Egypt. J. Chem. **Vol. 68,** SI: Z. M. Nofal, pp. 381- 390 (2025)

Egyptian Journal of Chemistry

http://ejchem.journals.ekb.eg/

Phytochemical Characterization and *In vitro* Hepatoprotective Effect of Egyptian *Litchi sinensis* Sonn. Leaves Against *tert*-butyl Hydroperoxide Induced Hepatotoxicity

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Abstract

Background: *Litchi sinensis* Sonn. is traditionally known as an efficient remedy for inflammation, liver, and skin diseases in Chinese medicine. Since most research has focused on the analysis of seeds and fruits. The current study aims to provide a quantitative metabolite profiling of the polyphenolic compounds of *Litchi* leaves and highlight their antioxidant and hepatoprotective activity, as no detailed metabolite characterization is reported for the cultivated Egyptian leaves.

Methods & Results: In our study, Cell-free screening of the antioxidant potential using DPPH method revealed that the ethyl acetate fraction was the most active among other fractions. Interestingly, a dose-dependent increment of Hepa1c1c7 cell viability was detected in cells pretreated with ethyl acetate fraction using the MTT method. However, the cytoprotecting activity was not accomplished through NAD(P)H dehydrogenase (NQO1) induction. Therefore, the potential activity may be ascribed to the 'direct' antioxidant effect. Total phenolics, flavonoids, and proanthocyanidins were quantitatively measured in ethyl acetate fraction to yield 77.3±0.42, 11.4±0.12, and 46.5±0.33%, respectively. HPLC analysis of the ethyl acetate fraction revealed the presence of protocatechuic acid (452.58±1.86 ug/g) as the major phenolic acid. Naringenin was identified for the first time in *Litchi* leaves, accounting for 2503.21±2.03 ug/g. Moreover, epicatechin gallate, catechin, and epigallocatechin gallate were the prominent identified proanthocyanidins monomers, accounting for 8050.8±13.6, 2385.75±1.56 & 2053.2±10.03 µg/g from the ethyl acetate fraction.

Conclusion: The results suggested that *Litchi sinensis* leaves phenolics exhibited a protective effect against tert-butyl hydroperoxide-induced hepatotoxicity through the direct antioxidant effect, recommending their use as a promising candidate for the development of hepatoprotective drugs.

Keywords: Antioxidant; Cytoprotective; *Litchi*; Procyanidins; Sapindaceae.

Introduction

Litchi chinensis Sonn. is a beautiful non-climacteric evergreen tree of the Sapindaceae family commonly known as Chinese Cherry, or Mountain Lychee [1]. It is extensively cultivated in the subtropical regions of America, and Asia [2]. The fruit has a characteristic bright red color with a sweet odor of rose and juicy pulp. It is consumed worldwide as a rich source of vitamins and minerals [1,3]. The cultivated *Litchi* in China is commonly known as the king of fruit [4]. Moreover, fruits and seeds demonstrate anti-hyperlipidemic, anti-platelet, hypoglycemic, and anticancer activities while the pericarp is described as anti-tussive, antibacterial, antipyretic, and analgesic [1,5]. Extensive reports highlight the nutritional value of *Litchi* fruit along with its processing by-products such as peel and seeds [1,6,7]. Recently, the anticancer potential of the seeds has been documented against prostate cancer through inhibition of the hepatocyte growth factor receptor/ Nuclear Factor Kappa B signaling pathway [8]. The traditional Chinese herbal medicine highlighted the hepatoprotective activity of phenolic compounds of Chinese *Litchi* pulp on alcohol-induced liver injury [9]. The phenolic compounds showed a good antioxidant potential and effectively decreased the hepatic expression of the nuclear factor erythroid 2-related factor 2 (Nrf2) genes. Liver diseases are considered one of the most important health problems that are responsible for about 2 million deaths per year. Recently, several risk factors were directly linked to their high incidence rate, ranging from excessive alcohol consumption, obesity, extensive use of pesticides, and exposure to environmental pollution. Despite the efficient strides in vaccination, liver-related diseases still represent a highly significant burden, especially in high-income countries. Unfortunately, most of

