Bangladesh Pharmaceutical Journal ***27****(2): 137-148, 2024 (July)*

In vitro Cytotoxic and Anti-ROS Potentials of the Aerial Part of *Pyrrosia lanceolata* on A549 Cells and Cell Free Condition

Azim Uddin[1], A.S.M. Ali Reza[2], Adeeba Anjum[1], Md. Aziz Abdur Rahman[1], Samina Yesmin[3], Mst Samima Nasrin[1], Lima Khatun[1], Md. Golam Mostofa[4], Md. Golam Sadik[1] and A.H.M. Khurshid Alam[1]

[1]Department of Pharmacy, University of Rajshahi, Rajshahi 6205, Bangladesh
[2]Department of Pharmacy, International Islamic University Chittagong, Chittagong, Bangladesh
[3]BTRI, Bangladesh Council of Scientific and Industrial Research, Dhaka, Bangladesh
[4]Department of Pharmacy, Gono Bishwabidyalay, Savar, Dhaka, Bangladesh

(Received: November 20, 2023; Accepted: February 15, 2024; Published (web): July 30, 2024)

Abstract

Antioxidants and cancer are somewhat related to each other. The study was designated to evaluate the antioxidant activities of the aerial parts of *Pyrosia lanceolata* (*P. lanceilata*, Fam. Polypodiaceae) and their cytotoxic potential in a lung cancer cell line (A549). The extraction of plant material was performed by using methanol (CME), followed by solvent-solvent partitioning with different solvents. The methanol (CME) and its subsequent fractions (pet-ether PEF, chloroform CHF, ethyl acetate EAF and aqueous AQF fractions) confirmed the presence of alkaloids, flavonoids, phenols, tannins and terpenoids at different degrees. The phenolic and flavonoid content were found to be 69.46, 37.93, 64.61, 126.74 and 60.64 mg of GAE/gm and 33.93, 4.11, 45.68, 146.95, and 44.36 mg of CAE/gm of dried CME, PEF, CHF, EAF and AQF, respectively. The overall antioxidant and iron reduction capacities of CME and all the fractions were in the following order: EAF > CHF > CME > AQF > PEF and EAF > CHF > CME > AQF > PEF, respectively. Among the fractions, EAF significantly scavenged DPPH (IC_{50} of 1.34 µg/ml) and OH radicals (IC_{50} of 4.38 µg/ml), which closely resembled those of standard BHT (IC_{50} of 0.9 µg/ml) and CA (IC_{50} of 2.73 µg/ml), respectively. Moreover, EAF (IC_{50} of 14.06 µg/ml) demonstrated a similar effect on inhibiting lipid peroxidation when compared to standard CA (IC_{50} of 13.86 µg/ml). In addition, CHF (24.25%) and EAF (14.9%) showed a potent cytotoxic effect in A549 cells. Our results suggest that *P. lanceolata* might be a good source of anticancer potential having antioxidant activity.

Key words: Antioxidant, free radical, *Pyrrosia lanceolata*, polypodiaceae, cytotoxicity, A549.

Introduction

Complementary alternative therapy is used for cancer treatment in addition to standard treatments such as acupuncture, dietary supplements, massage therapy, hypnosis and meditation (Giard *et al.*, 1973) though they have detrimental effects on general health. There has been an increase in research into using traditional herbal medicine to treat lung carcinomas in an effort to address these particular shortcomings of the available treatments. Traditional medicines are thought to have a wide pharmacological action that benefits several biological targets in numerous ways with little to no negative side effects (Simsek *et al.*, 2017) https://www.mdpi.com/2218-1989/13/4/480-B6-metabolites-13-00480. Thus, it is thought that the creation of these plant-based traditional medicines may result in the discovery of several unique compounds and their combinations that can have the desired effects on various cancer-related signaling

Corresponding author: AHM Khurshid Alam, E-mail: khurshid.jaist@gmail.com
DOI: https://doi.org/10.3329/bpj.v27i2.75182

pathways while also reducing side effects when compared to conventional chemotherapeutics.

According to contemporary theories, oxidative stress (OS) is intimately linked to every aspect of cancer, including carcinogenesis and the tumor-bearing stage (Noda and Wakasugi, 2001). OS can be minimized by increasing the levels of endogenous and exogenous antioxidants. Antioxidants that lower OS are abundant in plants (Rahman *et al.*, 2019). Plants have traditionally been employed to cure a number of ailments, including cancer. As a result of such ongoing research, many secondary plant metabolites, including vincristine, vinblastine, paclitaxel, docetaxel, etoposide, taniposide, camptothecin and irinotecan are functioning as anticancer drugs (Agarwal *et al.*, 2012). Well-stored plant kingdoms in Bangladesh can help in ethnopharmacological research on plants, which may offer helpful tips for finding new cancer medicines. The National Cancer Institute (NCI) has screened over thirty five thousand plant species for chemicals that may have anticancer properties; interestingly, roughly only three thousand plants (Desai *et al.*, 2008) exhibit cytotoxic potential. This was the rationale behind selecting *Pyrrosia lanceolata* (*P. lanceolata*) from *Pyrrosia* genus for the study.

