The Apoptosis-inducing Antiproliferative Activity and Quantitative Phytochemical Profiling of Polyphenol-rich Part of *Leea Aequata* L. Leaves

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Abstract

Aim/Objectives: Oxidative stress (OS) caused by the accumulation of free radicals in the body triggers many life-threatening diseases, including cancer. Plants are the rich source of safe and effective medicinal compounds like antioxidants that can prevent OS by scavenging free radicals. The aim of this study was to explore the chemical compositions of the methanolic extract of *Leea aequata* L. leaves possessed the highest antioxidant and cytotoxic activities.

Methods and Materials: The *Leea aequata* leaves (LAL) was extracted with 80% methanol and the crude methanolic extract of LAL (CME) was successively fractionated with petroleum ether, chloroform, ethyl acetate and finally with water to get petroleum ether (PEF), chloroform (CHF), ethyl acetate (EAF) and aqueous (AQF) fraction, respectively. The antioxidant activity was evaluated by the *in vitro* standard spectrophotometric methods. The MTT colorimetric method was used to measure cytotoxic activity. Apoptosis was measured using fluorescein annexin V-FITC/PI staining method. GC-MS was used to investigate phytochemical compositions.

Results: Among the fractions, the highest phenolic and flavonoid-rich fraction was EAF, which contained 267.05 ± 8.53 mg of gallic acid equivalent (GAE) and 152.98 ± 1.96 mg of catechin equivalent (CAE)/gm of dried extract, respectively. The EAF was found to be the highest antioxidant and reducing power activities. Also, EAF had the strongest free radical scavenging activity with IC_{50} values of 5.11, 36.54 and 38.96 $\mu\text{g/mL}$ for DPPH, hydroxyl and lipid peroxide free radicals, respectively. Moreover, EAF significantly reduced the HeLa cell proliferation and it was due to the induction of apoptosis of HeLa cells. Furthermore, GC-MS data revealed that EAF contained 17 compounds. Methyl esters of long chain fatty acid were the major chemical constituents. Interestingly, hexadecanoic acid, methyl ester; 9-octadecenoic acid (Z)-, methyl ester; 9,12-octadecadienoic acid, methyl ester (Z, Z) and phenol, 2,4-bis(1,1-dimethylethyl) are known to be antioxidant and cytotoxic. Antioxidant and anti-cancer activity were significantly correlated with the total content of phenolics and flavonoids, suggest that the fractions could be the good source of antioxidant and anti-cancer agents.

Conclusion: The EAF of *Leea aequata* has strong polyphenolic content compared to the other fractions. The cytotoxic and antioxidant activity of *Leea aequata* were due to the presence of different bioactive compounds.

Background

Living organisms continuously produce reactive oxygen species (ROS) during normal cellular metabolism [1]. At a low or moderate concentration, ROS can play some vital roles in that living organisms like synthesize some essential cellular structures, make stronger defense systems to protect from pathogens, act as a messenger of cell-to-cell, and is essential for the normal neural activity [2]. On the other hand, at a higher concentration, ROS can demolish and modify the many essential macromolecules like DNA, RNA, lipids, and proteins. Most potent reactive molecules can be generated by these modifications [3].

The lack of balance between generation and scavenging of free radicals by antioxidant is termed as oxidative stress (OS) [1]. Diet, smoking, concurrent disease status (e.g., diabetes), alcohol consumption, pollution, genetic composition, age, menopausal status, gender etc. are the major risk factors of OS development [4]. OS is responsible for many life-threatening conditions, including cancer and many other diseases [1]. According to the WHO (2018), globally cancer is the second leading cause of death which is responsible for 1 out of 6 deaths [5]. Around 9.6 million deaths were caused by cancer in 2018 [6]. The prevalence of cancer is increasing day by day. Current treatment options for cancer have some limitations. These drugs are highly toxic for both normal and cancer cells and thus cause health related other serious complications [7]. That's why searching a novel anticancer drug is an urgent need, which has no harmful effect on normal cells and cures cancer [8]. Cytotoxic drugs are the main treatment option for some types of cancer [9]. They are very effective for fast proliferating cancer like leukemia and lymphomas [3]. They kill cancer cells by damaging DNA and microtubules [10]. Many polyphenols include flavonoids, alkaloids, saponins, cardiac glycoside, and fatty acids were reported to have cytotoxic properties [1, 3, 6, 11]. The previous study showed that ROS scavenger can be used to suppress the cancer cells proliferation as ROS is responsible for the development, growth and proliferation of cancer cells [6]. Antioxidants can protect the cells from cancer and reduce the risks of cancer. Numerous studies reported that nutraceuticals rich in antioxidants killed cancer cells by showing pro-oxidative activity [12]. Antioxidants are the compounds, which are able to delay, prevent or cure the oxidative damage by the presence of it at lower concentration compared to that of the free radicals [13]. They play an important role to scavenge the free radicals, stop the propagation of free radical chain reaction and terminate the chain reaction. Phenolic and aromatic ring containing antioxidants are the most effective antioxidants as they can release $H^{\bullet}$ to stabilize the radical intermediate in free radical chain reaction [14]. Antioxidants protect living organisms by reducing OS, DNA mutation, malignant transformation, and other parameters of cell damage [15].

Recently, some toxicological studies reported that synthetic antioxidants are not a good source of antioxidants as they show unwanted or adverse side effects. Researchers are more interested in natural antioxidant sources due to easily available, economic, and non-toxic. Polyphenolic compounds are the main constituents of natural sources [16]. Therefore, antioxidant-enriched botanical supplements can confer protection by decreasing OS and inflammatory processes [17, 18]. Over 8,000 polyphenolic compounds and more than 6,000 flavonoids are reported in various plants. Most of the flavonoids showed the most powerful antioxidant activity [13, 19, 20]. Nature provides the major portion of anticancer drugs. Total 136 drugs were recorded as anticancer drugs in the world over the period from 1981 to 2014. Surprisingly, 83% of these anticancer drugs were natural sources [21]. For instance, drugs derived from plant compounds are curcumin (diferuloylmethane) [22] and paclitaxel which is a taxane diterpene present in the crude extract obtained from the bark of *Taxus brevifolia* Nutt [23]. Another example is Taxol (essentially all taxanes), which hinders microtubule disassembly by joining the microtubules that have been polymerized [23]. Moreover, pomiferin, an isoflavone isolated from Osage orange (*Maclura pomifera*), and sulforaphane, an isothiocyanate isolated from broccoli, have recently been identified to possess anticancer properties via HDACi [24]. Thymoquinone has been reported to have

potent anticancer and superoxide anion scavenging abilities in animal models and cell culture systems [25]. The thymoquinone has been isolated from seed of *Nigella sativa*.

Leea aequata (*L. aequata*) is a traditional medicinal plant belongs to the family of Leeaceae [26]. It is widely distributed in Bangladesh, Bhutan, Cambodia, China, India, Malaysia, Myanmar, Nepal, Philippines, Thailand, and Vietnam [27]. In Bangladesh, it is distributed in the forest of Chittagong and Sylhet [28]. It contains phenolics, flavonoids, saponin, tannins, glycosides, and steroids [7, 29]. *L. aequata* is used for treating carbuncle, gastric tumour, hydrocele, rheumatism, ureterolithiasis, vertigo, vomiting, bronchitis, dyspepsia, itching, leprosy and tuberculous ulcers [28]. Traditionally, the roots, tubers and stems are used as mucilaginous and astringent [30]. The barks and roots are used to treat indigestion, jaundice, chronic fever and malaria as astringent and anthelmintic [31]. The leaves and thigh are used as antiseptic and wound healing agent [30]. The paste of fresh leaves is externally used to treat skin disease and wound [27]. The essential oil obtained from the whole plant by steam distillation is traditionally used to treat tuberculosis [30]. Recent studies revealed that the seeds, stems and roots possessed antibacterial activity [7]. The leaves have anticonvulsant, antinociceptive and anthelmintic activity [28, 31]. The *L. aequata* has widely been used by local practitioner against various diseases which encouraged us to carry out the pharmacological activity of this plant in this study. In this study, we investigated the *in vitro* antioxidant and cytotoxic activity of the different fractions of *L. aequata* leaves (LAL). We also explored the chemical profiling of the most effective antioxidant and cytotoxic fraction.

Materials And Methods

Collection and Identification of Plant

Leaves of *Leea aequata* L. (*L. aequata*), LAL, were collected from Sylhet, Bangladesh, in November 2017 and were identified by an expert taxonomist at Bangladesh National Herbarium where a voucher specimen (DACB Accession Number 45396) was deposited.

Preparation of Plant's Leaves

Collected leaves were washed properly with fresh tap water to remove dirty materials followed by shade dried for several days with occasional sun drying. These were then dried in an oven for 24 hours at considerably low temperature for better grinding. The dried materials were ground into coarse powder by a grinding machine in the Department of Pharmacy, University of Rajshahi, Bangladesh and stored at room temperature for future use.

Extraction of Plant's Leaves

The dried powdered of LAL (about 500 gm) were taken in an amber-coloured extraction bottle (2.5 litres capacity) and soaked the leaves with 100% methanol (1L × 3 times). The sealed bottle was shaken and

stirred occasionally. The final extract was filtered separately through cotton and then Whatman No.1 filter papers followed by concentrated with a rotary evaporator (Bibby Sterlin Ltd, UK) under vacuum pressure at 45°C to afford 80 gm leaves extract. A solution of an aliquot (35 gm) of extract in methanol and water was fractionated by pet-ether, chloroform and ethyl acetate to get petroleum ether (PEF, 4.86 gm), chloroform (CHF, 3.79 gm), ethyl acetate (EAF, 9.45 gm) and aqueous (AQF, 15.1 gm) fractions.

Determination of Antioxidant Compounds

Determination of Total Phenolic

The content of total phenolic of different extractives was determined according to the method described by Wolfe *et al.*, 2003 [32] in which Folin-ciocalteu reagent (FCR) was used as oxidizing agent and gallic acid (GA) was used as standard. Briefly, 0.4 mL of plant extract / standard of different concentrations were mixed with 2 mL of FCR (diluted 10 times with deionized water) reagent solution in different test tubes. After adding 3 mL of sodium carbonate (7.5%) solution, the test tubes were incubated for 30 minutes at 25°C to complete the reaction. Then the absorbance of the solution of each test tube was recorded at 760 nm using a spectrophotometer against blank. The total content of phenolic compounds in different extracts was expressed as mg of gallic acid equivalent (GAE) per gram of dry extract and calculated using the calibration curve equation, $y = 0.102x + 0.0268$ ($R^2 = 0.9996$) by the formula, $C = (x \times V)/M$. Where, C is total content of phenolic compounds as mg of GAE in each gram of dried extract, x is GAE concentration in mg/mL present in that particular sample concentration, V is final volume of the solution in mL and M is mass of the sample in final solution in gm.

Determination of Total Flavonoids

The content of total flavonoids of different extractives was determined by colorimetric method [13] using Catechin (CA) as standard. Briefly, 0.5 mL of plant extract / standard of different concentrations was mixed with 150 μ L of 5% sodium nitrate and 2.5 mL of distilled water in different test tubes and incubated for 5 min at room temperature (RT). 0.3 mL of 10% $AlCl_3$ was added in each test tube and incubated for 6 min at RT. 1 mL of 4% NaOH was added to the mixture and incubated at RT for 15 minutes to complete the reaction. Then the absorbance of the solution of each test tube was measured at 510 nm using a spectrophotometer against blank. The total content of flavonoid compounds in different extracts was calculated as catechin equivalent (CAE) per gm of dry extract from the standard curve equation, $y = 0.0264x - 0.038$, $R^2 = 0.9994$ by the formula, $C = (x \times V)/M$. Where, C is total content of flavonoids compounds as mg of CAE in each gram of dried extract, x is CAE concentration in mg/mL present in that particular sample concentration, V is final volume of the solution in mL and M is mass of the sample in final solution in gm.

***In vitro* Antioxidant Assay**

Determination of Total Antioxidant Capacity

The total antioxidant capacity of different extractives was determined according to Prieto et al., 1999 [33] with some modifications. 0.5 mL of plant extract / standard of different concentrations was mixed with 3 mL of reaction mixture containing 0.6 M sulphuric acid, 28 mM sodium phosphate and 1% ammonium molybdate and then incubated at 95°C for 10 minutes to complete the reaction. The absorbance of the solution of each test tube was measured at 695 nm using a spectrophotometer against blank after cooling at RT. Increased absorbance of the reaction mixture indicates increased total antioxidant capacity. In this study, standard/samples at five different concentrations ranging from 12.5 to 150 µg/mL were taken for each antioxidant assay. Concentrations were selected on the basis of trial and error to fit the range of concentration that can fully represent the rational change of antioxidant activity with the increasing concentration of the samples. The experiment was repeated three times at each concentration.