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Received date 06 March 2025; Revised date 23 April 2025; Accepted date 13 May 2025
DOI: 10.21608/EJCHEM.2025.354441.11410

the conventional drugs that are commonly used for liver dysfunction have unanticipated side effects [10]. For example, long-term treatment with sorafenib, the first approved drug for hepatocellular carcinoma, causes dramatic renal and pancreatic failure [11]. Oxidative stress plays a crucial role in the structural and functional damage of the liver through the depletion of DNA, and stimulation of apoptotic pathways. *Tert*-butyl hydroperoxide (t-BHP) is a highly reactive hydroperoxide analogue that is commonly used as a pro-oxidant inducer of oxidative stress. Natural antioxidant compounds were reported to neutralize the generated reactive oxygen species (ROS) and reduce the oxidative damage induced by t-BHP and provide natural protection of hepatocyte cells. Extensive research was conducted to look for natural alternatives to ameliorate oxidative stress and improve liver functions with minimum side effects [12]. *Litchi* is well recognized as a wealthy provenance of different classes of secondary metabolites such as phenolic acids, flavonoids, proanthocyanidins, sterol, and triterpenes [7,13]. Its phenolic compounds demonstrate diverse biological activities as antioxidant, immunomodulatory, anti-inflammatory, antimicrobial, and anticancer activities specifically, the native procyanidin (PCs) are an important class of flavan-3-ol molecules that accumulate in all parts of the plant (pulp, peels, seeds, and leaves)[14,15]. Catechin and epicatechin are the principal building blocks of PCs. They consist mainly of oligomeric procyanidins dimer and trimer of type A and B2 [16]. These compounds were responsible for the potential antidiabetic activity of the Hemaoli cultivar of the pulp over the other cultivars [17]. Similarly, procyanidin A2 and epicatechin are the unique polyphenolic compounds of Chinese *Litchi* pericarp. They significantly ameliorated CCL4-induced liver injury and promoted the self‐repairing of hepatocytes even more [18]. Moreover, flavonoids such as kaempferol and luteolin glycosides were among the most abundant secondary metabolites in leaves, fruits, and seeds with antioxidant potential [19]. As far as we know, scientific studies about the phytochemical and therapeutic potential of *Litchi* leaves need further investigations. The present study implemented a quantitative investigation of several polyphenolic compounds and highlighted their antioxidant and protective activity against tert-butyl hydroperoxide-induced hepatotoxicity (t-BHP) induced hepatic damage.

Methods

Cell culture

Hepa1c1c7 (ATCC) cells were cultured in alpha MEM supplemented by 10% Fetal bovine serum, penicillin/streptomycin solution, and 2 mM l-glutamine. Cells were maintained in a humidified incubator with 5% CO_2 at 37 °C. Reagents for cell culture were purchased from Lonza (Germany).

Chemicals & reagents

All standards reported in this study were purchased from Sigma-Aldrich (St. Louis, MO, USA). Ethanol, dichloromethane, ethyl acetate, and *n*-butanol were analytical grade and obtained from El- Gomhouria Company for Trading Chemicals and Medical Appliances, Cairo, Egypt. Deionized water was used in all experiments and samples for HPLC and UV. All samples were filtered through a 0.22 µm membrane before injection.

Preliminary detection of secondary metabolites

Preliminary phytochemical investigation of the main secondary metabolites was established according to the method described by Venkatesh et al. 2008 [20] to check the existence of alkaloids, flavonoids, anthraquinone, saponins, sterols, and/or triterpenes, condensed tannins, and coumarins.

Quantitative determination of extractive yields

Fresh leaves of *Litchi chinensis* were air-dried and pulverized to a fine powder. About 100g of leaves were subjected to successive fractionation with solvents of increasing polarity (petroleum ether (60-80°C), 70% ethanol, dichloromethane, ethyl acetate, and water-saturated n-butanol) to isolate compounds based on their solubility.

Cell-free antioxidant activity using DPPH of *Litchi* fractions.

The antioxidant effect of different fractions was evaluated by measuring the scavenging power of free radicals using DPPH (2,2-diphenyl-1-picrylhydrazyl). Fractions were dissolved in DMSO and diluted into the assay reaction to give final concentrations of (6.25 -100 µg/ml). The reaction was initiated for 30 min in the dark after the addition of 180 µl of DPPH reagent in methanol (0.004% *w/v*). The percentage of antioxidant activity (scavenging) was calculated as previously described by Younis et al. 2023 [21]. The effective concentration that scavenges 50% of DPPH (EC_{50}) in the vehicle control was statistically derived from dose-response analysis using Graph Pad Prism software (San Diego, USA).

Hepatoprotective activity of *Litchi* leaves fractions.

The hepa1c1c7 cells were employed as previously described by Hamed 2012 [22], with some modifications. Briefly, cells were seeded onto 96-well plates (from the stock of 1×10^5 cells/ml). After 18 h, cells were subjected to either (DMSO, 0.1% *v/v*) or plant extracts /fractions serial dilutions (6.25-100 µg/ml) for 48 h after which *t*-BHP was added (75 µM). MTT assay was performed to determine the percentage of cell viability as previously described by Hegazy et al. 2017 [23].