P. lanceolata is a member of Polypodiaceae family and is locally called "Jibonti". There are about 100 species in the *Pyrrosia* genus. The plant is located in Bangladesh, India, Sri Lanka, Bhutan, China, Taiwan, Malaysia, New Guinea and Sumatra. In Bangladesh, it has traditionally been used to treat rheumatism, starvation and weariness (Anzumi *et al.*, 2014). It has a long history of use as a medicinal fern for sore throats and skin conditions. According to Gnana and Catharin (2014), the entire plant is used as an astringent and to cure diarrhea. It has been revealed that ashes and pieces of burnt plant are applied to newly cut or wounded areas to stop the bleeding and that frond juice is administered to treat diarrhea and burns (Quattrocchi 2012). Despite its impressive traditional and therapeutic uses for a variety of diseases, a little chemical and biological potentials are known. Therefore, present study aimed to investigate the possible cytotoxic effect of *P. lanceolata* on lung carcinoma cells, free radical scavenging and antioxidant properties as well as the phytochemical screening of the aerial part of the plant.

Methods and Materials

Plant collection: In January 2021, the aerial parts of *P. lanceolata* was collected from the University of Rajshahi campus. A taxonomist from the Department of Botany at University of Rajshahi recognized the plant and Bangladesh National Herbarium kept a voucher specimen for this collection, accession number DACB-51235. The plant was then cleaned with DM water, dried in the sun shade for a few days and the dried materials were further ground into coarse powder and stored for later use at room temperature.

Extraction and solvent-solvent partitioning: About 250 g of dried powder was immersed in 1.5 liters of pure methanol within an amber glass extractor bottle. The bottle was placed in a common location and subjected to intermittent agitation and stirring, prior to being strained through a series of filters, starting with cotton and subsequently utilizing filter paper (Whatman No. 1). The resulting liquid was then processed using a rotary evaporator (BIOBASE, BK-RE-1A) under controlled conditions of low pressure and temperature (40 °C) and obtained 30 gm of methanolic extract (abbreviated as CME). An aliquot was prepared by adding 90 ml methanol and 10 ml water in 30 gm of dried CME, which was then subjected to fractionate by modified Kupchan method (Kupchan *et al.*, 1973). The amounts of obtained petroleum ether fraction (PEF), chloroform fraction (CHF), ethyl acetate fraction (EAF) and aqueous fraction (AQF) are shown in table 1.

Determination of total phenolics: The total phenolic content was determined by using the Folin-Ciocalteu reagent (FCR) where gallic acid (GA) used as a standard, as previously reported by Mostofa *et al.* (2024). Here, 2 ml of the Folin-Ciocalteu reagent (10× dilution in deionized water) was mixed with different concentrations of 0.4 ml plant extracts or

standard followed by Na_2CO_3 solution (2 ml, 7.5%). Subsequently, absorbance was recorded with a UV spectrophotometer (Shimadzu, USA) at 760 nm. Gallic acid equivalent, or GAE, was used to represent the total phenolic contents as mg of GAE /g of dry extract.

Table 1. Weight (gm) of different fractions after partitioning of CME.

CME and its different fractions	Weight (gm) of CME and its different fractions
CME	110 gm
PEF	76 gm
CHF	14 gm
EAF	5 gm
AQF	15 gm

Determination of total flavonoids: A colorimetric assay for aluminum chloride ($AlCl_3$) was used to calculate the amount of the total flavonoids (Zhishen *et al.*, 1999). The absorbance of the solution was measured at 510 nm wavelength. Total flavonoid content was expressed in terms of catechin equivalent, CAE, mg of CAE/g of dry extract.

Determination of total antioxidants: The experiment was conducted using phosphomolybdate methodology where catechin was used as a standard (Prieto *et al.*, 1999). The test is based on samples reducing Mo (VI) to Mo (V) and the green phosphate/Mo (V) complex forming at an acidic pH. The absorbance was measured at 695 nm.

DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay: The DPPH radical-scavenging assay, as reported by Blois (1958), was used to examine the extracts' capacity to scavenge free radicals where BHT is used as standard. The plant extracts' capacity to donate hydrogen atoms was assessed by measuring the decolorization of a methanol solution of DPPH. When antioxidants exist, the violet or purple color that DPPH creates in methanol solutions become change to light yellow. Using spectrophotometry, the mixture's absorbance has been determined at 517 nm. The percentage of DPPH radical scavenging activity was calculated by the following equation:

$$\% \text{ DPPH radical scavenging activity} = [A_0 - A_1 / A_0] \times 100$$

where A_0 is the absorbance of the control and A_1 is the absorbance of the extracts or standard. After that, the IC_{50} was determined by graphing the percent of inhibition against concentration.

Ferric reducing power capacity: The ferric (Fe^{3+})-reducing power capacity of the extracts and standard was measured by using the method of Oyaizu (1986). By detecting the production of Perl's Prussian blue at 700 nm in comparison to a blank, the Fe^{2+} concentration can be measured. Increased absorbance is the indication of increased reducing capacity. The experiment was repeated three times at each concentration.