Ferric Reducing Power Capacity

The total antioxidant capacity of different extractives was estimated as described Oyaizu 1986 [34]. 0.25 mL of plant extract / standard of different concentrations was mixed with 0.625 mL of potassium buffer (0.2 M, pH 6.6) and 0.625 mL of 1% potassium ferricyanide [$K_3Fe(CN)_6$], and incubated for 20 min at 50°C to complete the reaction. 0.625 mL of 10% TCA was added in each of different test tubes and centrifuged at 3000 rpm for 10 min. 1.8 mL supernatant was withdrawn from each test tube and mix with 1.8 mL of distilled water followed by the addition of 0.36 mL of 0.1% ferric chloride ($FeCl_3$) solution to each diluted reaction mixture. Finally, the absorbance of each sample and standard was measured at 700 nm using a spectrophotometer against blank.

DPPH (1,1-diphenyl-2-picrylhydrazyl) Radical Scavenging Assay

The free radical-scavenging ability of the extracts/standard was tested using the DPPH radical-scavenging assay as described by Blois [35] and Desmarchelier *et al.* [36]. Briefly, 2.4 mL of methanol solution of 0.1 mM DPPH was mixed with 1.6 mL of plant extracts / standard dissolved in methanol at different concentrations and incubated at RT for 30 minutes in a dark place to complete the reaction. Then absorbance for test and standard solutions was measured at 517 nm using a spectrophotometer. A typical blank solution contained the same solution mixture without plant extracts and/or standard and it was incubated under the same conditions as the rest of the sample solution. The percentage (%) scavenging activity of DPPH radicals was calculated from the following equation:

$$\% I = \{(Ac-As)/Ac\} \times 100$$

Where, Ac is absorbance of the control, As is absorbance of the extract / standard. Then all the % scavenging was plotted against concentration and IC₅₀ was calculated.

Hydroxyl Radical Scavenging Assay

The hydroxyl radical-scavenging activity of the extracts/standard was determined by the method as described by Halliwell and Gutteridge [37]. Hydroxyl radical was generated by the Fe³⁺-ascorbate-EDTA-H₂O₂ system (Fenton reaction). The assay is based on the quantification of the 2-deoxy-D-ribose degradation product, which forms a pink chromogen upon heating with TBA at low pH. The reaction mixture contained 0.8 mL of phosphate buffer solution (50 mmol L⁻¹, pH 7.4), 0.2 mL of extracts/standard at different concentrations (6.25-100 µg/mL), 0.2 mL of EDTA (1.04 mmol L⁻¹), 0.2 mL of FeCl₃ (1 mmol L⁻¹), and 0.2 mL of 2-deoxy-D-ribose (28 mmol L⁻¹) was taken in the test tubes. The mixtures were kept in a water bath at 37 °C and the reaction was started by adding 0.2 mL of AA (2 mmol L⁻¹) and 0.2 mL of H₂O₂ (10 mmol L⁻¹). After incubation at 37 °C for 1 h, 1.5 mL of TBA (10 g L⁻¹) was added to the reaction mixture followed by adding 1.5 mL of HCl (25%). The mixture was heated at 100 °C for 15 min and then cooled down with water. The absorbance of solution was measured at 532 nm with a spectrophotometer. The hydroxyl radical scavenging activity was evaluated with the inhibition percentage of 2-deoxy-D-ribose oxidation on hydroxyl radicals. The percentage of HRSA (%HRSA) was calculated according to the following formula:

The percentage (%) scavenging activity of HO• radicals was calculated from the following equation:

$$\% \text{ hydroxyl radical-scavenging activity} = \{(Ac-As)/Ac\} \times 100$$

Where, Ac= Absorbance of the control, As= Absorbance of the extract/ standard. At the concentrations ranged from 6.25 to 100 µg/mL, the range of the absorbance values of the samples was 0.068–3.478, respectively. The experiment was repeated three times at each concentration.

In vitro Biological Studies on *L. aequata*

Lipid Peroxidation Inhibition Assay

The degree of lipid peroxidation was assayed by estimating the Thiobarbituric Acid Reactive Substances (TBARS) by using the standard method as described by Haenen and Bast 1983 [36]. The adult long Swiss albino mice weighing about 35 gm was anesthetized with chloroform. The excised mice livers were homogenized with a homogenizer in an ice-cold phosphate buffer (50 mm, pH 7.4) to produce a 1/10 homogenate. The homogenate was centrifuged at 12,000 rpm for 15 min at 4°C. The supernatant was

used as liposome for *in vitro* lipid peroxidation assay. 0.5 mL of supernatant and 0.3 mL of extract / standard at different concentrations were mixed with 1 mL of 0.15 M KCl and 200 μ L of 0.4 mM FeCl₃ and put in the incubator at 37°C for 30 minutes. After that, 2 mL of ice-cold TBA-TCA-HCl-BHT solution was immediately added to each test tube to stop the reaction and then heated at 90°C for 60 minutes. After cooling on ice and centrifugation at 3000 rpm for 5 min, the supernatants were removed and the absorbance of supernatants was measured on a spectrophotometer at 532 nm. A control experiment was performed in the presence of distilled water without the extract and / or standard. The percentage (%) scavenging activity of lipid peroxide radicals was calculated from the equation,

$$\% I = \{(Ac-As)/Ac\} \times 100$$

Where, Ac is absorbance of the control, As is absorbance of the extract / standard. Then % of scavenging was plotted against concentration and IC₅₀ was calculated.

Cytotoxicity Screening

Cell Culture

HeLa (cervical cancer cell) cells were purchased from American Type Culture Collection, USA. The cells were routinely cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum in an atmosphere with 5% CO₂ at 37°C. Exponential-growth cells were used in all the experiments.

Cell proliferation assay

Cell proliferation was assessed using the MTT assay as described by [3, 10]. Briefly, 5×10^3 cells were incubated in 96-well plates in the presence of various concentrations of the fractions for 48 h. At the end of the treatment, 20 μ L MTT (5 mg/mL dissolved in PBS) was added to each well and incubated for an additional 4 h at 37 °C. The purple-blue MTT formazan precipitate was dissolved in 200 μ L of DMSO and the optical density was measured at 570 nm using microplate reader (Varioskan Flash 2.4.3., Thermo Fisher Scientific) and percentage of cell viability was calculated by using the following formula: OD of samples/OD of controlsX100.

Measurement of Apoptosis of EAF

DAPI, annexin V-FITC and PI triple fluorescence staining for cancer cell apoptosis measurement were performed according to the method as described by Rahman *et al.*, 2021 [10]. Briefly, HeLa cells were cultured in medium containing 100 nM samples. After 48h treatment, the cells were washed twice with

0.01 M PBS and suspended in 200 μ L binding buffer. The cells were then incubated with 10 μ L DAPI, 10 μ L annexin V-FITC and 5 μ L PI for 30 min at 4°C in the dark. DAPI, annexin V-FITC and PI fluorescence were immediately observed under an FV1000 confocal laser scanning microscope (Olympus, Tokyo, Japan).

GC-MS Analysis of EAF

The bioactive compounds from the EAF were analyzed using the GC-MS with electron-impact ionization (EI) on a gas chromatograph (GC-17A, Shimadzu Corporation, Kyoto, Japan) coupled to a mass spectrometer (GC-MS TQ 8040, Shimadzu Corporation). A fused silica capillary column (Rxi-5 ms; 0.25-m film, 30-m long, 0.32-mm internal diameter) coated with DB-1 (J&W) was used. The inlet temperature was 260°C and the oven temperature was set at 70°C (0 min); 10°C, 150°C (5 min); 12°C, 200°C (15 min); and 12°C, 220°C (5 min) with a hold time of 10 min. The flow rate of the column was 0.6 mL/min helium gas at a constant pressure of 90 kPa. The aux (GC to MS interface) temperature was 280°C. The MS was set to scan mode with a scanning range of 40–350 amu, while the ionization mode was EI type and the mass range was set within 50–550 m/z. The sample (3 μ L) was injected in split fewer modes. The total GC-MS run time was set for 29.33 min and compounds in the peak areas were identified by comparison with those in the database of the GC-MS library version NIST 08-S.

In silico Molecular Docking

Protein Preparation

The 3D crystal structures of Caspase 3 (PDB: 5IAE) for HeLa [38, 39], structure of EGFR kinase domain (PDB: 2ITY) [40], glutathione reductase (PDB: 3GRS) [41], and urate oxidase (PDB: 1R4U) [42] were downloaded in PDB format from Protein Data Bank [43]. Then, the structures were prepared and refined through the process as described by Uddin *et al* [44, 45].

Ligand Preparation

Isolated compounds from EAF were collected from Pubchem databases in SDF format. The 3D configuration for these was built by using Ligprep wizard in Maestro Schrödinger (v11.1) with an OPLS3 [46] force field. Their ionization states were generated at pH 7.0 $\pm$ 2.0 using Epik in Schrödinger Suite. Up to 32 possible stereoisomers per ligand were retained.

Receptor Grid Generation

Receptor grids were calculated for prepared proteins so that various ligand poses bind within the predicted active site during docking. In Glide, Van der Waals radius scaling factor is 1 and the partial charge cutoff is 0.25, which are default parameters. A cubic box of specific dimensions centered on the centroid of the active site residues (Reference ligand active site) was generated for receptor. The bounding box was set to 14 Å × 14 Å × 14 Å for docking experiments.

Glide Standard Precision (SP) Ligand Docking

SP flexible ligand docking was carried out in the Glide of Schrödinger-Maestro (v11.1) [44, 47, 48] within which penalties were applied to non-cis/trans amide bonds. Van der Waals scaling factor and partial charge cutoff was selected to be 0.80 and 0.15, respectively for ligand atoms. Final scoring was performed on energy-minimized poses and displayed as glide score. The best docked pose with the lowest glide score value was recorded for each ligand.

***In Silico* Determination of Pharmacokinetic Parameters by SwissADME**

The pharmacokinetic parameters or drug-likeness properties of the isolated compounds were evaluated by SwissADME online tool (<http://www.swissadme.ch/>). ADME (Absorption, Distribution, Metabolism and Excretion) properties depicts the compound acceptability in the body which is determined by Lipinski's rule of five [49].

***In Silico* Toxicological Properties Prediction by AdmetSAR**

Toxicological characteristics of the isolated compounds were determined using the admetSAR online tool (<http://lmmd.ecust.edu.cn/admetsar1/predict/>) since toxicity imparts a major concern during the development of new drugs. In the present study, ames toxicity, carcinogenic properties, acute oral toxicity, and rat acute toxicity were predicted [50].

***In Silico* Prediction of Activity Spectra for Substances (PASS) Study by PASS Online**

The isolated compounds from EAF were examined for evaluating the anticancer, antiviral, free radical scavenging, lipid peroxidase inhibitor, and antioxidant activities by using PASS online (<http://www.way2drug.com/PassOnline/>) [51].

Statistical Analysis

All tests were carried out using the GraphPad Prism (version 8.4.3) and Microsoft Excel-2019 for the statistical and graphical evaluation. Data were presented as the mean $\pm$ standard deviation (SD) from triplicate experiments. The data for significant differences between the tests and control groups was described using one-way analysis of variance (ANOVA). P-values of <0.05 , <0.01 , <0.001 and <0.0001 were considered statistically significant.

Results

Determination of Antioxidant Compounds

Determination of Total Phenolic Content

The content of total phenols of CME and its four fractions were determined using the FCR (Fig. 1A). The results showed that EAF contains the highest phenolic content (267.05 ± 8.53 mg GAE/gm of dried sample). AQF (241.37 ± 17.38 mg of GAE/gm of dried extract) and CME (233.39 ± 17.01 mg of GAE/gm of dried extract) were also the rich sources of phenolic contents. On the other hand, CHF (39.41 ± 0.69 mg of GAE/gm of dried extract) and PEF (25.48 ± 0.3 mg of GAE/gm of dried extract) were comparatively poor sources of phenolic content.

Determination of Total Flavonoid Content

The content of total flavonoids of CME with its four fractions, such as PEF, CHF, EAF and AQF were determined using well known aluminum chloride colorimetric method. Fig. 1B represents the total flavonoid content of all the extractives as mg of CAE/gm of dried extract. The flavonoid content of CME, PEF, CHF, EAF and AQF were found to be 131.41 ± 1.58 , 60.96 ± 0.7 , 82.31 ± 3.15 , 152.98 ± 1.96 and 67.92 ± 2.43 mg of CAE/gm of dried extractives, respectively. Comparing the total flavonoid content among the different fractions, it was noticed that EAF contained the highest amount of flavonoids followed by CME, PEF, CHF and AQF.

In vitro Antioxidant Assay

Total Antioxidant Activity

The total antioxidant activity of the different extractives was assessed by phosphomolybdenum method based on the reduction of Mo (V1) to Mo (V). Total antioxidant activity of Me-LAL with its four fractions and standard (CA) was depicted in the Fig. 2A. Increased absorbance indicates increased antioxidants activity. EAF (1 ± 0.03 absorbance at $100 \mu\text{g/mL}$) had the highest antioxidant activity among all the

fractions and was closed to that of the standard (1.06 ± 0.09 absorbance at $100 \mu\text{g/mL}$). CME (0.89 ± 0.05 absorbance at $100 \mu\text{g/mL}$) and AQF (0.79 ± 0.01 absorbance at $100 \mu\text{g/mL}$) also possessed better antioxidant activity than CHF (0.23 ± 0.02 absorbance at $100 \mu\text{g/mL}$) and PEF (0.31 ± 0.05 absorbance at $100 \mu\text{g/mL}$), but their antioxidant activity was slightly less than that of the standard and EAF. These results demonstrated that all the extractives of CME possessed noticeable antioxidant activity, which was concentration dependent.