Treatment of the cell lysates for NQO1

The induction of the chemo-preventive marker NAD (P)H dehydrogenase [quinone] 1 (NQO1) in the murine hepatoma cell line was assessed. Briefly, Hepa-1C1C7 cells (1.5×10^5 cells/ml) were seeded onto 6-well plates and incubated overnight to

stick and create semi-confluent monolayers. Monolayers were subjected to either vehicle (final concentration 0.1% v/v DMSO) or *Lichi* total extract or ethyl acetate fraction (6.25, 25, and 100 µg/ml) for an additional 24 h. In parallel, 4'-bromoflavone (4-BF) was used as a positive control [24]. Following aspiration of treatment medium, ice-cold Dulbecco's PBS was used to wash the treated monolayers. Then, Cells were scrapped in ice-cold lysis buffer (pH 7.4, 25 mM Tris-Cl, 5 µM FAD and 250 mM sucrose). The cell suspensions were then sonicated on ice for five seconds (20% amplitude). Sonicates were then centrifuged (12,000 ×g for 10 min). The obtained supernatants were used for protein expression by Western blotting.

Western blotting analysis for NQO1.

This technique was used to detect specific proteins in cell lysates. Proteins in cell lysates (30 µg) were separated on 10% PAGE (Cleaver Scientific TETRAD system, UK) and transmitted onto PVDF membrane using a Trans-blot Turbo module (Bio-rad, USA). The membrane was blocked by using 5% skim milk for one hour at room temperature, followed by an overnight incubation at 4°C with either NQO1 (1:1000, Elabscience, USA) or β-actin (1:2000, Thermofisher Scientific, USA). Following three x 5 min washes using Tris buffer saline Tween 20 (TBST), the membranes were incubated with 1:5000 dilution of appropriate horseradish peroxidase-conjugated secondary antibody for 1 h at room temperature. After three TBST washes, membrane proteins were revealed using the Enzyme Chemiluminescence (ECL) western blotting detection substrate. Protein bands were imaged using a UVP Bio spectrum imaging system (Analytik Jena, UK).

Spectrophotometric assessment of the total polyphenolics

The total phenolic content was calculated as gallic acid equivalent using Folin–Ciocalteu reagent according to the method described by Miliauskas et al. 2004 [25].

Spectrophotometric assessment of the total flavonoids

The total flavonoids were calculated as a quercetin equivalent based on the reaction of flavonoids with aluminum chloride according to the reported method by Mohsen et al. 2020 [26].

Spectrophotometric assessment of the total proanthocyanidins

The total condensed tannins were calculated as catechin equivalent based on the reaction of proanthocyanidins with vanillin and hydrochloric acid according to the reported method by Ngamsuk et al. 2019 [27]**.**

HPLC quantitative assessment of flavonoids and phenolic acids in the ethyl acetate fraction

The High-Performance Liquid Chromatography technique was employed to identify and quantify the phenolic compounds against reference standards. The quantitative estimation of phenolic compounds was established using Agilent Technologies 1100 series liquid chromatograph equipped with an autosampler and a diode-array detector according to the prescribed method by Kim et al. 2006 [28]. The separation was performed using the Eclipse XDB-C18 column (150 X 4.6 µm; 5 µm) connected with a C18 guard column (Phenomenex, Torrance, CA). The mobile phase comprised of acetonitrile (solvent A) and 2% acetic acid in water (v/v) (solvent B); with gradient elution as follows: (B) 100% to 85 %→(0-30min), (B) 85% to 50 %→(30-50 min), 50% to 100%→(50-55 min). The flow rate was kept at 0.8 ml/min with a 10 µl injection volume. The peaks were monitored simultaneously at 280 and 320, 360 nm.

HPLC quantitative estimation of monomeric procyanidin in the ethyl acetate fraction

The analysis was carried out using HPLC Agilent1260 with a UV detector according to Desai et al. 2020 [29] with slight modification. The analytical column was Nucleosil C18, 5µm (4.6 x 250 mm), mobile phase (A) 0.1% phosphoric acid and (B) acetonitrile; with gradient elution, as follows: (B) 15% to 25 %→(0- 12 min), (B) 25%→(12-15min), (B) 25% to 15 %→(15-18 min); at a flow rate of 1ml/min, column temperature of 28°C, detection at 280 nm and injection volume 20 µl.

Statistical analysis

All the values were estimated as mean ± SD (n = 3). Statistically significant among the results were analyzed by one-way analysis of variance (ANOVA), followed by the Tukey test.