Hydroxyl radical scavenging assay: The capability of the extracts or standard to neutralize hydroxyl radicals was evaluated using a technique developed by Halliwell (1989). The Fe^{3+}-ascorbate-EDTA-H_2O_2 system (Fenton reaction) produced the hydroxyl radical. The test is based on measuring the 2-deoxy-D-ribose breakdown product, which, when heated with TBA at a low pH, develops a pink chromogen. Using a spectrophotometer, the absorbance of the sample has been measured at 532 nm. By measuring the % of inhibition of 2-deoxy-D-ribose oxidation on OH^-, the hydroxyl radical scavenging ability was assessed. The following formula was used to calculate the % of inhibition:

$$\% \text{ OH radicals scavenging activity} = [(A_1-A_2)/A_1]\times 100$$

Where A_1 denotes control's absorbance and A_2 denotes extract's or standard's absorbance. At each concentration, the experiment was conducted three times.

Ethics approval and consent to participate: The Institute of Biological Sciences, University of Rajshahi, Bangladesh accepted the set of guidelines that were followed for the experiment with rat, according to university guidelines (approval no: 249 (35)/320/IAMEBBC/IBSc).

Euthanasia method: We applied 70% (v/v) ethanol in 0.9% sterile saline in the ventral chest region for getting deep aesthesia (Underwood *et al.*, 2013, Allen-Worthington *et al.*, 2015).

Lipid peroxidation inhibition activity: The lipid peroxidation inhibition assay was calculated using the method as described by Liu and Ng (2000). The liver of a rat that was removed was blended in a buffer and then centrifuged to produce liposomes. The mixture of 0.5 ml of supernatant, 100 µl of 0.1 mM AA, 100 µl 10 mM $FeSO_4$, and 0.3 ml of extractives or standards at different concentrations (6.25-100 µg/ml) were combined to get the total volume up to 1 ml. After the incubation at 37 °C for 25 min, TCA (1 ml of 28%) and TBA (1.5 ml of 1%) were added immediately. Lastly, after being heated again at 100 °C for 15 min, the reaction mixture was cooled to RT and its absorbance was measured at 532 nm. The equation used to calculate the % of inhibition is as follows:

$$\% \text{ lipid peroxidation inhibition} = [(A_1-A_2)/A_2] \times 100$$

where A_1 is the absorbance of the control and A_2 is the absorbance of the extractives or standard. Plotting the percentage of inhibition against concentration allowed for the computation of the IC_{50}.

Cytotoxic assay: The cytotoxic activity was measured according to the method described by Strober (2015). A lung cancer cell line (A549) is originally obtained from American Type Culture Collection. A549 was cultured in DMEM medium with 5% CO_2 for 48 hours with and without samples. The number of viable cells present in a cell suspension was measured based on the principle that live cells with intact cell membranes reject trypan blue dye, whereas dead cells do not. The cells' ability to absorb or reject the dye is assessed visually by mixing the dye with a cell suspension, where the cytoplasm of a live cell is transparent, while that of a nonviable cell is blue. The total number of dead cells was counted in a counter chamber using a microscope. The following formula was used to get the percentage of viable cells:

$$\% \text{ of viable cells} = [(V_t-V_d)/V_t] \times 100$$

where V_t is the total number of cells and V_d is the number of death cells. Plotting the percentage of viable cells versus concentration allowed for the calculation of the IC_{50}.

Statistical analysis: The data, obtained from triple trials, are presented as the mean with standard deviation. Dunnett's test (OS Windows, GraphPad software) was used after one-way analysis of variance (ANOVA) to determine if there were any significant differences between the test and control groups. *P*-value of < 0.01 was considered statistically significant.

Results

Qualitative phytochemical analysis: The CME and its extractives (PEF, CHF, EAF, and AQF) derived from *P. lanceolata* were subjected to qualitative phytochemical analysis to detect the presence of different types of phytochemicals. The results for different phytoconstituents are shown in table 2. A significant number of flavonoids, glycosides, phenols and terpenoids was present in the EAF. Alkaloids, tannins and terpenoids were highly noticeable in CHF. Other fractions contained a mild to moderate amounts of phytoconstituents.

Determination of total phenolics: The total phenolics content in CME and its four fractions of *P. lanceolata* was measured using the standard curve of GA. Phenolic compounds were most abundant in EAF, followed by CME, CHF, AQF and PEF (Figure 1A), indicating that the plant might be a reliable source for polyphenols, including flavonoids.

Determination of total flavonoids: The total flavonoids content in CME and its four fractions of *P. lanceolata* was determined using the standard curve of CA. The concentrations of flavonoids in the different extracts were in the following order: EAF>CHF>AQF>CME>PEF (Figure 1B). Based on the findings of the phenolic and flavonoid content of the extractives, it is suggested that some of the fractions, particularly EAF and CHF, may have antioxidant activity (Kurutas, 2016).

Table 2. Qualitative tests of different fractions of CME and its four fractions.

Test for phytoconstituents	CME	PEF	CHF	EAF	AQF
Alkaloids	+	++	+++	+	+
Flavonoids	++	+	++	+++	++
Glycosides	+	+	+	+++	+
Phenols	++	+	++	+++	++
Tannins	++	++	+++	+	+
Terpenoids	++	++	+++	+++	+++

NB: (-) = Not present, + = Present in small amount, ++ = Present in moderate amount, +++ = Present in large amount.