Ferric Reducing Power Capacity

The reductive capabilities of the extractives and standard (AA) were shown in the Fig. 2B. It is well documented in several studies that higher the absorbance indicates higher reducing capacity and the increased absorbance follows concentration dependent manner [18]. The absorbance (2.77 ± 0.006) of EAF was closed to the absorbance (3.26 ± 0.002) of standard at a concentration of $50 \mu\text{g/mL}$ and EAF showed the highest reducing capacity among all the fractions. AQF (absorbance 1.78 ± 0.19) and CHF (absorbance 0.83 ± 0.09) possessed moderate reducing activity compared to the standard at a concentration of $50 \mu\text{g/mL}$. These results demonstrated that CME and its fractions had significant iron reducing capacity, which was concentration dependent.

DPPH Radical Scavenging Activity

DPPH radical scavenging activity is based on the ability of the extractives to scavenge the stable DPPH radical that contains an odd electron. The results of DPPH radical scavenging assay of CME and its four fractions and standard (BHT) were given in the Fig. 3A and 3B. Among the fractions, EAF showed the highest and significant ($p = 0.0001$) free radical scavenging activity. The IC_{50} values of EAF, CME and AQF were found to be 6.92 ± 0.19 , 6.09 ± 0.13 and $5.11 \pm 0.04 \text{ mg/mL}$, respectively which were higher than the standard BHT ($\text{IC}_{50} 7.93 \pm 0.19 \text{ mg/mL}$). On the other hand, CHF and PEF showed moderate scavenging activity with IC_{50} values of $24.97 \pm 1.2 \text{ mg/mL}$ and $9.52 \pm 0.17 \text{ mg/mL}$, respectively. These observations demonstrated that all the extractives of CME possessed DPPH free radical scavenging activity.

Hydroxyl Radical Scavenging Activity

In the hydroxyl radical scavenging assay, the ability of the extractives of CME to remove the hydroxyl radical in solution was evaluated quantitatively by colorimetric method using CA as standard and % of scavenging activity was calculated. The results of hydroxyl radical scavenging activity of different extractives and standard were shown in the Fig. 4A and 4B. The IC_{50} values of CME, PEF, CHF, EAF, AQF and standard CA for hydroxyl radical scavenging activity were 121, 475, 325, 37.5, 165 and $170 \mu\text{g/mL}$, respectively. Our results demonstrated that the EAF, AQF and CME showed higher activity when compared to the standard. Among the extracts, the EAF showed the significant ($p < 0.0001$) radical scavenging

activity and the IC₅₀ value of EAF was more than four times lower than that of the standard. Our results therefore clearly demonstrated that the fractionated extractives of CME possessed highly potent radical scavenging activity.

In vitro* Biological Studies of *L. aequata

Lipid Peroxidation Inhibition Assay

The results of lipid peroxidation inhibition of all the extractives of CME and standard CA were shown in the Fig. 5A and 5B. CME, PEF, CHF, EAF, AQF and CA showed 57.75±0.43, 51.5±0.39, 53.54±0.15, 62.83±0.58, 59.11±0.72 and 68.76±0.59 percentage of lipid peroxidation inhibition, respectively at a concentration of 100 µg/mL. Among the extractives, EAF ($p = 0.0025$) showed the highest inhibitory activity with IC₅₀ value of 40 µg/mL, which was closely similar to that of the CA (35 µg/mL). On the other hand, AQF had moderate inhibitory activity with IC₅₀ value of 53 µg/mL. The CHF and PEF showed comparatively less inhibitory activities. However, the inhibitory activity was concentration dependent.

Cytotoxicity Assays

In-vitro anticancer activity of all the extractives of CME and its four fractions and standard vincristin sulfate (VS) and 5-Fluorouracil (5-FU) on HeLa cell line was shown in the Fig. 6. The EAF showed the highest cytotoxic activity with 22.54% cell viability, whereas the standards VS and 5-FU showed 15.19% and 16.45% cell viability, respectively at a concentration of 500 µg/mL. CME and CHF also showed moderate cytotoxicity with cell viability 27.74% and 32.01%, respectively. However, the cytotoxic activity of EAF was in a concentration-dependent manner.

Determination of HeLa cell apoptosis

We evaluated the effect of EAF on HeLa cell apoptosis using DAPI, annexin–V FITC and PI triple fluorescence staining. Annexin V–FITC and PI signals were barely detectable in the control cells (without treatment), while treated cells exhibited strong fluorescence densities, indicating that EAF has capacity for inducing HeLa cell apoptosis (Fig. 7). Therefore, EAF was selected for GC-MS profiling to find out the compounds those might be responsible for apoptosis of HeLa cells.

GC-MS Analysis

GC-MS profiling was carried out on EAF because it was the most antioxidant rich and effective cytotoxic fraction among all the fractions. GC-MS profiling of EAF was shown in the Table 1 and it confirmed 17 different compounds (Fig. 8). The nature and the structure of the compounds were identified by the

retention time. The compounds were of different chemical classes. Hexadecanoic acid, methyl ester (42.5%), 9-Octadecenoic acid (Z)-, methyl ester (22.08%), 9,12-Octadecadienoic acid, methyl ester (11.74%), E-15-Heptadecenal (4.7%), 1-Heneicosanol (4.28%), E-14-Hexadecenal (3.22%), Methyl stearate (2.56%), Phenol, and 2,4-bis(1,1-dimethylethyl)- (2.21%) were the major constituents of EAF. 1-Tetradecene; 9-Hexadecenoic acid, methyl ester, (Z)-; Dodecanoic acid, methyl ester; Nonacos-1-ene; Tridecanoic acid, 12-methyl-, methyl ester; 11-Octadecenoic acid, methyl ester; Cyclopropane, nonyl- and n-Heptadecanol-1 were also present in the EAF.

***In silico* Molecular Docking for Antioxidant Activity**

The outcomes for molecular-docking simulation for sixteen selective compounds were represented in the Table 2. In case of antioxidant property, the compounds were subjected against urate oxidase (PDB: 1R4U) and glutathione reductase (PDB: 3GRS). The orders for docking scores when bonded with urate oxidase were given below: Phenol, 2,4-bis(1,1-dimethylethyl)- > 1-Heneicosanol > Nonacos-1-ene > Tridecanoic acid, 12-methyl-, methyl ester > 9,12-Octadecadienoic acid, methyl ester > 9-Hexadecenoic acid, methyl ester, (Z)- > E-14-Hexadecenal > 11-Octadecenoic acid, methyl ester > 9-Octadecenoic acid (Z)-, methyl ester > Methyl stearate > Dodecanoic acid, methyl ester > Hexadecanoic acid, methyl ester > n-Heptadecanol-1 > Cyclopropane, nonyl- > 1-Tetradecene. On the other hand, when interacted with glutathione reductase, the docking scores were in the following orders: Phenol, 2,4-bis(1,1-dimethylethyl)- > 1-Heneicosanol > Nonacos-1-ene > 9-Hexadecenoic acid, methyl ester, (Z)- > 9,12-Octadecadienoic acid, methyl ester > Tridecanoic acid, 12-methyl-, methyl ester > 9-Octadecenoic acid (Z)-, methyl ester > E-15-Heptadecenal > Hexadecanoic acid, methyl ester > Methyl stearate > 11-Octadecenoic acid, methyl ester > E-14-Hexadecenal > Dodecanoic acid, methyl ester > Cyclopropane, nonyl- > n-Heptadecanol-1 > 1-Tetradecene. The figures expressing the 3D conformation of ligand-receptor interactions for urate oxidase were given in the Fig. 9 and the interactions for glutathione reductase were given in the Fig. 10. Furthermore, hydrogen and hydrophobic bonds between each compound and protein, which is associated with amino acid residues was expressed in the Table 3 and Table 4.

***In silico* Molecular Docking for Anti-cancer Activity**

Two protein structures namely EGFR kinase domain (PDB: 2ITY) and caspase-3 (PDB: 5IAE) for HeLa cells were used in order to assess the anticancer activity of the selective compounds. When the compounds interacted with EGFR kinase domain, they showed docking scores in the following ordered: Phenol, 2,4-bis(1,1-dimethylethyl)- > 1-Heneicosanol > Nonacos-1-ene > 9,12-Octadecadienoic acid, methyl ester > 9-Hexadecenoic acid, methyl ester, (Z)- > 11-Octadecenoic acid, methyl ester > Methyl stearate > Tridecanoic acid, 12-methyl-, methyl ester > Hexadecanoic acid, methyl ester > 9-Octadecenoic acid (Z)-, methyl ester > n-Heptadecanol-1 > E-14-Hexadecenal > Cyclopropane, nonyl- > Dodecanoic acid, methyl ester > E-15-Heptadecenal > 1-Tetradecene. On the other hand, when the compounds interacted with caspase-3 the docking scores were in the following ordered: Phenol, 2,4-bis(1,1-dimethylethyl)- > 1-

Heneicosanol ◻ Nonacos-1-ene ◻ 11-Octadecenoic acid, methyl ester ◻ 9,12-Octadecadienoic acid, methyl ester ◻ Tridecanoic acid, 12-methyl-, methyl ester ◻ Methyl stearate ◻ 9-Hexadecenoic acid, methyl ester, (Z)- ◻ Dodecanoic acid, methyl ester ◻ Hexadecanoic acid, methyl ester ◻ 9-Octadecenoic acid (Z)-, methyl ester ◻ E-15-Heptadecenal ◻ Cyclopropane, nonyl- ◻ E-14-Hexadecenal ◻ n-Heptadecanol-1 ◻ 1-Tetradecene. The 3D representations for ligand-receptor complex associated with EGFR kinase domain were given in the Fig. 11 and the representations regarding caspase-3 were given in the Fig. 12. Also, the bond corresponding to the interactions are given in Table 5 and Table 6.

Determination of Pharmacokinetic Parameters by Swiss ADME and Toxicological Properties Prediction by AdmetSAR

The ADME (Absorption, Distribution, Metabolism and Excretion) properties of the selected compounds were evaluated on the basis of Lipinski rule of five. The data regarding each of the parameters were retrieved from swissADME online server, which was given in the Table 7. Three of the compounds were violated more than one parameter of the standard basis. This evaluation suggested that all of the compound have drug-like property and favorable oral bio-availability except Hexadecanoic acid, methyl ester, 11-Octadecenoic acid, methyl ester and Nonacos-1-ene. Also, the toxicological characteristics for each of the selective compounds were evaluated through AdmetSAR online server and have given in the Table 8. It depicted that all of the compounds were Non Ames Toxic and have little or insignificant rat acute toxicity which indicates that all of the compounds are safe for administration in the body.

PASS Prediction Study by PASS Online

Pass prediction study was carried out through PASS online server. All the compounds exhibited greater Pa value than Pi (Table 9).

Correlation and Regression of Phenolic and Flavonoid Content with Antioxidant and Cell Viability Potential

Excellent positive association was seen through the correlation and regression study for phenolic and flavonoid content with radical scavenging activity and cell viability of the CME extractives. Table 10 and Table 11 represented the correlation and regression ($P < 0.05$, $P < 0.01$, $P < 0.001$, and $P < 0.0001$) of phenolic and flavonoid content with the antioxidant and cell viability potential.

Discussions

Phenolic compound represents the largest group of phytochemicals and finds commonly in plants as free form or as glycosides. They show lots of biological activities, including anticancer, antimicrobial, antiulcer, antiarthritic, antiangiogenic, protein kinase inhibition and mitochondrial adhesion inhibition [7]. Flavonoids are the largest group of naturally occurring phenolic. They possess many biological activities, including antimicrobial, mitochondrial adhesion inhibition, antiulcer, antiarthritic, antiangiogenic, anticancer, protein kinase inhibition, etc. [52]. Phenolic and flavonoid compounds possess strong antioxidant property [16, 20]. Plants containing large amounts of phenolic and flavonoid show strong antioxidant activity. In this study, CME extractives possessed a significant amount of phenolic and flavonoid. Interestingly, EAF and PEF contained the highest amount of phenolic and flavonoid, respectively. Based on these observations, we summarized that the extracts' radical-scavenging nature might depend on their polyphenolic content, i.e., phenolics and flavonoids. It is evident that total phenols and flavonoids are potential antioxidants and free radical scavengers [11, 18, 45]. Moreover, polyphenolic compounds have a wide spectrum of chemical and biological activity, including antioxidant and anti-cancer properties [3, 13]. Free radicals produced in our body due to aerobic respiration and substrate oxidation, can cause oxidative stress which may contribute to the development of several diseases including cancer [3]. Overproduction of free radicals initiated oxidative stress in biological system. This oxidative stress causes serious damage to important cellular macromolecules such as protein and DNA. However, the production of free radicals can be balanced by antioxidant actions of endogenous enzymes as well as natural and synthetic antioxidants [3]. Antioxidants exert its action through several mechanisms including prevention of chain initiation, chelating of transition metal ion catalysts, decomposition of peroxidases, prevention of continued hydrogen abstraction and radical scavenging [15, 53].