Results

Preliminary detection of secondary metabolites

Phytochemical screening of the biologically active metabolites revealed the presence of flavonoids, sterols, and/or triterpenes, and condensed tannins. While Anthraquinones, saponins, coumarins, and alkaloids were absent.

Quantitative estimation of extractive yield

Ethyl acetate fraction with its orange color represented the highest yield (4.6%) of the fractionated alcoholic extract (9%) followed by a dark olive-green fraction of petroleum ether (3.7%) then the dark orange color of *n*- butanol (1.86%). Dichloromethane with its green color gave the least extractive yield (0.94 %).

Cell free-antioxidant activity using DPPH.

As shown in **Fig. (1) and Table (1)**, the EtOAc fraction (EC50 = 4.31±0.14 µg/ml) exhibited the most active radical scavenging effect against DPPH in a concentration-dependent manner. Lower activity was observed with other fractions as revealed with higher values of their calculated EC_{50}. Specifically, petroleum ether showed the least antioxidant activity (EC_{50} was 47.77±1.54 µg/ml).

Cell-based anti-TBHP activity

In the present study, t-BHP was established as an oxidative stress inducer in Hepa1c1c7 cells to investigate the hepatoprotective activity. As revealed from the anti-t-BHP activity assay, the total extract and EtOAc fraction gave a concentration-dependent increase of Hepa1c1c7 cell viability over that of t-BHP only (used near its IC_{50}) when the cells were pretreated with the extract and its fractions and then challenged with t-BHP cytotoxicity. The ethyl acetate fraction was the most active in protecting the cells against t-BHP induced-cytotoxicity (**Table 2**) which completely restored cell viability near vehicle control levels at the highest used concentration of 100 µg/ml, achieving 96.13±0.8% viability compared to the vehicle control. Unfortunately, petroleum ether fraction did not show any response towards t-BHP cytotoxicity.

NQO1 induction test by Western blotting

As illustrated in **Fig. (2),** the Western blot of NQO1, no induction activity was observed by treating Hepa1c1c7 cells with increasing concentrations of total extract and EtOAc fraction.

Table (1): Calculated EC50 values of *Litchi* total extract and fractions.

No.	Sample	EC50±SD
1	Quercetin	2.37±0.07
2	Total alcoholic extract	6.41±0.21
3	Pet. Ether	47.77±1.54
4	Dichloromethane fraction	13.43±0.37
5	EtOAC fraction	4.31±0.14
6	*n*-butanol fraction	5.85±0.12

Data are means±SEM

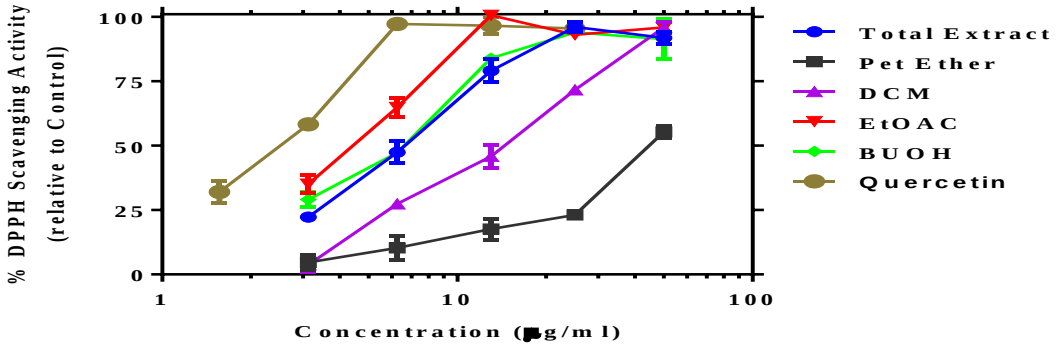

Figure (1): Antioxidant activity of total extract and different fractions of *Litchi* leaves, Pet Ether; petroleum ether, DCM; dichloromethane, EtOAC; Ethyl acetate, BUOH; Butanol.