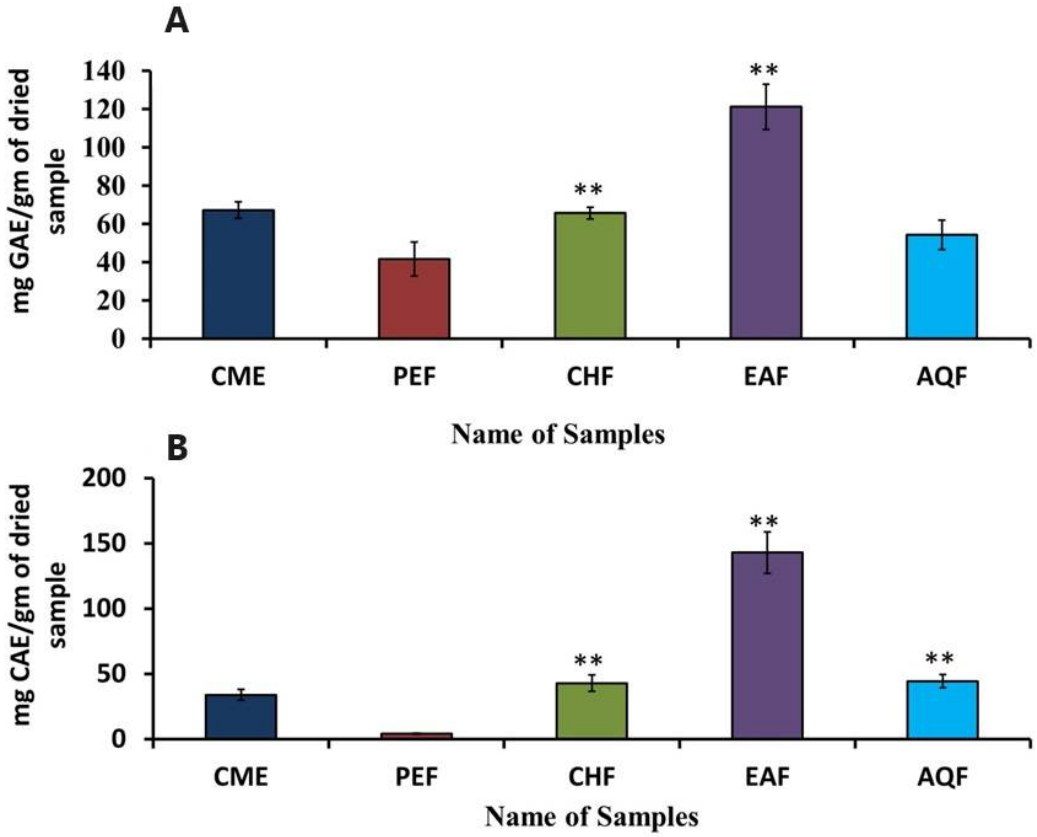

Figure 1. Comparison of (A) total phenolics content and (B) total flavonoids content of CME and four fractions of *P. lanceolata*. Here, CME = Crude methanolic extract, PEF= Petroleum ether fraction, CHF = Chloroform fraction, EAF = Ethyl acetate fraction and AQF = Aqueous fraction. Values with **$p < 0.01$ were considered significant. All data are expressed as the mean ± SD (n=3).

Determination of total antioxidant activity: The total antioxidant activity of the extractives of *P. lanceolata* and CA was determined at a number of concentrations ranging from 3.125-100 µg/ml. In this experiment, increased absorbance is the indication of increased total antioxidant capacity. The total antioxidant activity of CA, CME, and its four fractions was found to be 0.786 ± 0.008, 0.367 ±

0.018, 0.169 ± 0.015, 0.429 ± 0.006, 0.402 ± 0.006, and 0.377 ± 0.016, respectively at a concentration of 100 µg/ml (Figure 2). The fractions CHF, EAF and AQF possessed good antioxidant activity, indicating that the plant may have radical scavenging activity as antioxidants suppress OS caused by free radicals (Rahman *et al.*, 2021).

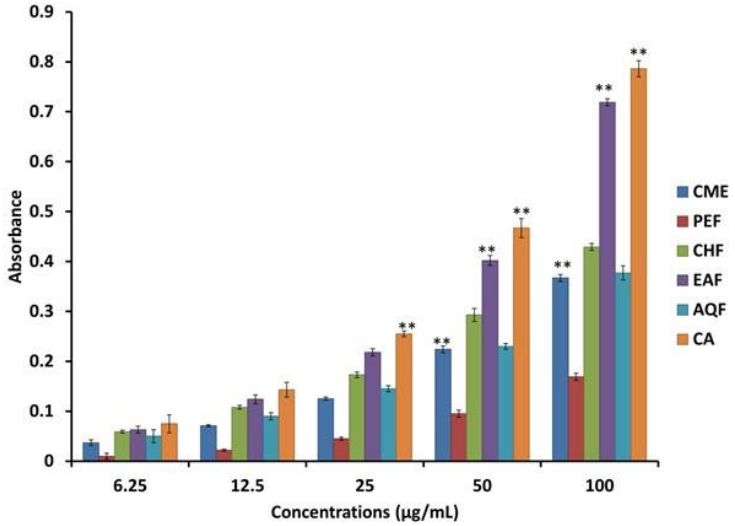

Figure 2. Total antioxidant activity of CME and four fractions of *P. lanceolata*. Here, CME = Crude methanolic extract, PEF= Petroleum ether fraction, CHF = Chloroform fraction, EAF = Ethyl acetate fraction and AQF = Aqueous fraction. Values with **p < 0.01 were considered significant. All data are expressed as the mean ± SD (n=3).