The total antioxidant activity of different extractives of CME was determined by measuring their reduction power of Mo (VI) to Mo (V) and subsequent formation of a green phosphate/Mo (V) complex. Dietary recommendations in recent years suggest increasing the consumption of foods containing polyphenolics, which have great impact on preventing chronic diseases, particularly cancer [54]. The evidence has brought the attention of scientists to an appreciation of antioxidants for prevention and treatment of diseases, and maintenance of human health [55]. In this study, the antioxidant capacity (in terms of absorbance at 700 nm) of the extractives was in the range of 0.032 ± 0.014 – 0.997 ± 0.026 at the concentration ranges from 6.25–100 $\mu\text{g/mL}$. Recent studies have shown that many flavonoid and related polyphenols contribute significantly to the phosphomolybdate scavenging activity of medicinal plants [3]. All the extracts showed good antioxidant activities that were gradually increased with increasing concentration. Interestingly, EAF contained the highest antioxidant activity, which was similar to the standard (CA). The highest antioxidant activity of EAF was due to the presence of polyphenolics. Previously, many researchers have proven that the antioxidant capacity of citrus was due to the presence of phenolics, flavonoids, and ascorbic acid [56]. Abdurahman NH *et al.* reported that phenolic compounds, β -Glucogallin and apocynin showed antioxidant activity in *Vernonia cinerea* leaves [57].

The ferric reducing power capacity is considered as another prominent indicator of anti-radical activity [58]. The mechanism of FRA capacity is associated with the presence of a reductant that donates a

hydrogen atom after ROS breakdown. In the current study, EAF showed the highest ferric reducing power capacity through its reduction of the Fe^{3+} -ferricyanide complex into the ferrous form, which was monitored through the blue-green complex at 700 nm. The ferric reducing power capacity of the CME and all the extractives were in the range of 0.073 ± 0.015 – 3.264 ± 0.01 at the concentration ranges from 6.25–100 $\mu\text{g/mL}$ shown in the Fig. 2B. This finding denoted that the reducing power of all extractives was dose dependent which supports the finding of the previous studies [7, 53, 59]. The significant reducing power of EAF ($p < 0.05$) was possibly due to the presence of phenolic constituents, which might act as electron donors. The absorbance (2.77 ± 0.006) of EAF was close to the absorbance (3.26 ± 0) of standard, AA at a concentration of 50 $\mu\text{g/mL}$. This result indicates that LAL contains a higher number of potent antioxidants which could stabilize and block free radical chain reactions.

DPPH radical scavenging activity is the most common method used for determining antioxidant activity. The antioxidant activity depends on the donating hydrogen or electron to stabilize the purple coloured DPPH free radical into reduced form of DPPH which is pale yellow colour [53]. A stable diamagnetic molecule is formed by the DPPH radical by accepting an electron or hydrogen radical, which is followed by the solution changing color from blue to yellow. This color-changing technique is a widely accepted *in vitro* method because of its simplicity, stability, and reproducibility [60]. All CME fractions showed significant DPPH scavenging activity ($p < 0.01$). The order of the IC_{50} of CME, PEF, CHF, EAF, AQF, and the standard BHT was as follows: $\text{EAF} > \text{CME} > \text{AQF} > \text{BHT} > \text{CHF} > \text{PEF}$. EAF had a significant IC_{50} value ($p < 0.01$) that was almost identical to that of BHT. Duan *et al.* (20007) reported that phenolics and flavonoids can reduce DPPH radicals by donating hydrogen ions [61]. Our results are consistent with that of the data published previously [3, 13, 62].

Hydroxyl radicals are the most highly ROS causing noxious effects on protein, lipids and nucleic acids. This can cause damage to cellular components by reacting with the polyunsaturated fatty acid of cell membrane phospholipids [18]. The hydroxyl radicals attack the fatty acid side chains of the membrane phospholipids and add a double bond to DNA bases [63]. The modification of the DNA bases may initiate different cellular process malfunctions, which are characterized by the primary pace of carcinogenesis [64]. In addition, the hydroxyl radicals are generated in the biological system by Fenton reaction with subsequent degradation of deoxyribose to TBARS which generates a pink chromogen on heating at low pH with thiobarbituric acid (TBA) [65]. In our investigation, CME and its extractives showed promising hydroxyl radical scavenging activity and were capable of protecting deoxyribose in a dose dependent manner. There is evidence that the hydroxyl radical activity of the extract is directly proportioned to its antioxidant activity [66]. Our results demonstrated that EAF, AQF and CME possessed higher hydroxyl radical scavenging activity than that of the CA (standard). The IC_{50} value of the most potent radical scavenger, EAF was four times lower than that of the standard CA. So, EAF can serve a potent hydroxyl radical scavenger and may protect cell and cellular components. Previous research finding showed that extracts which are able to scavenge hydroxyl radical can have ability to inhibit lipid peroxidation and break free radical chain reaction [62].

Lipid peroxides are highly reactive and more stable than ROS. They can diffuse from where they generate and show detrimental effects to the remote location as well as the site of generation. High concentration of lipid peroxide in the body is responsible for many life threatening diseases, including cancer. Therefore, limiting the formation of lipid peroxide generation is beneficial for controlling the life threatening diseases [7]. Antioxidant potential of extract or fraction is directly related to the scavenging of hydroxyl radical and consequently the inhibition of lipid peroxidation [67]. The erythrocytes intrinsically are more prone to peroxidation due to the heavy accumulation of polyunsaturated fatty acids and haemoglobin. During respiration erythrocytes are continuously exposed to high tension of oxygen, which can induce oxidative damage [68]. Our finding showed that the EAF showed the highest inhibitory activity with IC_{50} value (40.00 $\mu\text{g/mL}$) among all the extractives which was close to that of CA (35.00 $\mu\text{g/mL}$). CHF and AQF also had moderate inhibitory activity with the IC_{50} value of 82 and 53 $\mu\text{g/mL}$, respectively, was comparable to the standard, CA. The extract's ability to inhibit lipid peroxidation could be attributed to the presence of lipid-soluble antioxidant compounds, hence, could donate protons to stabilize lipid radicals or exquisitely scavenges lipid peroxy radicals, thereby, terminating subsequent chain propagation reactions [69]. Current evidence suggests that, *L. aequata* has antioxidant potential and the capacity to combat ROS associated damage because of its reducing power capacity as well as radical-scavenging activity.

Cancer is developed by cellular damage. Free radicals are responsible for cellular damage. The main characteristic of cancer cells is uncontrolled rapid proliferation. These cells are less prone to death [10]. Many naturally occurring compounds such as alkaloids, phenolics, flavonoids and saponins exhibit anticancer activity by inhibiting cell proliferation and angiogenesis, causing cell cycle arrest, and/or inducing cell apoptosis [10, 70]. Cancer can be stimulated by chromosomal irregularities and oncogene initiation determined by oxidative stress [71]. A byproduct termed hydrolyzed DNA is formed by DNA oxidation [72], and this byproduct initiates transcriptomic changes, and genes are mutated. Furthermore, reactive oxygen species are responsible for modifying DNA-protein cross-links, base and sugar lesions, strand breaks, and base-free sites [2]. Antioxidants are a class of compounds that mop up reactive oxygen species and prevent cancer. Our finding showed that EAF possessed more profound cytotoxic activity which is very close to that of standard VS and 5-FU on HeLa cell line. This cytotoxic activity may be due to the presence of high number of flavonoids, saponins and other compounds. The dose dependent activity showed that the presence of a high concentration of compounds exhibited less percentage of HeLa cancer cell viability.

Furthermore, the effect of EAF on HeLa cell apoptosis was evaluated using DAPI, annexin V-FITC and PI triple fluorescence staining. DAPI is used as a marker of cell membrane permeability seen in very late apoptotic cells; whereas, annexin V-FITC staining can identify apoptosis at an earlier stage [6]. In the present study, DAPI, annexin V-FITC, and PI signals were barely detectable in the control cells (without treatment), while treated cells had strong fluorescence densities, indicating that the EAF has the capacity for inducing HeLa cell apoptosis through the early and late stages (Fig. 7).

EAF is the most antioxidative and cytotoxic fraction among all the fractions of CME. GC-MS data of EAF explored the chemical constituents and its proportion in EAF. Hexadecanoic acid, methyl ester was

present as 42.5% of EAF which was reported as antioxidant and for cancer prevention [29]. 9-Octadecenoic acid (Z)-, methyl ester which is the second major constituent (22.08%) of EAF, has been reported for the prevention of cancer, antioxidant and anticancer activity [49]. 9,12-octadecadienoic acid, methyl ester (Z, Z) have been showed to be powerful anticancer activity with an IC_{50} value of 10.60 ± 1.12 against human lung cancer cell line A549. It has been previously proven that unsaturated fatty acid ester possessed significant antioxidant properties [73]. It has also been reported that fatty acid esters are deposited on the lipid layer of cell membrane and mitochondria. Thus, they showed cytotoxicity by increasing the permeability of cells due to the lack of integrity of cellular structure. Phenol, 2,4-bis(1,1-dimethylethyl)- was reported for antioxidant and antitumor activity [7].

Molecular docking is an integral segment in the structural molecular biology discipline which is designed to evaluate the probable ligand-protein synergy through computational approach [74]. Besides, it helps revealing the mechanism of action of the investigational compounds extrapolated from various natural sources and provides an efficient way to discovery of new active medicinal agent in order to serves a remedy for various diseases [75]. In this study, molecular docking simulation was performed to associate and reciprocate the findings with the current in vitro study. Sixteen compounds from *L. aequata* were selected in order to carry out molecular docking simulation for antioxidant and anticancer activity. Two protein namely urate oxidase (PDB: 1R4U) and glutathione reductase (PDB: 3GRS) were selected as ligand target for antioxidant activity. When compounds were subjected to docking simulation against urate oxidase, Phenol, 2,4-bis(1,1-dimethylethyl)- possessed the highest score (-4.196). Moreover, when the compounds were in complex with glutathione reductase, the same compound found to have the best outcome (-5.262). Phenol, 2,4-bis(1,1-dimethylethyl)- when interacted with urate oxidase it was formed two pi-alkyl bond with ARG-176 and HIS-256 and a conventional hydrogen bond with LYS-171. Again, when this compound interacted with glutathione reductase it has formed only a conventional hydrogen bond with THR-156. The docking score for this compound when compared to the standard drug ascorbic acid, it was seen that the result was very close. Which suggested that the compound to be a potential antioxidant agent. On the other hand, EGFR kinase domain (PDB: 2ITY) and caspase 3 (PDB: 5IAE) were used as a receptor for the experimental compound to determine their anticancer activity. After docking simulation, phenol, 2,4-bis(1,1-dimethylethyl)- found to have the best docking score (-5.512) when interacted with EGFR kinase domain and in case of caspase 3, the same compound were found to have the best result (-5.631). After comparing with the standard drug 5-fluorouracil, the compound possessed better score than the standard for both proteins. Phenol, 2,4-bis(1,1-dimethylethyl)- has formed a conventional hydrogen bond with ASP-855 and an alkyl bond LEU-718. Also, two pi-alkyl bond with LEU-844 and VAL-726 when it was in interaction with active site of EGFR kinase domain. This compound when interacted with caspase 3 receptors, it was formed one conventional hydrogen bond with ARG-207, a pi-pi stacked bond with TRP-206 and a pi-alkyl bond with TYR-204. Moreover, other compounds also shown good affinity with each of these four distinctive proteins for anticancer and antioxidant activity. Furthermore, each of these experimental compounds was evaluated for ADME and toxicological analysis. According to Lipinski rule of five orally administered agents should satisfy the following criteria: Molecular weight ≤ 500 , Hydrogen bond donor ≤ 5 , Hydrogen bond acceptor ≤ 10 , LogP ≤ 5 , Molar

refractivity 40–130 [49]. This rule depicted that one compound or possible medicinal agent cannot breach all of the parameter in order to have good oral bioavailability [50, 76, 77]. ADME analysis on the basis of Lipinski rule of five revealed that all of the compounds satisfied the given criteria except Hexadecanoic acid, methyl ester, 11-Octadecenoic acid, methyl ester and Nonacos-1-ene. Furthermore, toxicity evaluation of the selective compounds demonstrated that all of the compounds were Non AMES toxic and possess insignificant level of toxic property. Total phenolic and flavonoid contents of the *L. aequata* showed significant and strong positive correlation ($p\text{-value} < 0.001$ and 0.05) with antioxidants (DPPH) and cell viability assay. Our results are consistent with those found by Islam et al., [3] who reported that there is existence of a strong relationship between total phenolic contents and DPPH radical scavenging. Henceforth, it is evident from the molecular docking analysis that the isolated compounds from *L. aequata* possessed significant antioxidant and anticancer property and can be a potential source of probable medicinal agent to treat cancer and reactive oxygen species related complications.