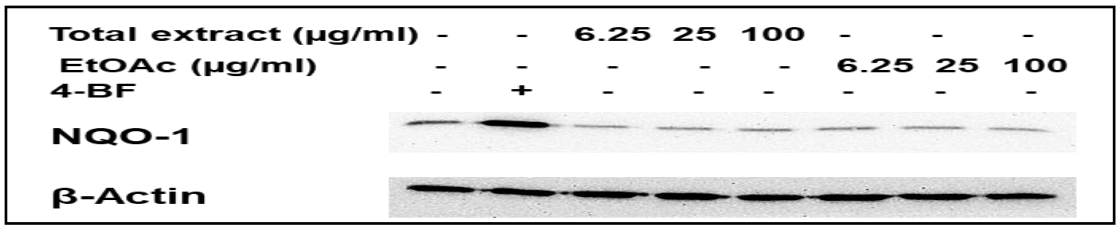

Figure (2): Western blotting of NQO1 protein expression. Hepa1c1c7 cells were treated with either vehicle,4 Bromo flavone (4-BF) or increasing concentrations of total extract /EtOAc fraction from *Litchi* leaves as described in the methods section.

Table (2): Protection of Hepa1c1c7 cell viability by *Litchi* total extract and its fractions against *t*-BHP Cytotoxicity.

	%Viability (MTT)				
Samples(µg/ml)	6.25	12.5	25	50	100
Total alcoholic extract	47.22±1.2	51.32±1.1	58.98±0.3*	63.9±0.4*	76.62±0.6*
Dichloromethane fraction	51.75±0.6	54.64±1.5*	61.37±1.5*	67.8±1.8*	68.02±1.8*
Ethyl acetate(EtOAc) fraction	47.79±0.9	53.22±1.5	59.12±1.5*	65.75±1.2*	96.13±0.8*
n-butanol fraction	53.50±0.7	56.17±0.7*	64.92±1.5*	67.68±1.6*	94.69±2.3*

* denotes significantly different % viability compared to *t*-BHP control, one-way ANOVA.
t-BHP (75 µM) was 46.66±0.4.

Spectrophotometric determination of total phenolics, total flavonoids, and procyanidins in the active fractions

The spectrophotometric technique was employed to quantitatively determine the total phenolics, flavonoids, and proanthocyanidins of the most active fractions (*n*-butanol, and ethyl acetate fractions) and compared with those in the total alcoholic extract. In terms of percentage, the ethyl acetate fraction showed the highest percentage of phenolics, flavonoids, and procyanidins. The phenolic content was 77.3±0.42% expressed as gallic acid equivalent, while flavonoids and procyanidins comprised 11.4% and 46.5%, respectively (**Table 3**).

Table (3): Total phenolic, flavonoids, and procyanidins content of *Litchi* total extract and active fractions

Content (%)	Total alcoholic extract	Ethyl acetate fraction	*n*-butanol fraction
Total phenolics	57.5±0.31*	77.3±0.42*	55.2±0.65*
Total flavonoids	8.8±0.04**	11.4±0.12**	10.8±0.32**
Total proanthocyanidins	42.9±0.23***	46.5±0.33***	39.5±0.41***

∗Denotes significant differences between groups

HPLC quantitative assessment of flavonoids and phenolic acids in ethyl acetate fraction

Quantitative estimation of twenty-two polyphenolic compounds in the most active fraction ethyl acetate (LETA) was performed as represented in **Fig. (3).** The analysis provided an excellent separation and quantification of the different phenolic compounds. Naringenin, apigenin-7-glucoside, catechin, and rutin in LETA represented the unique *Litchi* major bioactive metabolites by concentrations of 2503.21, 2323.55, 2385.75, and 1705.82 µg/g, respectively **Fig. (4)**. In addition, several minor phenolic compounds such as protocatechuic, *p*-coumaric, *p*-hydroxybenzoic, vanillic, rosmarinic, and cinnamic acids were also identified, as presented in **Table (4).**

Table (4): Identified phenolics and flavonoids in *Litchi* EtOAc fraction.

t_R (min)	**Polyphenolic compound in EtOAc fraction**	**Concentration (µg/g) extract**
Phenolic acids		
4.483	Gallic acid	128.14±0.41
7.429	Protocatechuic acid	452.58±1.86
10.745	*P*-hydroxybenzoic acid	190.01±0.43
11.561	Gentisic acid	ND
13.297	Chlorogenic acid	ND
14.193	Caffeic acid	ND
15.04	Syringic acid	ND
16.797	Vanillic acid	130.72±0.38
25.664	Ferulic acid	ND
27.556	Sinapic acid	ND
30.231	*P*-coumaric acid	195.24±0.62
37.515	Rosmarinic acid	133.31±0.29
40.809	Cinnamic acid	27.31±0.41

Flavanol compounds		
12.286	Catechin	2385.75±1.56
Flavonol compounds		
32. 33	Rutin	1705.82±1.47
48.601	Kaempferol	ND
Flavanone compounds		
34.369	Naringenin	2503.21±2.03
Flavone compounds		
36.322	Apigenin-7-glucoside	2323.55±2.28
47.911	Apigenin	ND
44.329	Luteolin	ND
55.053	Chrysin	ND
Hydroxycoumarin compounds		
24.416	Scopoletin	ND

