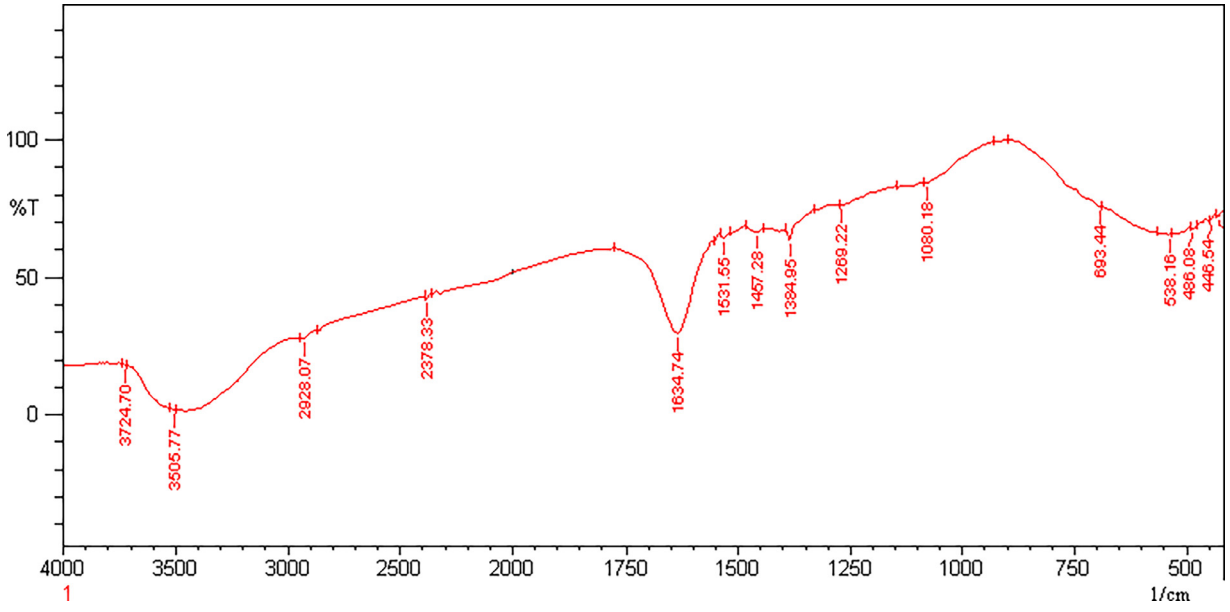

Fig. 5. FTIR chromatogram of methanolic extract (crude) of *G. cyanocarpa*.