Conclusion

The present study indicates that EAF fraction of *L. aequata* was the potential source of polyphenols, which showed significant antioxidant and cytotoxic activities. EAF also induced HeLa cell apoptosis. GC-MS data revealed that EAF contained different important phytoconstituents. Our study suggests that many bioactive compounds of EAF can be used to develop the beneficial drugs to manage various diseases like cancer.

Declarations

Ethics declarations

The Animal Ethical Committee, Rajshahi University (27/08/RUBCMB) and the Committee of Cell Research of Rajshahi Medical College, Bangladesh (ref. RMC/ER/2010-2013/01) approved the current research.

Consent for publication

Not applicable.

Availability of data and materials

All data related to this research are available within the manuscript.

Conflict of Interests

The author(s) declare(s) that there is no conflict of interests regarding the publication of this article.

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Tables

Board 1

GC-MS profiling of EAF of *L. aequata* leaves.

Name	R. Time	m/z	Area	Concentration (%)
Cyclopropane, nonyl-	8.103	55	9221	0.22%
1-Tetradecene	13.337	55	57746	1.40%
Phenol, 2,4-bis(1,1-dimethylethyl)-	16.291	191	91164	2.21%
Dodecanoic acid, methyl ester	16.724	74	32031	0.78%
E-14-Hexadecenal	18.908	55	132956	3.22%
Tridecanoic acid, 12-methyl-, methyl ester	24.056	74	18405	0.45%
E-15-Heptadecenal	26.307	55	193904	4.70%
9-Hexadecenoic acid, methyl ester, (Z)-	29.318	55	38562	0.93%
Hexadecanoic acid, methyl ester	29.912	74	1754372	42.50%
1-Heneicosanol	31.551	55	176606	4.28%
n-Heptadecanol-1	33.535	55	9258	0.22%
9,12-Octadecadienoic acid, methyl ester	33.681	67	484592	11.74%
9-Octadecenoic acid (Z)-, methyl ester	33.843	55	911366	22.08%
11-Octadecenoic acid, methyl ester	33.95	55	11021	0.27%
Methyl stearate	34.407	74	105541	2.56%
1-Heneicosanol	35.768	57	81769	1.98%
Nonacos-1-ene	39.436	57	19057	0.46%

Table 2

Molecular docking scores for the selected compounds and standard drugs

No.	Compounds	1R4U	3GRS	2ITY	5IAE
1.	Cyclopropane, nonyl-	+2.695	+0.909	+0.448	+0.996
1.	1-Tetradecene	+4.084	+3.067	+2.694	+3.548
1.	Phenol, 2,4-bis(1,1-dimethylethyl)-	-4.196	-5.262	-5.512	-5.631
1.	Dodecanoic acid, methyl ester	+1.391	+0.475	+0.584	-0.154
1.	E-14-Hexadecenal	+1.157	+0.066	+0.178	+1.212
1.	Tridecanoic acid, 12-methyl-, methyl ester	+0.68	-1.381	-0.28	-0.614
1.	E-15-Heptadecenal	+0.955	-0.479	+1.14	+0.935
1.	9-Hexadecenoic acid, methyl ester, (Z)-	+0.901	-1.449	-0.765	-0.195
1.	Hexadecanoic acid, methyl ester	+1.507	-0.447	-0.001	-0.029
1.	1-Heneicosanol	-2.202	-3.942	-3.189	-2.342
1.	n-Heptadecanol-1	+1.612	+1.01	+0.115	+1.486
1.	9,12-Octadecadienoic acid, methyl ester	+0.691	-1.427	-1.284	-0.672
1.	9-Octadecenoic acid (Z)-, methyl ester	+1.137	-0.856	+0.068	+0.002
1.	11-Octadecenoic acid, methyl ester	+1.022	-0.312	-0.497	-1.088
1.	Methyl stearate	+1.332	-0.447	-0.319	-0.586
1.	Nonacos-1-ene	-1.219	-2.462	-1.365	-1.994
1.	Ascorbic acid	-4.655 (Ascorbic acid)	-5.965 (Ascorbic acid)	-	-
	5-Fluorouracil	-	-	-5.218	-5.2

Table 3

Interaction and bond distances of selected compounds with urate oxidase (PDB: 1R4U) active site

Protein	Ligands	Hydrogen bond interactions		Hydrophobic Interactions	
		Amino acid residue	Distance (Å)	Amino acid residue	Distance (Å)
1R4U	Cyclopropane, nonyl-			ALA-225	5.17
				PHE-159	4.60
				PHE-162	4.55
	1-Tetradecene			ARG-176	4.42
				PHE-159	5.30
				ILE-288	5.71
				VAL-227	4.00
	Phenol, 2,4-bis(1,1-dimethylethyl)-	LYS-171	5.48	ARG-176	5.22
				HIS-256	4.68
	Dodecanoic acid, methyl ester	ASN-254	5.21	ARG-176	4.67
		HIS-256	3.68	TYR-257	6.15
				PHE-159	5.12
				VAL-227	4.26
	E-14-Hexadecenal	ARG-176	7.32	PHE-159	3.41
		VAL-227	3.39	SER-226	4.05
				GLN-228	4.59
				ARG-176	6.57
				PHE-278	6.22
	Tridecanoic acid, 12-methyl, methyl ester	TYR-257	5.48	TYR-257	6.33
				ILE-177	4.32
				PHE-278	6.24
				ARG-176	4.95
				HIS-256	4.15
				PHE-159	5.13
				VAL-227	4.05
				ILE-288	4.52

E-15-Heptadecenal	ARG-176	6.99	PHE-278	6.20
	VAL-227	3.93		
9-Hexadecenoic acid, methyl ester, (Z)-	HIS-256	3.71	TYR-257	4.43, 5.83
	ASN-254	5.15	ILE-177	3.94, 3.72
	TYR-257	5.14	ARG-176	4.84
			ILE-288	5.34
			PHE-159	5.47
			VAL-227	4.36
Hexadecanoic acid, methyl ester	ARG-176	6.46	PHE-278	5.45
			ILE-177	3.78
			ARG-176	5.55
			VAL-227	3.91
			PHE-159	5.74
			GLN-228	4.31
			ILE-228	5.24
			ASN-254	4.49
1-Heneicosanol	ILE-177	3.71	TYR-257	6.91
	TYR-257			
n-Heptadecanol-1	TYR-257	5.34	VAL-227	4.31
	ILE-177	3.76	PHE-159	4.61
9,12-Octadecadienoic acid, methyl ester	HIS-256	3.61	ILE-177	4.21, 4.41
			TYR-257	5.08
			ARG-176	4.76
9-Octadecenoic acid (Z)-, methyl ester	ASN-254	5.19	ARG-176	4.73
	HIS-256	3.73	TYR-257	6.24, 5.86
11-Octadecenoic acid, methyl ester	ASN-254	5.16	TYR-257	5.81, 4.49
	HIS-256	3.69	ARG-176	4.87

	TYR-257	0.12	ILE-177	3.91, 3.67
Methyl stearate	ASN-254	5.22	ILE-177	3.92, 4.08
	HIS-256	3.76	TYR-257	5.63, 4.34
			ARG-176	4.66
			LEU-163	4.15
Nonacos-1-ene	TRP-160	4.92, 2.78	HIS-256	4.06
Ascorbic acid	HIS-256	4.03	TYR-257	6.50
	ILE-177	4.43, 3.63		
	TYR-257	5.46		
	GLU-259	4.18		

Table 4

Interaction and bond distances of selected compounds with glutathione reductase (PDB: 3GRS) active site

Protein	Ligands	Hydrogen bond interactions		Hydrophobic Interactions	
		Amino acid residue	Distance (Å)	Amino acid residue	Distance (Å)
3GRS	Cyclopropane, nonyl-			LYS-66	4.48
				ILE-198	5.13
				PHE-181	6.68
				MET-202	6.47
				CYS-58	4.28
	1-Tetradecene			ILE-198	4.41
				MET-202	5.52
				LYS-66	4.56
				ALA-342	4.87
				ALA-155	4.37
	Phenol, 2,4-bis(1,1-dimethylethyl)-	THR-156	4.70		
	Dodecanoic acid, methyl ester	THR-57	3.66	ALA-155	4.34
		HOH-490	3.23	LYS-66	4.01
	E-14-Hexadecenal	THR-57	3.74	MET-202	4.81
				ILE-198	4.93
				LYS-66	4.67
				PHE-181	6.20
	Tridecanoic acid, 12-methyl, methyl ester	HOH-482	2.74	PHE-181	6.21
		ASP-331	3.52	LYS-66	4.77
				MET-202	4.65
				ILE-198	4.37
				GLY-157	3.72
				GLY-330	4.71
				ALA-155	4.06
	E-15-Heptadecenal	THR-57	3.80	ILE-198	4.95
		HOH-490	3.15	PHE-181	5.91

			LYS-66	5.24
			MET-202	4.73
9-Hexadecenoic acid, methyl ester, (Z)-	HOH-484	2.51	PHE-181	6.27
	SER-30	3.94	MET-202	6.01
	GLY-31	4.05	LYS-66	4.17
			GLY-157	3.53
			ALA-155	4.50
Hexadecanoic acid, methyl ester	THR-339	3.86	CYS-63	6.07
			LEU-337	5.53
			LEU-338	3.98
			LEU-33	4.45
			TYR-114	5.22
1-Heneicosanol	THR-97	3.63	VAL-370	5.16
	HOH-490	3.22	LEU-444	4.46
n-Heptadecanol-1	THR-339	3.98	TYR-114	4.86
			LEU-33	4.11
9,12-Octadecadienoic acid, methyl ester	THR-339	3.71	CYS-63	4.79
			TYR-197	6.22, 6.63, 5.92
			LEU-338	5.16, 4.00
			PRO-340	4.98
9-Octadecenoic acid (Z)-, methyl ester	LYS-66	5.27	LEU-338	5.88
			TYR-197	4.64
			LEU-33	4.11
			TYR-114	4.83
11-Octadecenoic acid, methyl ester	THR-339	4.20	LEU-338	4.12
			CYS-63	5.80
			VAL-59	5.39
			TYR-114	5.45
			LEU-33	6.61

Methyl stearate	THR-57	4.10	PHE-181	5.52
			ILE-198	5.20
			MET-202	4.89
			GLU-50	4.62, 5.27
			GLY-56	3.89
Nonacos-1-ene			LYS-114	5.85
			GLY-29	4.58
			ALA-34	4.33
Ascorbic acid	THR-57	3.67		
	GLU-50	4.60, 4.15, 4.93		
	HOH-490	3.11		
	ALA-155	4.10		

Table 5

Interaction and bond distances of selected compounds with EGFR kinase domain (PDB: 2ITY) active site

Protein	Ligands	Hydrogen bond interactions		Hydrophobic Interactions	
		Amino acid residue	Distance (Å)	Amino acid residue	Distance (Å)
2ITY	Cyclopropane, nonyl-			LYS-745	4.50
				LEU-788	5.31
				MET-766	6.76
				LEU-718	4.75
	1-Tetradecene			LEU-718	5.02
				LYS-745	3.96
	Phenol, 2,4-bis(1,1-dimethylethyl)-	ASP-855	4.01	LEU-718	5.60
				VAL-726	5.78, 5.65
				LEU-844	6.15, 5.63
	Dodecanoic acid, methyl ester			THR-854	3.78
				ASP-855	3.76
				LYS-745	3.14
	E-14-Hexadecenal	LYS-716	5.37	LYS-728	5.45
				LYS-745	4.18
				LEU-788	3.84
	Tridecanoic acid, 12-methyl, methyl ester			MET-766	5.82
				LEU-788	2.64, 3.65, 4.22
				LYS-745	2.85
				LEU-792	4.93
				LEU-718	5.14, 4.92
	E-15-Heptadecenal	LYS-716	5.50	LEU-788	3.91
				LYS-745	3.89
	9-Hexadecenoic acid, methyl ester, (Z)-	LYS-716	5.50	LEU-788	3.62
				LYS-745	4.09
				MET-766	6.43
	Hexadecanoic acid, methyl	LYS-716	5.42	LYS-728	5.98

ester			LEU-788	3.47
			MET-766	5.97
1-Heneicosanol	GLU-804	4.20	GLU-804	4.13
			HIS-805	4.61
			LYS-745	3.95
			LEU-788	3.84
n-Heptadecanol-1	LYS-745	4.77	THR-854	3.78
	ASP-855	3.37	TYR-801	4.20
			HIS-805	5.72
9,12-Octadecadienoic acid, methyl ester	LYS-716	5.42	LYS-728	5.19
			PRO-794	4.71, 5.68
			LYS-745	4.14
			MET-766	6.14
			LEU-788	4.04
9-Octadecenoic acid (Z)-, methyl ester			LEU-718	5.01
			LEU-792	5.15
			GLY-724	3.19
			GLY-721	3.99
			ALA-722	4.99
			GLY-719	4.19
			VAL-726	5.12
11-Octadecenoic acid, methyl ester	LYS-716	5.50	LYS-728	5.63
			ALA-743	3.84
			LYS-745	4.35
Methyl stearate	LYS-728	5.69	GLU-1004	3.91, 5.24
	LYS-716	4.91	MET-766	5.71
			LEU-788	5.14
Nonacos-1-ene			LYS-728	3.44
			ILE-715	4.32