ND: Not detected

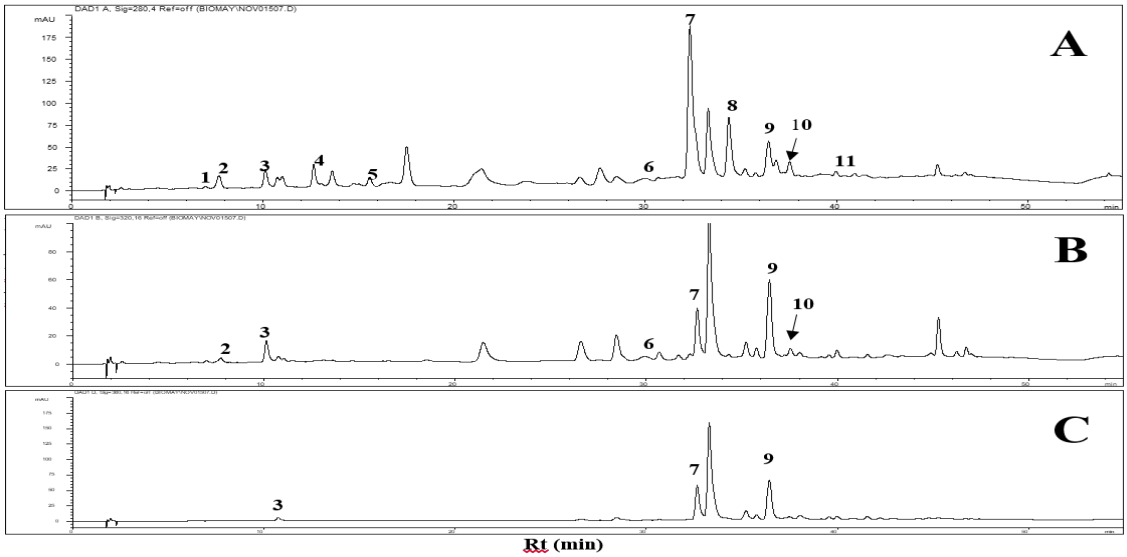

Figure (3): Representative HPLC chromatograms of the identified compounds of EtOAc fraction of *Litchi* leaves, A: at 280 nm, B: at 320 nm, C: at 360 nm. 1: gallic acid, 2: Protocatechuic acid, 3 *p*-hydroxybenzoic, 4: Catechin, 5: Vanillic acid, 6: *p*-coumaric, 7: Rutin, 8: Naringenin, 9: Apigenin-7-glucoside, 10: Rosmarinic acid, 11: Cinnamic acid.

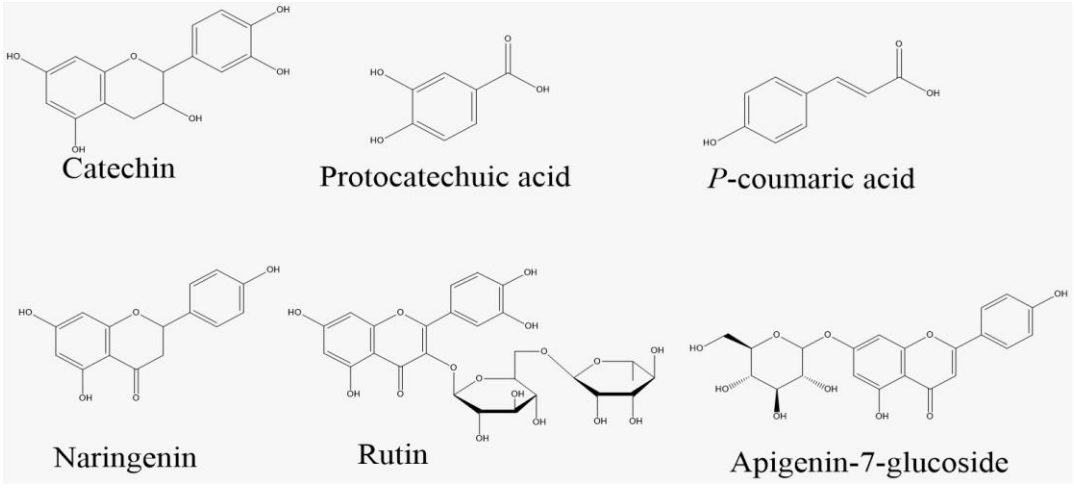

Figure (4): Chemical structures of the most abundant compounds in ethyl acetate fraction of *Litchi* leaves.