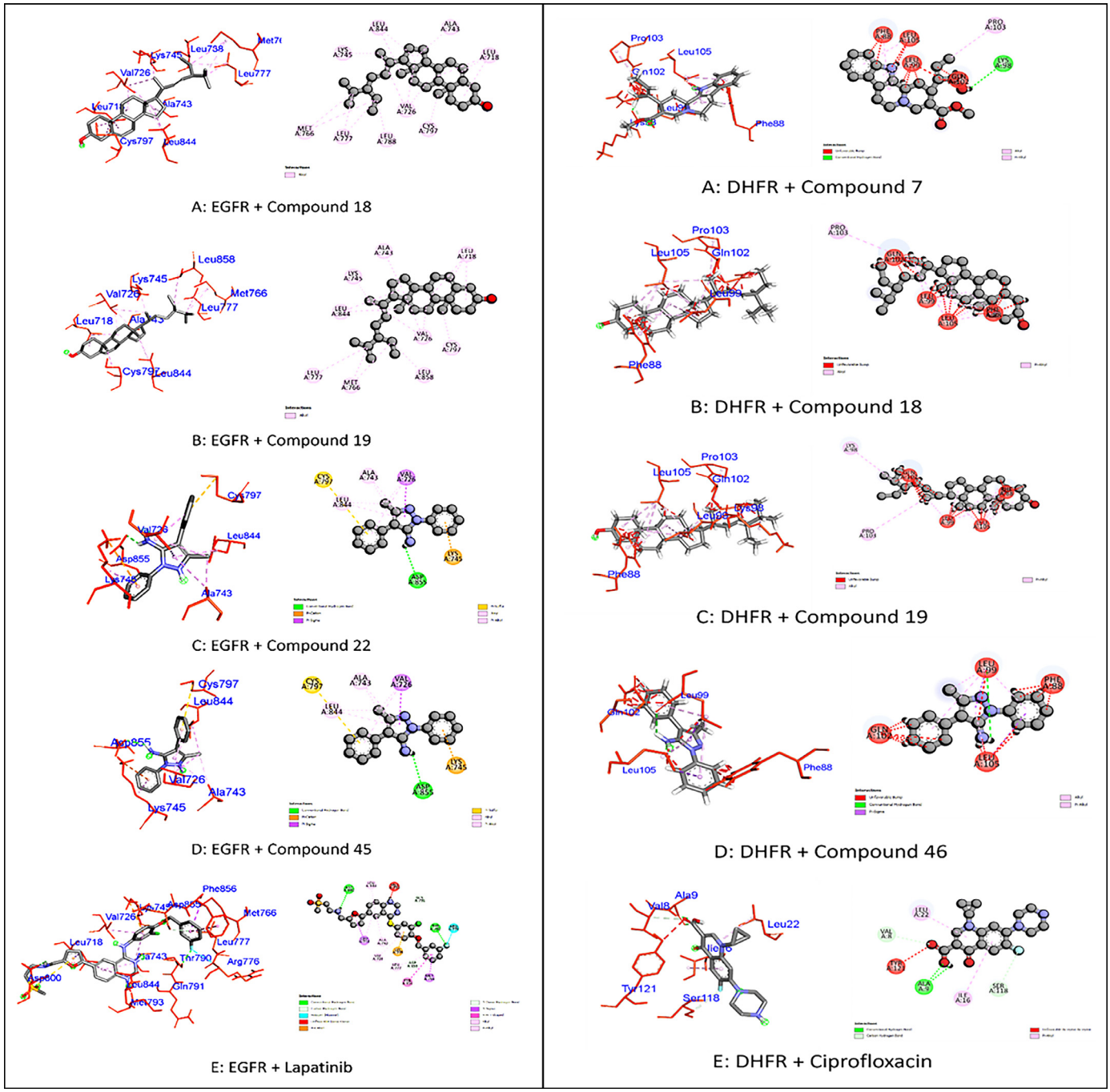

Fig. 6. Visual illustration of the molecular interactions of the most prominent phytocompounds with the (a) EGFR (PDB ID: 1XKK) enzyme with 3D visualization (Compound **18** = A, Compound **19** = B, Compound **22** = C, Compound **45** = D, Compound **67** = E, Standard Lapatinib = F.) and (b) DHFR (PDB ID: 4M6J) enzyme with 3D visualization (Compound **7** = A, Compound **18** = B, Compound **19** = C, Compound **46** = D, Standard Ciprofloxacin = E.).

and LEU 105 was observed in Figure-. Figure- also showed that compound **18** interacted with the same amino acids except LYS 98 which was replaced by PHE 88. The interacted amino acids for the standard Ciprofloxacin were VAL 8, ALA 9, ILE 16, LEU 22, SE18, and TYR 121.

EGFR regulates cell functions such as proliferation and death. Ligand interaction, such as EGF binding, causes conformational changes that result in tyrosine phosphorylation in the C-terminal domain. This activates downstream pathways including MAPK, PI3K/AKT, and STAT3/STAT5, which prevent apoptosis and promote cancer-related activities [88]. Our findings show that some of the compounds of Table 5 exhibited a strong affinity towards DHFR. Especially the highest active two compounds namely **18** and **19** strongly bound with the nine amino acids of DHFR through alkyl bonds and showed binding affinity scores of −9.7 and −9.2 kcal/mol, respectively (Fig. 6 and Table 6). However, the standard interacted with fourteen amino acids by two hydrogen bonds, four alkyl bonds, five pi bonds, and other three bonds.

DHFR is a protein that plays a crucial role in DNA synthesis during bacterial cell growth. In the folate pathway, the enzyme dihydrofolate reductase (DHFR) transforms dihydrofolate acid (DHF) into tetrahydrofolic acid (THF)). The production of amino acids and nucleic acids, which are necessary for cell development and proliferation, depends on THF. Damage to the folate system causes unchecked cell growth, which in turn causes a variety of malignancies [89].