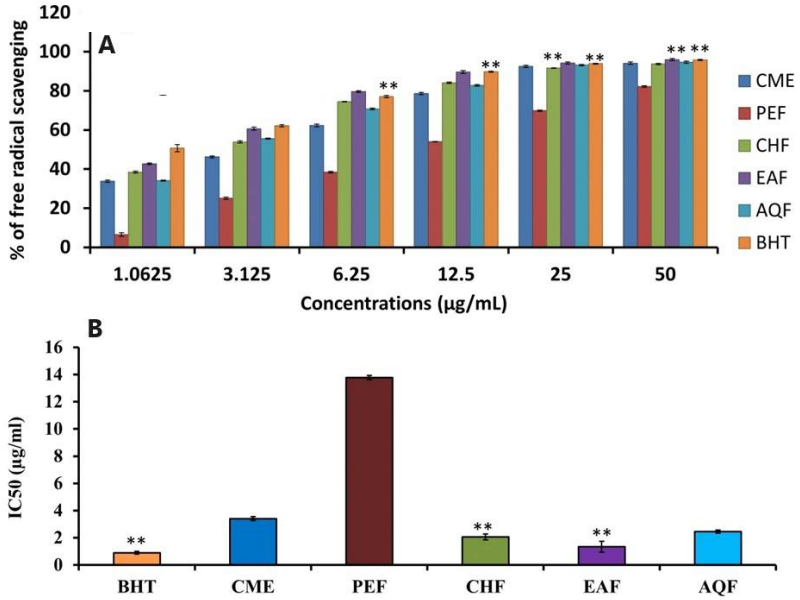

Figure 3. (A) DPPH free radical scavenging activity of BHT, CME and four fractions of *P. lanceolata* and (B) Comparison of IC_{50} values of standard BHT, CME and four fractions. The data is expressed as mean ± SD (n = 3) for all dosages tested. Values with **p < 0.01 were considered significant. All data are expressed as the mean ± SD (n=3).

Determination of DPPH radical scavenging activity: The DPPH radical scavenging activity of different extractives of *P. lanceolata* and BHT as a standard was determined using a number of concentrations ranging from 3.125-100 µg/ml. The findings of the DPPH radical scavenging assay of BHT, CME, PEF, CHF, EAF and AQF are shown in figure 3A (Concentration vs % of free radical scavenging). EAF displayed notable antioxidant activity, with its scavenging ability being comparable to that of BHT (standard). Among the extractives, the IC_{50} values of EAF and CHF were 1.34 and 2.06 µg/ml, respectively; while the IC_{50} value of standard BHT was 0.9 µg/ml, demonstrating that the extractives exhibited varying degrees of potent radical scavenging action (Figure 3B).

Determination of iron reducing power: The values of Fe^{3+} reducing power of the different extractives of *P. lanceolata* and CA are shown in figure 4. The extractives and standard have the following orders of reducing power capacities: CA> EAF>CHF>AQF>CME>PEF at a concentration of 100 µg/ml. The EAF demonstrated considerable decreases in power capacity, with the magnitude of reduction being almost identical to that of the standard (CA).