HPLC quantitative estimation of monomeric procyanidin in the ethyl acetate fraction

The analysis of monomeric procyanidins as illustrated in **Table (5)** and **Fig. (5)** revealed that epicatechin gallate (ECG) as well as epigallocatechin gallate (EGCG) are the major monomeric procyanidins, accounting for 8050.8 & 2053.2 µg/g from the ethyl acetate fraction. The detected catechin, EGCG, and epicatechin monomers in *Litchi* leaves were previously reported in *Litchi* fruits [30].

Table (5): Identified procyanidins monomers in *Litchi* EtOAc fraction.

Rt (min)	Proanthocyanidins monomers	Conc. (µg/g) extract
4.8	Epicatechin (EC)	1732.1± 2.05
6.4	Epigallocatechin gallate (EGCG)	2053.2±10.03
7.6	Gallocatechin gallate (GCG)	353.4±1.01
10.4	Epicatechin gallate (ECG)	8050.8 ±13.6

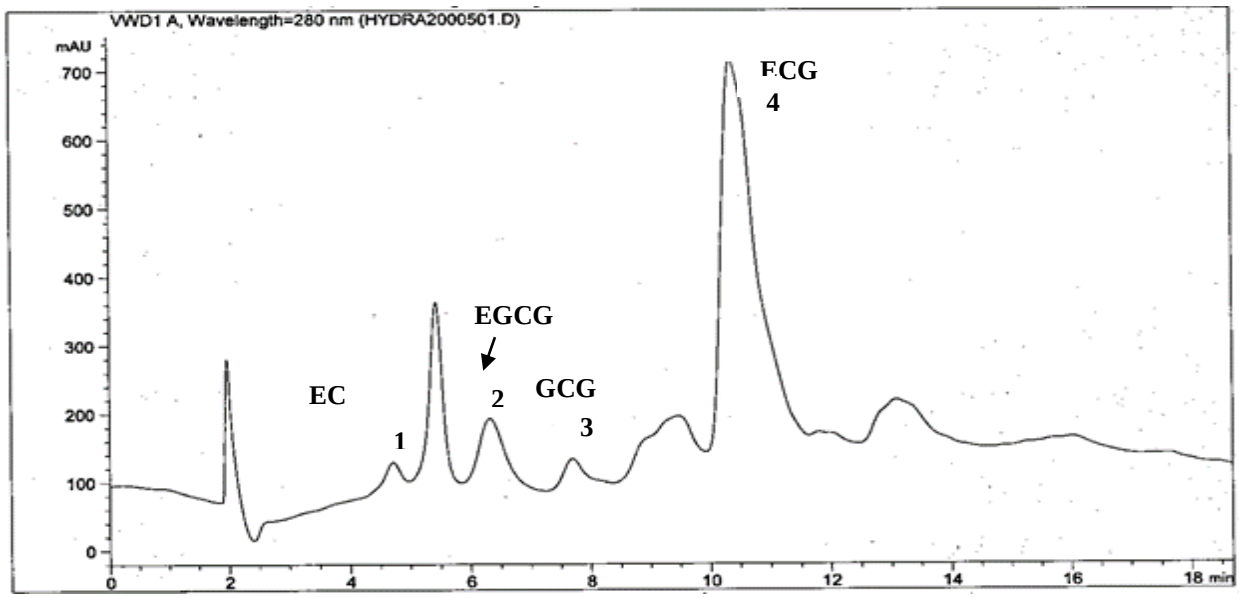

Figure (5): Representative HPLC chromatogram of the identified proanthocyanins monomers in EtOAc fraction of *Litchi* leaves. Compounds 1; Epicatechin(EC), 2; Epigallocatechin gallate (EGCG), 3; Gallocatechin gallate (GCG), 4; Epicatechin gallate (ECG).

Discussion

Several *in vitro* and *in vivo* studies demonstrated the beneficial bioactivities of *litchi* seed & fruit phytochemicals, such as antioxidant, anti-inflammatory, and anti-cancer activities [31]. However, there are few biological and phytochemical studies on *litchi* leaves. A recent study by Kanwal *et al.* 2024 [32] stated that the inhibitory effect of the methanolic extract of *litchi* leaves on the viability of liver cancer cells was greater than the extracts from the seeds and bark. According to the comprehensive review reported by Bishayee *et al.* 2024 [15], few studies were reported in *litchi* leaves metabolites, mainly epicatechin, cinnamtannin B1, and proanthocyanidin B2. These findings supported our research, as concerned mainly with *litchi* leaves, proving the relation between their secondary metabolites and the hepatoprotective effect.