5-fluorouracil	ASP-855	3.60	LYS-745	4.42
	LYS-745	4.30	VAL-726	5.89

Table 6

Interaction and bond distances of selected compounds with Caspase 3 (PDB: 5IAE) active site

Protein	Ligands	Hydrogen bond interactions		Hydrophobic Interactions	
		Amino acid residue	Distance (Å)	Amino acid residue	Distance (Å)
5IAE	Cyclopropane, nonyl-			TYR-204	2.89
				TRP-206	4.80
	1-Tetradecene			MET-61	4.37
				PHE-128	6.49
	Phenol, 2,4-bis(1,1-dimethylethyl)-	ARG-207	3.53	TRP-206	5.90, 4.71
				TYR-204	4.62
	Dodecanoic acid, methyl ester	ASN-208	3.79	GLU-248	5.92
		TRP-214	5.23	TRP-206	4.29
		PHE-250	5.33	TYR-204	3.46
				PHE-256	5.22
				LEU-168	5.51
	E-14-Hexadecenal	ARG-64	4.21	SER-205	4.08
		ARG-207	3.74	PHE-256	4.91
	Tridecanoic acid, 12-methyl, methyl ester	TRP-214	5.18	TRP-214	5.45
		ASN-208	4.07	GLU-248	5.32
		PHE-250	5.85	SER-249	4.01
		PHE-256	5.14		
		LEU-168	5.27		
		TYR-204	3.48, 4.74		
		TRP-206	3.46, 4.21		
	E-15-Heptadecenal	ARG-64	3.93	PRO-133	5.54
		ARG-207	3.90		
	9-Hexadecenoic acid, methyl ester, (Z)-	PHE-250	5.35	GLU-248	5.46, 5.34
		ASN-208	3.71	HIS-121	4.14

	TRP-214	5.28	CYS-163	4.26
Hexadecanoic acid, methyl ester	TRP-214	3.74	TRP-214	4.76
	ASN-208	5.02	PHE-247	4.52
			PHE-256	5.12
			TYR-204	3.50
			LEU-168	5.20
			TRP-206	4.17
1-Heneicosanol	ARG-64	4.66	SER-120	5.18
n-Heptadecanol-1	ARG-64	4.81	SER-205	3.61
	SER-120	4.75		
9,12-Octadecadienoic acid, methyl ester	ASN-208	3.49	SER-249	4.44
	TRP-214	5.49	CYS-163	4.20
	PHE-250	5.50		
9-Octadecenoic acid (Z)-, methyl ester	ASN-208	3.73	GLU-248	5.75
	TRP-214	5.07	SER-249	4.20
11-Octadecenoic acid, methyl ester	PHE-250	5.49	SER-249	4.30
	TRP-214	5.35	MET-61	4.78
	ASN-208	3.54	PHE-128	5.20
			HIS-121	4.82
Methyl stearate	PHE-250	5.44	TRP-214	6.45
	TRP-214	5.19	GLU-248	5.36, 5.29
			MET-61	4.78
			PHE-128	6.32
Nonacos-1-ene			CYS-161	4.76
5-fluorouracil	SER-120	4.67	HIS-121	3.51
	GLN-161	3.89	CYS-163	5.84
	ARG-64	4.89		

Table 7

Physicochemical properties of the isolated compound from EAF for good oral bioavailability.

Compound	Lipinski Rules					Lipinski's Violations
	MW	HBA	HBD	Log P	MR	
	≤ 500	≤ 10	≤ 5	≤ 5	40–130	≤ 1
Cyclopropane, nonyl-	168.32	0	0	4.73	57.68	0
1-Tetradecene	193.37	0	0	5.71	68.94	1
Phenol,2,4-bis(1,1-dimethylethyl)-	206.32	1	1	3.09	67.01	0
Dodecanoic acid, methyl ester	214.34	2	0	4.10	65.89	0
E-14-Hexadecenal	238.41	1	0	5.13	78.75	1
Tridecanoic acid, 12-methyl-, methyl ester	242.40	2	0	4.75	75.50	0
E-15-Heptadecenal	252.44	1	0	5.50	83.56	1
9-Hexadecenoic acid, methyl ester, (Z)-	268.43	2	0	5.26	84.64	1
Hexadecanoic acid, methyl ester	270.45	2	9	5.54	85.12	2
1-Heneicosanol	312.57	1	1	7.25	104.22	1
n-Heptadecanol-1	256.5	1	1	7.8	82.34	1
9,12-Octadecadienoic acid, methyl ester	294.47	2	0	5.69	93.98	1
9-Octadecenoic acid (Z)-, methyl ester	296.49	2	0	5.95	94.26	1
11-Octadecenoic acid, methyl ester	296.49	2	9	5.95	94.26	2
Methyl stearate	298.50	2	0	6.24	94.73	1
Nonacos-1-ene	406.77	0	0	11.21	141.04	2
MW, Molecular weight (g/mol); HBA, Hydrogen bond acceptor; HBD, Hydrogen bond donor; Log P, Lipophilicity; MR, Molar refractivity.						

Table 8 Toxicological properties identified compounds from EAF fraction.

Parameters				
Compound	Ames toxicity	Carcinogens	Acute oral	Rat Acute Toxicity
Cyclopropane, nonyl-	NAT	NC	III	1.5283
1-Tetradecene	NAT	Carcinogens	III	1.3452
Phenol, 2,4-bis(1,1-dimethylethyl)-	NAT	NC	III	2.2064
Dodecanoic acid, methyl ester	NAT	Carcinogens	III	1.4915
E-14-Hexadecenal	NAT	NC	III	1.6100
Tridecanoic acid, 12-methyl-, methyl ester	NAT	NC	III	1.5702
E-15-Heptadecenal	NAT	NC	III	1.6100
9-Hexadecenoic acid, methyl ester, (Z)-	NAT	Carcinogens	III	1.7357
Hexadecanoic acid, methyl ester	NAT	Carcinogens	III	1.4915
1-Heneicosanol	NAT	NC	III	1.5561
n-Heptadecanol-1	NAT	NC	III	1.5561
9,12-Octadecadienoic acid, methyl ester	NAT	Carcinogens	III	1.7357
9-Octadecenoic acid (Z)-, methyl ester	NAT	Carcinogens	III	1.7357
11-Octadecenoic acid, methyl ester	NAT	Carcinogens	III	1.7357
Methyl stearate	NAT	Carcinogens	III	1.4915
Nonacos-1-ene	NAT	Carcinogens	III	1.3452
NAT, Non Ames toxic; NC, Non-carcinogenic; Category-I ($LD_{50} \leq 50\text{mg/kg}$); Category-II ($50\text{mg/kg} < LD_{50} < 500\text{mg/kg}$); Category-III ($500\text{mg/kg} < LD_{50} < 5000\text{mg/kg}$); Category-IV ($5000\text{mg/kg} < LD_{50}$)				

Table 9

Biological activities predicted for identified compounds by PASS online.

Name	Characteristics	Biological Properties Predicted by Pass Online	P _a	P _i
Cyclopropane, nonyl-	Antioxidant	Free radical scavenger	0.181	0.093
		Peroxidase substrate	0.400	0.020
		Reductant	0.461	0.033
		Lipid peroxidase inhibitor	0.322	0.057
		Antiviral (Parainfluenza)	0.065	0.012
		Antiviral (Influenza)	0.572	0.015
		Antiviral (Rhinovirus)	0.595	0.007
		Antiviral (Picornavirus)	0.699	0.005
		Antiviral (Adenovirus)	0.395	0.031
		Antiviral (CMV)	0.376	0.007
		Antiviral (Herpes)	0.396	0.037
		Antiviral (Influenza A)	0.261	0.081
		Antiviral (Hepatitis)	0.111	0.084
		Antiviral (Poxvirus)	0.333	0.045
	Anticancer	Antineoplastic , Alkylator	0.156	0.033
		Antineoplastic (endocrine cancer)	0.178	0.073
		Antineoplastic (bone cancer)	0.283	0.010
		Antineoplastic (thyroid cancer)	0.181	0.053
		Antineoplastic (non-Hodgkin's lymphoma)	0.381	0.126
		Antineoplastic (multiple myeloma)	0.271	0.115
		Antineoplastic antimetabolite	0.119	0.073
		Antineoplastic (sarcoma)	0.166	0.113
		Antineoplastic (insulinoma)	0.014	0.008
1-Tetradecene	Antioxidant	Antioxidant	0.282	0.027
		Free radical scavenger	0.286	0.034
		Reductant	0.461	0.033

	Antiviral	Lipid peroxidase inhibitor	0.364	0.046
		Antiviral	0.197	0.099
		Antiviral (Influenza)	0.396	0.048
		Antiviral (Rhinovirus)	0.651	0.004
		Antiviral (Picornavirus)	0.542	0.035
		Antiviral (Adenovirus)	0.417	0.023
		Antiviral (CMV)	0.512	0.002
		Antiviral (Herpes)	0.417	0.028
		Antiviral (Hepatitis B)	0.173	0.127
		Antiviral (Poxvirus)	0.279	0.071
		Antiviral (Herpesvirus 3, Human)	0.023	0.003
		Antiviral (Hepatitis)	0.169	0.023
		Antiviral (Hepatitis C)	0.144	0.020
		Antiviral (Parainfluenza)	0.076	0.008
	Anticancer	Antineoplastic (endocrine cancer)	0.216	0.037
		Antineoplastic (thyroid cancer)	0.214	0.023
		Antineoplastic, alkylator	0.128	0.049
		Antineoplastic antimetabolite	0.134	0.059
		Antineoplastic (liver cancer)	0.263	0.035
Phenol, 2,4-bis(1,1-dimethylethyl)-	Antioxidant	Antioxidant	0.525	0.006
		Free radical scavenger	0.532	0.009
		Peroxidase substrate	0.703	0.003
		Reductant	0.808	0.004
		Lipid peroxidase inhibitor	0.629	0.008
	Antiviral	Antiviral	0.181	0.119
		Antiviral (Influenza)	0.471	0.028
		Antiviral (Rhinovirus)	0.299	0.254
		Antiviral (Picornavirus)	0.425	0.091

			Antiviral (Adenovirus)	0.369	0.043
			Antiviral (CMV)	0.274	0.049
			Antiviral (Herpes)	0.275	0.110
			Antiviral (Poxvirus)	0.274	0.075
			Antiviral (Parainfluenza)	0.039	0.037
		Anticancer	Antineoplastic (squamous cell carcinoma)	0.133	0.043
			Antineoplastic (bladder cancer)	0.165	0.097
			Antineoplastic (bone cancer)	0.199	0.160
			Antineoplastic, alkylator	0.102	0.074
			Antineoplastic alkaloid	0.166	0.149
			Antineoplastic (cervical cancer)	0.103	0.095
Dodecanoic acid, methyl ester	Antioxidant		Antioxidant	0.210	0.050
			Free radical scavenger	0.332	0.025
			Peroxidase substrate	0.424	0.017
			Reductant	0.523	0.020
			Lipid peroxidase inhibitor	0.292	0.067
	Antiviral		Antiviral	0.176	0.125
			Antiviral (Influenza)	0.417	0.041
			Antiviral (Influenza A)	0.227	0.145
			Antiviral (Rhinovirus)	0.616	0.005
			Antiviral (Picornavirus)	0.554	0.031
			Antiviral (Adenovirus)	0.425	0.020
			Antiviral (CMV)	0.438	0.004
			Antiviral (Herpes)	0.392	0.039
			Antiviral (Herpesvirus 3, Human)	0.012	0.008
			Antiviral (Hepatitis B)	0.219	0.070
			Antiviral (Parainfluenza)	0.072	0.009
	Anticancer		Antineoplastic (non-Hodgkin's lymphoma)	0.409	0.099

			Antineoplastic (endocrine cancer)	0.214	0.038
			Antineoplastic (thyroid cancer)	0.222	0.019
			Antineoplastic, alkylator	0.218	0.016
			Antineoplastic antimetabolite	0.205	0.032
			Antineoplastic (liver cancer)	0.215	0.069
			Antineoplastic (solid tumors)	0.238	0.154
			Antineoplastic (sarcoma)	0.165	0.117
			Antineoplastic (insulinoma)	0.012	0.009
			Antineoplastic (bladder cancer)	0.156	0.117
E-14-Hexadecenal	Antioxidant		Antioxidant	0.164	0.086
			Free radical scavenger	0.185	0.089
			Peroxidase substrate	0.471	0.012
			Reductant	0.346	0.068
			Lipid peroxidase inhibitor	0.202	0.131
	Antiviral		Antiviral	0.177	0.124
			Antiviral (Influenza)	0.434	0.036
			Antiviral (Influenza A)	0.233	0.132
			Antiviral (Rhinovirus)	0.497	0.025
			Antiviral (Picornavirus)	0.445	0.078
			Antiviral (Adenovirus)	0.334	0.064
			Antiviral (CMV)	0.343	0.012
			Antiviral (Herpes)	0.390	0.040
			Antiviral (Herpesvirus 3, Human)	0.015	0.004
			Antiviral (Poxvirus)	0.225	0.123
			Antiviral (Parainfluenza)	0.042	0.032
	Anticancer		Antineoplastic (brain cancer)	0.223	0.089
			Antineoplastic antibiotic	0.139	0.035
			Antineoplastic (bone cancer)	0.192	0.182