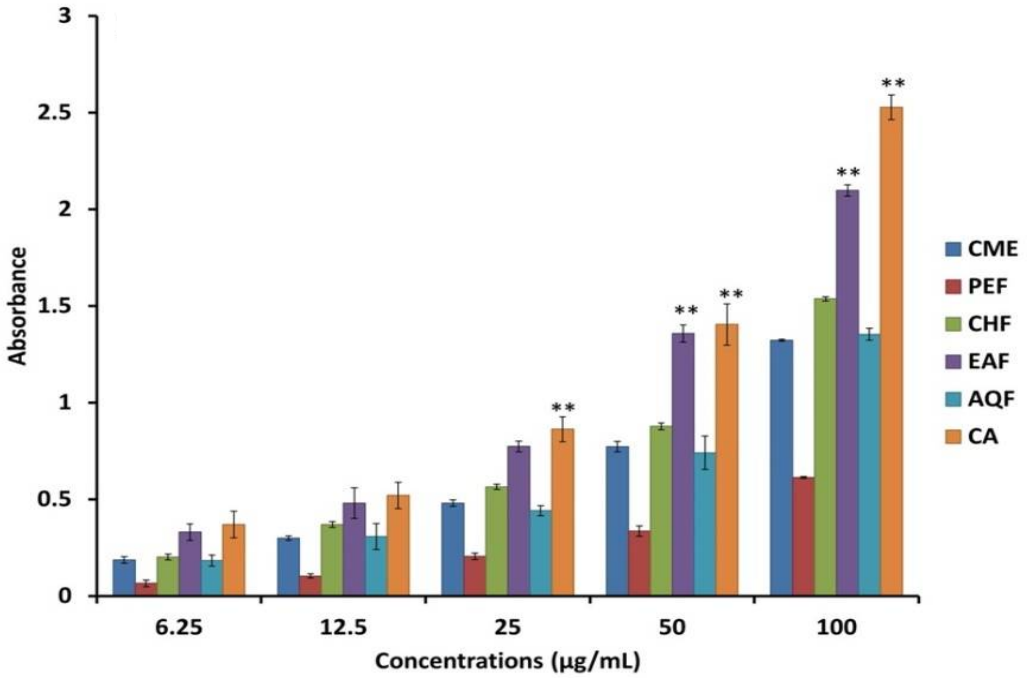

Figure 4. Comparison of the reducing power capacity of standard CA, CME and four fractions of *P. lanceolata* at different concentrations. The data is expressed as mean ± SD (n = 3) for all dosages tested. Values with **p < 0.01 were considered significant. Here, CME = Crude methanolic extract, PEF= Petroleum ether fraction, CHF = Chloroform fraction, EAF = Ethyl acetate fraction and AQF = Aqueous fraction. All data are expressed as the mean ± SD (n=3).

Determination of hydroxyl radical scavenging activity: The hydroxyl radical scavenging activity was evaluated using different concentrations of the extractives and CA, ranging from 3.125-100 µg/ml. The percentage of hydroxyl radical scavenging of CA, CME, PEF, CHF, EAF and AQF was 92.53 ± 1.068, 84.46 ± 1.438, 62.4 ± 1.605, 80.49 ± 1.266, 87.74 ± 1.075, and 88.63 ± 1.206, respectively at a concentration of 100 µg/ml with IC_{50} values of 2.73, 7.53, 29.65, 8.38, 4.38, and 5.26 µg/ml, respectively

(Figure 5A and 5B). The radical scavenging activity of all the extractives was significant except PEF (62.4 ± 1.605). Importantly, EAF scavenged hydroxyl radicals significantly, which was comparable to standard CA.

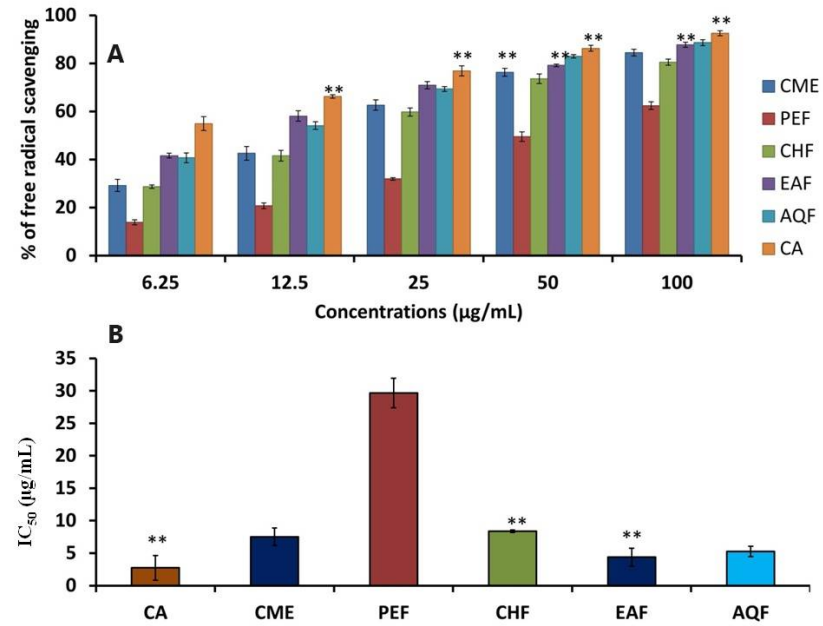

Figure 5. (A) Comparison of hydroxyl radical scavenging activity of CA, CME and four fractions of *P. lanceolata*. (B) Comparison of IC_{50} (µg/mL) values of CA, CME and its four fractions of *P. lanceolata*. Values with (**p < 0.01) were considered significant. Here, CME = Crude methanolic extract, PEF= Petroleum ether fraction, CHF = Chloroform fraction, EAF = Ethyl acetate fraction and AQF = Aqueous fraction. All data are expressed as the mean ± SD (n=3).