In this study, we have employed a biologically guided fractionation for *Litchi sinensis* leaves by different polarity solvents. The preliminary Cell-free screening of the antioxidant potential using DPPH method proved that the ethyl acetate fraction of *litchi* leaves had antioxidant potential over other fractions **(**petroleum ether, dichloromethane, and butanol). The hepatoprotective activity was investigated revealing for the first time that the ethyl acetate fraction was the most active to protect the cells against t-BHP-induced-cytotoxicity in Hepa1c1c7 cells. The Western blot of NQO1 indicated that the cytoprotecting activity against t-BHP on pretreatment with those fractions was not accomplished through NAD(P)H dehydrogenase(NQO1). These results suggested that the potential activity may be ascribed to the 'direct' antioxidant effect.
The previous biological results agreed with the chemical analysis in which total phenolics, flavonoids, and proanthocyanidins in the ethyl acetate fraction were higher than n-butanol as well as the total extract. Therefore, quantitative metabolite profiling of the ethyl acetate fraction was proceeded.

As the characterization of the phenolic profile was the key step to identify the potential antioxidant activity, several scientific studies have been established about the phenolic metabolites of *Litchi* pulp and its by-products such as peel and seeds. However, little is concerned with the investigation of its leaves as a valuable source of biologically active metabolites.

Our study revealed the presence of several flavonoids mainly naringenin, apigenin-7-glucoside, and rutin. Naringenin glycoside and its aglycone had been previously identified in Chinese *Litchi* seeds [33,34]. To the best of our knowledge, this study represents the first to report on its presence in the leaves. Other flavonoids such as luteolin and kaempferol were previously detected in *Litchi* seeds and leaves [14,35], but they were not identified through this study in *Litchi* leaves. Differences in geographic origin and part used in this study may be responsible for the variation of active metabolites [21]. Interestingly, naringenin and apigenin exhibited a hepatoprotective effect attributed to their potential antioxidant activities [36]. In the same context, apigenin ameliorated the hepatic function by up-regulating the apoptotic pathway of BCL-2(B-cell leukemia/ lymphoma2 protein) and suppressed the oxidative signaling of Nrf2 (nuclear factor erythroid2) [11].

In this study, the derivatives of benzoic and cinnamic acids were the most abundant phenolic acids identified in LETA. Structurally, they exhibited multispectral pharmacological actions due to their diverse chemical structures, mainly the phenolic moiety. This is in good agreement with the literature [37], which highlighted the antioxidant and hepatoprotective potential of trans-cinnamic acid in Thailand *Litchi* pulp.

In this study, several monomeric procyanidins have been quantified as catechin, epicatechin, GCG, EGCG, and ECG. The presence of catechin, EGCG, ECG and epicatechin monomers was reported in *Litchi* fruits and seeds [30,31]. Catechins were reported as antioxidants by direct mechanisms (scavenging ROS and chelating metal ions) and indirect mechanisms [38]. All catechin diastereoisomers are common in chemical structures through phenolic hydroxyl groups that stabilize free radicals and act as direct antioxidants [39]. The arrangement and number of hydroxyl groups in the molecule have a positive correlation with the antioxidant activity of the phenolic compounds thus it was reported that EGCG was more active than catechin & epicatechin [40]. Interestingly, the literature highlighted the potential activity of the phenolic secondary metabolites of *Litchi* pulp as a potent hepatoprotective especially procyanidin-A, epicatechin, and quercetin glycosides *via* the online knockout method [41]. Recently, procyanidin-A identified in *Litchi* fruit showed excellent anti-inflammatory and anti-proliferative activities [42].

Conclusion

For the first time, we report the hepatoprotective activity of *Litchi sinensis* Sonn. leaves against tert-butyl hydroperoxide-induced toxicity. Our obtained results reveal that the anti-oxidant effect can be attributed to the 'direct' antioxidant effect against the t-BHP radical responsible for oxidative cytotoxicity in the tested model, as well as the direct antioxidant activities shown by DPPH scavenging results. The activity was emphasized by the identified phenolic compounds, mainly procyanidins (catechins), which were reported to act as an antioxidant by the direct mechanism. In addition to naringenin, which was reported for the first time in *Litchi* leaves. Moreover, several antioxidant metabolites were quantitatively estimated. These findings recommended further *in vivo* studies on *litchi* leaves in the future as a natural economic by-product source, rich in antioxidants, for the development of hepatoprotective drugs.

Conflict of interest

No potential conflict of interest was reported by the authors.

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