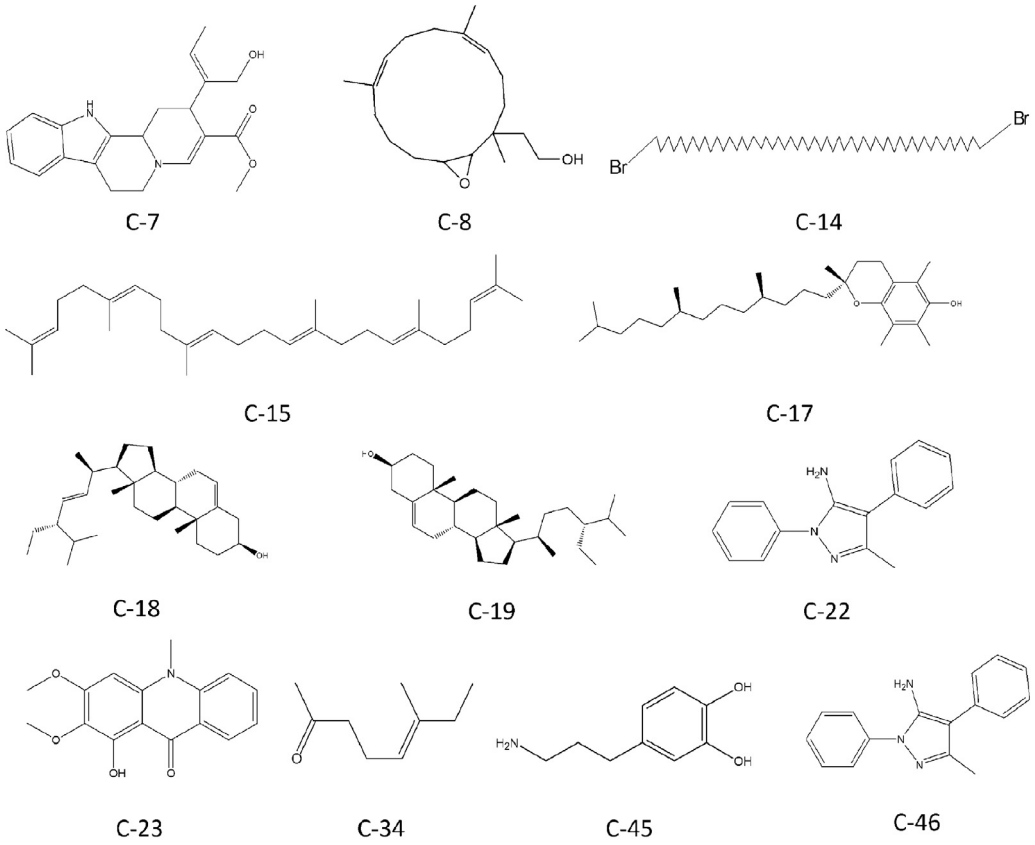

Fig. 7. Structure of compounds which illustrated high affinity towards EGFR and DGFR.

Table 7
ADME/T studies of best bonded compounds from leaves of *Glycosmic cyanocarpa* against DHFR and EGFR.

Compounds	H-bond Donor	H-bond acceptors	Lipophilicity - log P (o/w)	GI absorption	AMES toxicity	Hepatotoxicity	Drug likeliness	Bioavilability score
7	2	3	2.67	High	No	Yes	No; 1 violation: MW > 350	0.55
8	1	2	3.98	High	No	No	No; 1 violation: XLOGP3 > 3.5	0.55
14	0	0	20.48	Low	No	No	No; 3 violations: MW > 350, Rotors > 7, XLOGP3 > 3.5	0.17
17	1	2	8.27	Low	No	No	No; 3 violations: MW > 350, Rotors > 7, XLOGP3 > 3.5	0.55
18	1	1	6.97	Low	No	No	No; 2 violations: MW > 350, XLOGP3 > 3.5	0.55
19	1	1	7.19	Low	No	No	No; 2 violations: MW > 350, XLOGP3 > 3.5	0.55
22	1	1	3.13	High	Yes	Yes	No; 2 violations: MW < 250, XLOGP3 > 3.5	0.55
23	1	4	2.4	High	Yes	No	Yes	0.55
45	3	3	0.78	High	Yes	Yes	No; 1 violation: MW < 250	0.55
46	1	1	3.13	High	Yes	Yes	No; 2 violations: MW < 250, XLOGP3 > 3.5	0.55
67	0	3	11.42	Low	No	Yes	No; 2 violations: MW > 500, MLOGP > 4.15	0.17

However, several compounds identified in this article exhibited promising activity against DHFR. Especially compounds **7** and **18** surpassed the standard by the mean of lowest binding affinity. It appears that the binding strength of the ligands increases with decreasing binding energy [88,90]. Compound **7** unfavorably bumped with four amino acids, as well as bound by a single hydrogen and alkyl bond with two amino acids of DHFR. However, four unfavorable bumps and a single alkyl bond were observed for compound **18** with five amino acids Fig. 6(b). In contrast, Ciprofloxacin illustrates two hydrogen bonds (one conventional, one carbon-hydrogen bond), two alkyl bonds, and one unfavorable acceptor bond with six amino acids of DHFR.