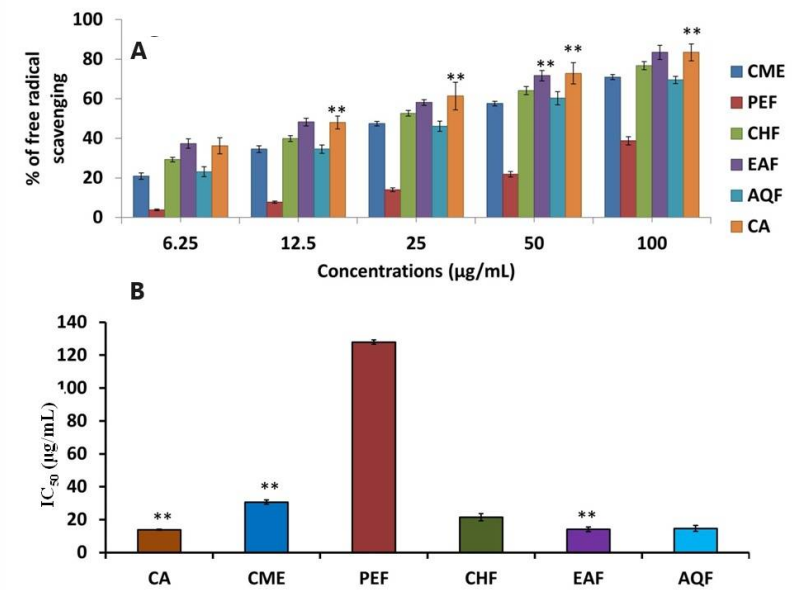

Figure 6. (A). Comparison of lipid peroxidation inhibition activity of standard CA, CME, and four fractions of *P. lanceolata* at different concentrations and (B) Comparison of IC_{50} values of CA, CME, and four fractions of *P. lanceolata*. Values with **p < 0.01 were considered significant. Here, CME = Crude methanolic extract, PEF= Petroleum ether fraction, CHF = Chloroform fraction, EAF = Ethyl acetate fraction and AQF = Aqueous fraction. All data are expressed as the mean ± SD (n=3).

Lipid peroxidation inhibition activity: The results of lipid peroxidation inhibition of the extractives are shown in figures 6A and 6B. At a concentration of 100 µg/ml, % of inhibition for CA, CME, PEF, CHF, EAF and AQF was 83.44 ± 4.25, 70.91 ± 1.29, 38.72 ± 2.08, 76.73 ± 2.08, 83.43 ± 3.64 and 69.52 ± 1.83, respectively. The IC_{50} values were 13.87, 30.62, 127.96, 21.53, 14.04, and 14.66 µg/ml, respectively. The inhibition of lipid peroxides by EAF was similar to that of standard CA (Figures 6A and 6B).

In vitro cytotoxic activity: Different concentrations ranging from 0 to 40 µg/ml of the tested samples were applied to the A548 cancer cell line. The percent of viable cells was found to be 94.8, 91.5, 75.5, 85.1, and 89.1 (Figure 7). Among the extractives, EAF and CHF showed significant inhibition of the growth of A549 cells.

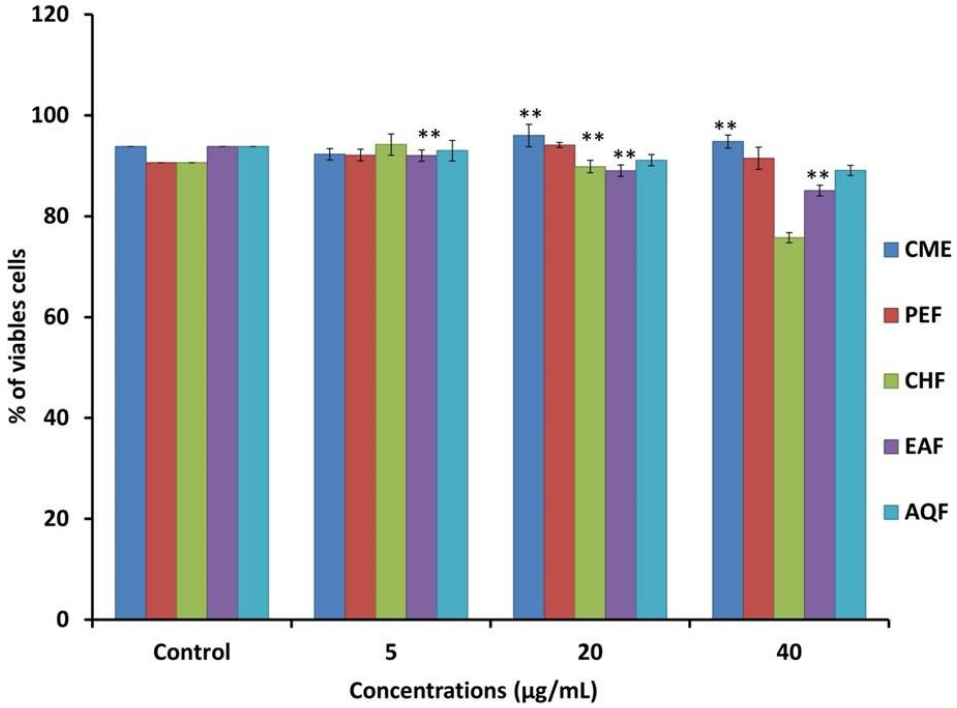

Figure 7. Percentage (%) of viable cells of CME and four fractions from *P. lanceolata* on lung cancer cell line (A549). The data is expressed as mean ± SD (n = 3) for all dosages tested. Values with (**p < 0.01) were considered significant. Here, CME = Crude methanolic extract, PEF= Petroleum ether fraction, CHF = Chloroform fraction, EAF = Ethyl acetate fraction and AQF = Aqueous fraction.