Tridecanoic acid, 12-methyl-, methyl ester		Antineoplastic, alkylator	0.161	0.031
		Antineoplastic antimetabolite	0.119	0.073
	Antioxidant	Antioxidant	0.243	0.038
		Free radical scavenger	0.312	0.028
		Peroxidase substrate	0.396	0.020
		Reductant	0.384	0.055
		Lipid peroxidase inhibitor	0.302	0.063
	Antiviral	Antiviral	0.234	0.070
		Antiviral (Influenza)	0.388	0.050
		Antiviral (Influenza A)	0.282	0.056
		Antiviral (Rhinovirus)	0.595	0.007
		Antiviral (Picornavirus)	0.482	0.058
		Antiviral (Adenovirus)	0.404	0.028
		Antiviral (CMV)	0.379	0.006
		Antiviral (Herpes)	0.394	0.038
		Antiviral (Hepatitis B)	0.243	0.054
		Antiviral (Poxvirus)	0.253	0.093
		Antiviral (Parainfluenza)	0.056	0.017
	Anticancer	Antineoplastic (non-Hodgkin's lymphoma)	0.478	0.047
		Antineoplastic (endocrine cancer)	0.252	0.025
		Antineoplastic (bone cancer)	0.255	0.027
		Antineoplastic (thyroid cancer)	0.209	0.025
		Antineoplastic, alkylator	0.171	0.027
		Antineoplastic antimetabolite	0.159	0.044
		Antineoplastic (liver cancer)	0.186	0.103
		Antineoplastic (solid tumors)	0.227	0.170
		Antineoplastic antibiotic	0.078	0.063
		Antineoplastic (insulinoma)	0.017	0.005

E-15-Heptadecenal	Antioxidant	Antioxidant	0.164	0.086
		Free radical scavenger	0.185	0.089
		Peroxidase substrate	0.471	0.012
		Reductant	0.346	0.068
		Lipid peroxidase inhibitor	0.202	0.131
	Antiviral	Antiviral	0.177	0.124
		Antiviral (Rhinovirus)	0.497	0.025
		Antiviral (Picornavirus)	0.445	0.078
		Antiviral (Adenovirus)	0.334	0.064
		Antiviral (CMV)	0.343	0.012
		Antiviral (Herpes)	0.390	0.040
		Antiviral (Herpesvirus 3, Human)	0.015	0.004
		Antiviral (Poxvirus)	0.225	0.123
		Antiviral (Parainfluenza)	0.042	0.032
	Anticancer	Antineoplastic (brain cancer)	0.223	0.089
		Antineoplastic, alkylator	0.161	0.031
		Antineoplastic antimetabolite	0.119	0.073
		Antineoplastic (bone cancer)	0.192	0.182
		Antineoplastic antibiotic	0.139	0.035
9-Hexadecenoic acid, methyl ester, (Z)-	Antioxidant	Antioxidant	0.269	0.030
		Free radical scavenger	0.380	0.019
		Peroxidase substrate	0.548	0.007
		Reductant	0.594	0.012
		Lipid peroxidase inhibitor	0.326	0.056
	Antiviral	Antiviral	0.165	0.141
		Antiviral (Influenza)	0.492	0.024
		Antiviral (Influenza A)	0.208	0.200
		Antiviral (Rhinovirus)	0.639	0.004
		Antiviral (Picornavirus)	0.497	0.052

		Antiviral (Adenovirus)	0.387	0.035
		Antiviral (CMV)	0.488	0.003
		Antiviral (Herpes)	0.415	0.029
		Antiviral (Herpesvirus 3, Human)	0.013	0.006
		Antiviral (Hepatitis B)	0.181	0.114
		Antiviral (Parainfluenza)	0.049	0.023
	Anticancer	Antineoplastic (non-Hodgkin's lymphoma)	0.425	0.086
		Antineoplastic (endocrine cancer)	0.163	0.102
		Antineoplastic (thyroid cancer)	0.197	0.034
		Antineoplastic, alkylator	0.189	0.021
		Antineoplastic antimetabolite	0.148	0.050
		Antineoplastic (liver cancer)	0.273	0.030
		Antineoplastic (solid tumors)	0.244	0.145
		Antineoplastic (lymphoma)	0.137	0.125
		Antineoplastic antibiotic	0.090	0.054
Hexadecanoic acid, methyl ester	Antioxidant	Antioxidant	0.210	0.050
		Free radical scavenger	0.332	0.025
		Peroxidase substrate	0.424	0.017
		Reductant	0.523	0.020
		Lipid peroxidase inhibitor	0.292	0.067
	Antiviral	Antiviral	0.176	0.125
		Antiviral (Influenza)	0.417	0.041
		Antiviral (Influenza A)	0.227	0.145
		Antiviral (Rhinovirus)	0.616	0.005
		Antiviral (Picornavirus)	0.554	0.031
		Antiviral (Adenovirus)	0.425	0.020
		Antiviral (CMV)	0.438	0.004
		Antiviral (Herpes)	0.392	0.039

			Antiviral (Herpesvirus 3, Human)	0.012	0.008
			Antiviral (Hepatitis B)	0.219	0.070
			Antiviral (Poxvirus)	0.352	0.039
			Antiviral (Parainfluenza)	0.072	0.009
		Anticancer	Antineoplastic (non-Hodgkin's lymphoma)	0.409	0.099
			Antineoplastic (endocrine cancer)	0.214	0.038
			Antineoplastic (bone cancer)	0.220	0.094
			Antineoplastic (thyroid cancer)	0.222	0.019
			Antineoplastic, alkylator	0.218	0.016
			Antineoplastic antimetabolite	0.205	0.032
			Antineoplastic (liver cancer)	0.215	0.069
			Antineoplastic (solid tumors)	0.238	0.154
			Antineoplastic (sarcoma)	0.165	0.117
			Antineoplastic (bladder cancer)	0.156	0.117
			Antineoplastic (insulinoma)	0.012	0.009
	1-Heneicosanol	Antioxidant	Antioxidant	0.229	0.043
			Free radical scavenger	0.265	0.040
			Peroxidase substrate	0.630	0.004
			Reductant	0.731	0.005
			Lipid peroxidase inhibitor	0.504	0.016
		Antiviral	Antiviral	0.202	0.094
			Antiviral (Influenza)	0.516	0.020
			Antiviral (Influenza A)	0.286	0.052
			Antiviral (Rhinovirus)	0.642	0.004
			Antiviral (Picornavirus)	0.666	0.009
			Antiviral (Adenovirus)	0.528	0.004
			Antiviral (CMV)	0.489	0.003
			Antiviral (Herpes)	0.442	0.020

			Antiviral (Herpesvirus 3, Human)	0.022	0.003
			Antiviral (Hepatitis B)	0.265	0.044
			Antiviral (Poxvirus)	0.431	0.025
			Antiviral (Parainfluenza)	0.231	0.002
		Anticancer	Antineoplastic (non-Hodgkin's lymphoma)	0.521	0.030
			Antineoplastic (multiple myeloma)	0.244	0.167
			Antineoplastic (lymphoma)	0.135	0.128
			Antineoplastic, alkylator	0.281	0.010
			Antineoplastic antimetabolite	0.216	0.030
			Antineoplastic (liver cancer)	0.294	0.022
			Antineoplastic (bone cancer)	0.257	0.025
		Antioxidant	Antioxidant	0.296	0.024
			Free radical scavenger	0.332	0.025
			Peroxidase substrate	0.591	0.005
			Reductant	0.637	0.009
			Lipid peroxidase inhibitor	0.354	0.048
9,12-Octadecadienoic acid, methyl ester		Antiviral	Antiviral (Influenza)	0.441	0.035
			Antiviral (Rhinovirus)	0.627	0.005
			Antiviral (Picornavirus)	0.458	0.071
			Antiviral (Adenovirus)	0.362	0.047
			Antiviral (CMV)	0.466	0.003
			Antiviral (Herpes)	0.401	0.035
			Antiviral (Herpesvirus 3, Human)	0.011	0.009
			Antiviral (Hepatitis B)	0.168	0.139
			Antiviral (Poxvirus)	0.257	0.089
			Antiviral (Parainfluenza)	0.043	0.030
		Anticancer	Antineoplastic (non-Hodgkin's lymphoma)	0.389	0.118

		Antineoplastic (thyroid cancer)	0.189	0.042
		Antineoplastic, alkylator	0.167	0.028
		Antineoplastic antimetabolite	0.132	0.060
		Antineoplastic (liver cancer)	0.291	0.023
		Antineoplastic (solid tumors)	0.231	0.164
		Antineoplastic (lymphoma)	0.181	0.069
		Antineoplastic antibiotic	0.089	0.055
9-Octadecenoic acid methyl ester, (E)-	Antioxidant	Antioxidant	0.269	0.030
		Free radical scavenger	0.380	0.019
		Peroxidase substrate	0.548	0.007
		Reductant	0.594	0.012
		Lipid peroxidase inhibitor	0.326	0.056
	Antiviral	Antiviral	0.165	0.141
		Antiviral (Influenza)	0.492	0.024
		Antiviral (Influenza A)	0.208	0.200
		Antiviral (Rhinovirus)	0.639	0.004
		Antiviral (Picornavirus)	0.497	0.052
		Antiviral (Adenovirus)	0.387	0.035
		Antiviral (CMV)	0.488	0.003
		Antiviral (Herpes)	0.415	0.029
		Antiviral (Herpesvirus 3, Human)	0.013	0.006
		Antiviral (Hepatitis B)	0.181	0.114
		Antiviral (Poxvirus)	0.279	0.071
		Antiviral (Parainfluenza)	0.049	0.023
	Anticancer	Antineoplastic (non-Hodgkin's lymphoma)	0.425	0.086
		Antineoplastic (endocrine cancer)	0.163	0.102
		Antineoplastic (thyroid cancer)	0.197	0.034
		Antineoplastic, alkylator	0.189	0.021

11-Octadecenoic acid, methyl ester		Antineoplastic antimetabolite	0.148	0.050
		Antineoplastic (liver cancer)	0.273	0.030
		Antineoplastic (solid tumors)	0.244	0.145
		Antineoplastic (lymphoma)	0.137	0.125
		Antineoplastic antibiotic	0.090	0.054
	Antioxidant	Antioxidant	0.269	0.030
		Free radical scavenger	0.380	0.019
		Peroxidase substrate	0.548	0.007
		Reductant	0.594	0.012
		Lipid peroxidase inhibitor	0.326	0.056
	Antiviral	Antiviral	0.165	0.141
		Antiviral (Influenza)	0.492	0.024
		Antiviral (Influenza A)	0.208	0.200
		Antiviral (Rhinovirus)	0.639	0.004
		Antiviral (Picornavirus)	0.497	0.052
		Antiviral (Adenovirus)	0.387	0.035
		Antiviral (CMV)	0.488	0.003
		Antiviral (Herpes)	0.415	0.029
		Antiviral (Herpesvirus 3, Human)	0.013	0.006
		Antiviral (Hepatitis B)	0.181	0.114
		Antiviral (Poxvirus)	0.279	0.071
		Antiviral (Parainfluenza)	0.049	0.023
	Anticancer	Antineoplastic (non-Hodgkin's lymphoma)	0.425	0.086
		Antineoplastic (endocrine cancer)	0.163	0.102
		Antineoplastic (thyroid cancer)	0.197	0.034
		Antineoplastic, alkylator	0.189	0.021
		Antineoplastic antimetabolite	0.148	0.050

Methyl stearate	Antioxidant	Antineoplastic (liver cancer)	0.273	0.030
		Antineoplastic (solid tumors)	0.244	0.145
		Antineoplastic (lymphoma)	0.137	0.125
		Antineoplastic antibiotic	0.090	0.054
		Antineoplastic antibiotic	0.639	0.004
	Antioxidant	Antioxidant	0.210	0.050
		Free radical scavenger	0.332	0.025
		Peroxidase substrate	0.424	0.017
		Reductant	0.523	0.020
		Lipid peroxidase inhibitor	0.292	0.067
	Antiviral	Antiviral	0.176	0.125
		Antiviral (Influenza)	0.417	0.041
		Antiviral (Influenza A)	0.227	0.145
		Antiviral (Rhinovirus)	0.616	0.005
		Antiviral (Picornavirus)	0.554	0.031
		Antiviral (Adenovirus)	0.425	0.020
		Antiviral (CMV)	0.438	0.004
		Antiviral (Herpes)	0.392	0.039
		Antiviral (Herpesvirus 3, Human)	0.012	0.008
		Antiviral (Hepatitis B)	0.219	0.070
		Antiviral (Poxvirus)	0.352	0.039
		Antiviral (Parainfluenza)	0.072	0.009
	Anticancer	Antineoplastic (non-Hodgkin's lymphoma)	0.409	0.099
		Antineoplastic (endocrine cancer)	0.214	0.038
		Antineoplastic (thyroid cancer)	0.222	0.019
		Antineoplastic, alkylator	0.218	0.016
		Antineoplastic antimetabolite	0.205	0.032
		Antineoplastic (liver cancer)	0.215	0.069