Table 7 displays the outcomes of ADMET analyses and predictions for drug likeliness concerning specific compounds with notable affinities for the mentioned receptors. Except for compound **23**, all other compounds violated Lipinski's rule of five, suggesting that compound **23** holds a higher potential for oral bioavailability. Compounds **7**, **8**, and **45** breached one rule, while compounds **18**, **19**, **22**, **46**, and **67** violated two rules, and compounds **14** and **1** also broke two rules, indicating a descending order of oral bioavailability. However, with the exception of compounds **14** and **67**, all compounds in Table 6 exhibited a substantial bioavailability score of 0.55, while the latter two compounds showed a lower score of 0.17. Compounds **7**, **22**, **45**, **46**, and **67** ex-

hibit a propensity for inducing hepatotoxicity, whereas compounds **22**, **23**, **45**, and **46** display a strong affinity for AMES toxicity, indicating potential carcinogenic properties.

Our study indicates that some of the compounds identified from leaves extract of *G. Cyanocarpa* could become probable hits against the abovementioned pathways. Moreover, further elaborated research should be done to find out the pharmacological and toxicological properties of these compounds.

4. Conclusions

Phytochemical screening of the plant *G. cyanocarpa* through gas chromatography mass spectrometry has shown a lot of potential compound(s) useful for various biochemical impact and medicinal uses. In computer aided molecular docking study, some of our compounds exhibited antimicrobial and cytotoxic potentiality which is supported by previously reported information about the compounds. Previous study showed that the plant *G. cyanocarpa* showed significant cytotoxic activity brine shrimp lethality bioassay [91]. Scholars also identified the antifungal activity of the plant extracts [92].

Sitosterol is a common plant sterol that has various pharmacological and nutritional benefits, such as lowering cholesterol levels, modulating immune responses, and preventing oxidative stress. It also has some antimicrobial properties against bacteria, fungi and viruses. It is noted that derivatized sterols can play a significant role in determining of epidermal growth factor receptor–overexpressing cancer cells by raman imaging [93].

The organisms *Staphylococcus aureus, Escher P. aeruginosa, B. subtilis, Candida albicans*, the fungus *Aspergillus niger*, and *Trichophyton mentagrophytes*, among others, have all been shown to be inhibited by sitosterol. The mechanisms of action may involve disruption of cell membrane integrity, interference with cell wall synthesis, inhibition of enzyme activity, or modulation of gene expression. Additionally, hepatitis C virus (HCV), human immunodeficiency virus type 1 (HIV-1), and herpes simplex virus type 1 (HSV-1) are all susceptible to the antiviral effects of sitosterol. The mechanisms of action may involve inhibition of viral entry, replication, or assembly [94]. According to a research article, some 1-(4-methoxybenzyl)-3-cyclopropyl-1*H*-pyrazol-5-amine products have been developed and evaluated for the effectiveness of their antimicrobial properties against standard strains of Gram-positive and Gram-negative bacteria by using standard disc diffusion method. As compared to the common antibiotic ciprofloxacin, the findings revealed that several of the molecules had moderate to strong antibacterial activity. The authors hypothesized that the inclusion of cyclopropyl group and substituents with electron donations on the phenyl ring may increase the antibacterial activity of pyrazol-5-amine derivatives [95]. Another research suggests that the presence of cyclopropyl group and electron-donating substituents on the phenyl ring may enhance the anticancer activity of the pyrazol-5-amine derivatives [96].

In tests with the following bacteria: *Staphylococcus aureus, E. coli*, *P. aeruginosa*, *B. subtilis, Candida albicans*, and *Aspergillus niger*, previously isolated stigmasterol demonstrated the strongest antimicrobial effect, with the smallest inhibitory values ranging from 1.95 to 125 g/mL. Using the MTT assay, the cytotoxicity investigations revealed that the separated stigmasterol has significant potential against the Hela (mammalian cancer) cell line, with an IC50 value of 9.8 g/mL [97]. A study that reported that 1,2-benzenediol,4-(2-aminopropyl)-18,19-secoyohimban-19-oic acid was one of the compounds present in the methanol extract of *Chlorococcum humicola*, a green alga that exhibited anticancer and antioxidant activities [98]. Finally, it can be inferred that, the plant *G. cyanocarpa* has the potentiality to act as a natural source to develop anticancer and antimicrobial drug if further research is done properly.

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Institutional review board statement

Not applicable.

Informed consent statement

Not applicable.

Data availability statement

The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

CRediT authorship contribution statement

Mohammad Abdullah TAHER: Conceptualization, Methodology, Formal analysis, Investigation, Supervision, Writing – original draft, Writing – review & editing. **Aysha Akter LABONI:** Formal analysis, Investigation. **Suriya Akter SHOMPA:** Investigation. **Md Mashiur RAHMAN:** Writing – original draft. **Mohammad Mahmudul HASAN:** Investigation, Writing – original draft. **Hasin HASNAT:** Writing – review & editing. **Mala KHAN:** Conceptualization, Supervision.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.cjac.2023.100336.

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