Discussion

Qualitative phytochemical screening, which establishes the presence or absence of phytochemicals, is the initial stage in phytochemical analysis. Alkaloids, flavonoids, glycosides, tannins and terpenoids were present in CME and its other fractions. In contrast to EAF, which indicates a modest number of flavonoids, glycosides, phenols, and terpenoids, CHF suggests a significant presence of alkaloids, tannins and terpenoids. In addition, terpenoids, tannins and flavonoids were mildly to moderately prevalent in CME, PEF, and AQF.

Quantitative phytochemical analysis takes into account the quantity or concentration of phytochemicals contained in the plant. Quantitative phytochemical analysis is a more thorough and useful technique than qualitative phytochemical analysis because the results of the studies can be used to develop new drugs, standardize herbal medicines, explain plant medicinal potentials and estimate plant

toxicity levels (Egbuna *et al.*, 2018). The hydroxyl groups in phenolic compounds play a vital role to neutralize free radicals, thereby contributing to antioxidant activity (Aryal *et al.*, 2019). In this study, overall phenolic contents varied significantly among fractions, but CHF possessed a notable concentration of phenolic compounds, indicating that the fraction may be a promising source of antioxidants. Another important plant metabolite is flavonoids, which have antioxidant activity. Like phenolics, the amount and position of free hydroxyl groups of flavonoids affect its antioxidant activity (Reza *et al.*, 2018). The total amount of flavonoids in each extract varied but EAF contained a high number of flavonoids showing that EAF is a rich source of polyphenols (phenolics and flavonoids).

The quantity of free radicals that a test solution can neutralize is measured to determine the total antioxidant activity. This is done to gauge the biological samples' ability to act as antioxidants (Rubio *et al.*, 2016). All the extracts displayed varying levels of activity in the phosphomolybdenum assay. Increased absorbance is a sign of increased antioxidant activity. The EAF has the highest antioxidant activity. This study showed that antioxidant-rich botanical supplements can lessen free radicals, which are known to cause a variety of diseases in humans (Alam *et al.*, 2016). The reducing power of plant polyphenols can be used to assess their antioxidant activity. The reducing power is generally associated with the presence of reductants, which donates a hydrogen atom and exert antioxidant action by breaking the free radical chains (Reza *et al.*, 2021). The polyphenolics riched fraction (EAF) showed the highest reducing power capacity suggesting that EAF would be a valuable source of antioxidants (Reza *et al.*, 2018; Tareq *et al.*, 2023).

Scavenging free radicals is a vital process that helps to protect against the harmful effects of free radicals in various diseases, including cancer (Rahman *et al.*, 2015). Antioxidants are thought to exert their effect on DPPH radicals by donating hydrogen, which is eagerly accepted by DPPH, thereby quenching its reactivity. When DPPH accepts an electron or hydrogen atom, it loses its absorption spectrum band at 515-528 nm and becomes neutral with a pale yellow to colorless appearance (Hossain *et al.*, 2018). Hydrogen atoms donating ability of EAF considerably reduced the number of DPPH free radicals, indicating its potential in the treatment of diseases. According to a recent investigation (REF), free radicals play a role in the development of numerous persistent health issues, including heart disease, inflammation, cataracts and cancer (Lobo *et al.*, 2010, Martemucci *et al.*, 2022).

Hydroxyl radical ($HO^{\bullet}$) is the most reactive oxygen-based molecule and can extensively damage nearby biological molecules (Pavithra and Vadivukkarasi, 2015). The "deoxyribose assay" is commonly used to assess $HO^{\bullet}$ scavenging. CHF and EAF having significant polyphenolic compounds showed the strongest $HO^{\bullet}$ scavenging activity by donating hydrogen atoms. The capability of the plant extracts to quench HO• might directly relate to the prevention of lipid peroxidation. Moreover, the most crucial measure of antioxidant activity is the ability to prevent lipid peroxidation (Kurutas, 2016).

Conclusions

P. lanceolata, a promising candidate for polyphenols, alkaloids, flavonoids, tannins and terpenoids in varying concentrations. The plant is also a good source of antioxidants, which inhibit lipid peroxidation and scavenge free radicals. Moreover, it shows potential cytotoxic activities. Therefore, it is advised to investigate the molecular mechanisms by which the plant scavenges free radicals and exhibits cytotoxic activity.

Ethics approval and consent to participate

All methods for animal experiments were carried out by the institutional guideline and regulations, and ethical permission was taken from Institute of Biological Sciences (IBSc), University of Rajshahi, Bangladesh (license no: 249 (35)/320/IAMEBBC/ IBSc).

Funding

Faculty of Science, University of Rajshahi (RU), Rajshahi. Grant/Award Number: 911/5/52/RU/Science-39/20-21.

Acknowledgments

The authors wish to thank the Department of Pharmacy, University of Rajshahi for kind support to progress the research. The authors gratefully acknowledge the financial support from the faculty of science, University of Rajshahi, Bangladesh.

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