Nonacos-1-ene	Antioxidant	Antineoplastic (solid tumors)	0.238	0.154
		Antineoplastic (insulinoma)	0.012	0.009
		Antineoplastic (bone cancer)	0.220	0.094
		Antineoplastic (sarcoma)	0.165	0.117
		Antineoplastic (Bladder cancer)	0.156	0.117
	Antioxidant	Antioxidant	0.282	0.027
		Free radical scavenger	0.286	0.034
		Peroxidase substrate	0.468	0.012
		Reductant	0.461	0.033
		Lipid peroxidase inhibitor	0.364	0.046
	Antiviral	Antiviral	0.197	0.099
		Antiviral (Influenza)	0.396	0.048
		Antiviral (Rhinovirus)	0.651	0.004
		Antiviral (Picornavirus)	0.542	0.035
		Antiviral (Adenovirus)	0.417	0.023
		Antiviral (CMV)	0.512	0.002
		Antiviral (Herpes)	0.417	0.028
		Antiviral (Herpesvirus 3, Human)	0.023	0.003
		Antiviral (Hepatitis)	0.169	0.023
		Antiviral (Poxvirus)	0.279	0.071
		Antiviral (Parainfluenza)	0.076	0.008
	Anticancer	Antineoplastic (ovarian cancer)	0.150	0.059
		Antineoplastic (pancreatic cancer)	0.281	0.074
		Antineoplastic (thyroid cancer)	0.214	0.023
		Antineoplastic (endocrine cancer)	0.216	0.037
		Antineoplastic (cervical cancer)	0.177	0.050
		Antineoplastic, alkylator	0.128	0.049

n-Heptadecanol-1		Antineoplastic (pancreatic cancer)	0.281	0.074
		Antineoplastic antimetabolite	0.134	0.059
		Antineoplastic (liver cancer)	0.263	0.035
	Antioxidant	Antioxidant	0.229	0.043
		Free radical scavenger	0.265	0.040
		Peroxidase substrate	0.630	0.004
		Reductant	0.731	0.005
		Lipid peroxidase inhibitor	0.504	0.016
	Antiviral	Antiviral	0.202	0.094
		Antiviral (Influenza)	0.516	0.020
		Antiviral (Influenza A)	0.286	0.052
		Antiviral (Rhinovirus)	0.642	0.004
		Antiviral (Picornavirus)	0.666	0.009
		Antiviral (Adenovirus)	0.528	0.004
		Antiviral (CMV)	0.489	0.003
		Antiviral (Herpes)	0.442	0.020
		Antiviral (Herpesvirus 3, Human)	0.022	0.003
		Antiviral (Hepatitis B)	0.265	0.044
		Antiviral (Poxvirus)	0.431	0.025
		Antiviral (Parainfluenza)	0.231	0.002
	Anticancer	Antineoplastic (non-Hodgkin's lymphoma)	0.521	0.030
		Antineoplastic (multiple myeloma)	0.244	0.167
		Antineoplastic (lymphoma)	0.135	0.128
		Antineoplastic, alkylator	0.281	0.010
		Antineoplastic antimetabolite	0.216	0.030
		Antineoplastic (liver cancer)	0.294	0.022
		Antineoplastic (bone cancer)	0.257	0.025

Table 10

Correlation coefficients between the total phenolic contents and antioxidant as well as HeLa cell proliferation.

Total Phenolic Contents (Correlation R ²)						
Assays	DPPH	Hydroxyl	Lipid Peroxide	TAC	FRPC	MTT
CME	0.55856	0.93479 ^{**}	0.89227 [*]	0.9888 ^{***}	0.90741 [*]	0.99414 [*]
PEF	1.9483	0.97567 ^{**}	0.9105 [*]	0.92046 ^{**}	0.97598 ^{**}	0.92154
CHF	0.14652 ^{**}	0.95742 ^{**}	0.88646 [*]	0.97608 ^{**}	0.99748 ^{****}	0.99996 ^{**}
EAF	0.0808	0.66511	0.8493 [*]	0.99566 ^{***}	0.88279 [*]	0.97599
AQF	0.8171	0.94858 ^{**}	0.85996 [*]	0.99387 ^{***}	0.99265 ^{***}	0.97742
Indicates significance at [] <i>P</i> <0.05, ^{**} <i>P</i> <0.01, ^{***} <i>P</i> <0.001, ^{****} <i>P</i> <0.0001. Where, DPPH assay =2,2-diphenyl-1-picrylhydrazyl assay, TAC = Total Antioxidant Capacity, FRPC = Ferric Reducing Power Capacity and MTT Assay = 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide Assay						

Table 11

Correlation coefficients between the total flavonoid contents and antioxidant as well as HeLa cell proliferation.

Total flavonoid contents (Correlation R ²)						
Assays	DPPH	Hydroxyl	Lipid Peroxide	TAC	FRPC	MTT
CME	0.55856	0.93479 ^{**}	0.89227 [*]	0.9888 ^{***}	0.90741 [*]	0.99414 [*]
PEF	0.66765	0.97567 ^{**}	0.9105 [*]	0.92046 ^{**}	0.97598 ^{**}	0.92154
CHF	0.97345 ^{**}	0.95742 ^{**}	0.88646 [*]	0.97608 ^{**}	0.99748 ^{****}	0.99996 ^{**}
EAF	0.4918	0.66511	0.8493 [*]	0.99566 ^{***}	0.88279 ^{**}	0.97599
AQF	0.55222	0.94858 ^{**}	0.85996 [*]	0.99387 ^{***}	0.99265 ^{***}	0.97742
Indicates significance at [] <i>P</i> <0.05, ^{**} <i>P</i> <0.01, ^{***} <i>P</i> <0.001, ^{****} <i>P</i> <0.0001. where, DPPH assay =2,2-diphenyl-1-picrylhydrazyl assay, TAC = Total Antioxidant Capacity, FRPC = Ferric Reducing Power Capacity and MTT Assay = 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide Assay						

Figures

Figure 1

Polyphenolic contents of CME and its different fractions. (A) Total phenolic (mg of GAE/gm of dried sample) and (B) flavonoid contents (mg of CAE/gm of dried sample) of CME and it's four fractions (PEF, CHF, EAF and AQF). Data expressed as mean $\pm$ SD (n = 3) for all tested dosages. Where, methanolic extract of *L. aequata* (CME), petroleum ether (PEF), chloroform (CHF), ethyl acetate (EAF), and aqueous (AQF) fractions.

Figure 2

Figure 2

Comparative antioxidant activity of CME and its different fractions. (A) Total antioxidant and (B) Ferrous reducing antioxidant capacity of CME and its four fractions (PEF, CHF, EAF and AQF). Data expressed as mean $\pm$ SD (n = 3) for all tested dosages. Where, methanolic extract of *L. aequata* (CME), petroleum ether (PEF), chloroform (CHF), ethyl acetate (EAF), and aqueous (AQF) fractions.

Figure 3

Figure 3

Comparative DPPH free radical scavenging activity of CME and its different fractions. (A) DPPH free radical scavenging and (B) IC₅₀ (µg/mL) values of CME and its four fractions (PEF, CHF, EAF and AQF). Data expressed as mean ± SD (n = 3) for all tested dosages. Values with (*p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001) were considered significant. Where, methanolic extract of *L. aequata* (CME), petroleum ether (PEF), chloroform (CHF), ethyl acetate (EAF), and aqueous (AQF) fractions.

Figure 4

Figure 4

Comparative hydroxyl radical scavenging activity of CME and its different fractions. (A) Hydroxyl radical scavenging and (B) IC₅₀ (µg/mL) values of CME and its four fractions (PEF, CHF, EAF and AQF). Data expressed as mean ± SD (n = 3) for all tested dosages. Values with (*p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001) were considered significant. Where, methanolic extract of *L. aequata* (CME), petroleum ether (PEF), chloroform (CHF), ethyl acetate (EAF), and aqueous (AQF) fractions.

Figure 5

Figure 5

Comparative lipid peroxidation inhibition of CME and its different fraction. (A) Lipid peroxidation inhibition and (B) IC₅₀ (µg/mL) values CME and its four fractions (PEF, CHF, EAF and AQF). Data expressed as mean ± SD (n = 3) for all tested dosages. Values with (*p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001) were considered significant. Where, methanolic extract of *L. aequata* (CME), petroleum ether (PEF), chloroform (CHF), ethyl acetate (EAF), and aqueous (AQF) fractions.

Figure 6

Figure 6

Percent (%) of viable cells of CME and its four fractions (PEF, CHF, EAF and AQF) of *L. aequata* leaves on HeLa cells. Data expressed as mean $\pm$ SD (n = 3) for all tested dosages.

Figure 7

A

Untreated HeLa Cells

B

EAF-treated Cells

Figure 7

Measurement of apoptosis of EAF of *L. aequata* leaves on HeLa cells.

Figure 8

Figure 8

Gas chromatography-mass spectrometry profile of EAF was obtained from GC-MS with electron impact ionization (EI) method on a gas chromatograph (GC-17A, Shimadzu Corporation, Kyoto, Japan) coupled to a mass spectrometer (GC-MS TQ 8040, Shimadzu Corporation, Kyoto, Japan).

Figure 9

3D representation of molecular docking simulation between urate oxidase (PDB:1R4U) and (A) Cyclopropane, nonyl-; (B) 1-Tetradecene; (C) Phenol, 2,4-bis(1,1-dimethylethyl)-; (D) Dodecanoic acid, methyl ester; (E) E-14-Hexadecenal; (F) Tridecanoic acid, 12-methyl-, methyl ester; (G) E-15-Heptadecenal; (H) 9-Hexadecenoic acid, methyl ester, (Z)-; (I) Hexadecanoic acid, methyl ester; (J) 1-Heneicosanol; (K) n-Heptadecanol-1; (L) 9,12-Octadecadienoic acid, methyl ester; (M) 9-Octadecenoic acid (Z)-, methyl ester; (N) 11-Octadecenoic acid, methyl ester; (O) Methyl stearate; (P) Nonacos-1-ene; (Q) Ascorbic acid.

Figure 10

3D representation of molecular docking simulation between glutathione reductase (PDB: 3GRS) and (A) Cyclopropane, nonyl-; (B) 1-Tetradecene; (C) Phenol, 2,4-bis(1,1-dimethylethyl)-; (D) Dodecanoic acid, methyl ester; (E) E-14-Hexadecenal; (F) Tridecanoic acid, 12-methyl-, methyl ester; (G) E-15-Heptadecenal; (H) 9-Hexadecenoic acid, methyl ester, (Z)-; (I) Hexadecanoic acid, methyl ester; (J) 1-Heneicosanol; (K) n-Heptadecanol-1; (L) 9,12-Octadecadienoic acid, methyl ester; (M) 9-Octadecenoic acid (Z)-, methyl ester; (N) 11-Octadecenoic acid, methyl ester; (O) Methyl stearate; (P) Nonacos-1-ene; (Q) Ascorbic acid.

Figure 11

3D representation of molecular docking simulation between EGFR kinase domain (PDB: 2ITY) and (A) Cyclopropane, nonyl-; (B) 1-Tetradecene; (C) Phenol, 2,4-bis(1,1-dimethylethyl)-; (D) Dodecanoic acid, methyl ester; (E) E-14-Hexadecenal; (F) Tridecanoic acid, 12-methyl-, methyl ester; (G) E-15-Heptadecenal; (H) 9-Hexadecenoic acid, methyl ester, (Z)-; (I) Hexadecanoic acid, methyl ester; (J) 1-Heneicosanol; (K) n-Heptadecanol-1; (L) 9,12-Octadecadienoic acid, methyl ester; (M) 9-Octadecenoic acid (Z)-, methyl ester; (N) 11-Octadecenoic acid, methyl ester; (O) Methyl stearate; (P) Nonacos-1-ene; (Q) 5-fluorouracil

Figure 12

3D representation of molecular docking simulation between caspase 3 (PDB: 5IAE) and (A) Cyclopropane, nonyl-; (B) 1-Tetradecene; (C) Phenol, 2,4-bis(1,1-dimethylethyl)-; (D) Dodecanoic acid, methyl ester; (E) E-14-Hexadecenal; (F) Tridecanoic acid, 12-methyl-, methyl ester; (G) E-15-Heptadecenal; (H) 9-Hexadecenoic acid, methyl ester, (Z)-; (I) Hexadecanoic acid, methyl ester; (J) 1-Heneicosanol; (K) n-Heptadecanol-1; (L) 9,12-Octadecadienoic acid, methyl ester; (M) 9-Octadecenoic acid (Z)-, methyl ester; (N) 11-Octadecenoic acid, methyl ester; (O) Methyl stearate; (P) Nonacos-1-ene; (Q) 5-fluorouracil