Comparative Study on Some Biological Activities of *Dimocarpus Longan* Lour. (Longan) And *Litchi Chinensis* Sonn. (Lychee) Seeds

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Abstract

This research deals with the study of some biological activities of *Dimocarpus longan* Lour. (Longan, LGS) and *Litchi chinensis* Sonn. (Lychee, LS) seeds. Preliminary phytochemical screening by test tube methods revealed that alkaloids, α-amino acids, carbohydrates, glycosides, organic acids, phenolic compounds, reducing sugars, saponins, starch, steroids, tannins, terpenoids, and flavonoids are present, and cyanogenic glycosides are absent in both samples. The total phenolic content was determined by the Folin-Ciocalteu Reagent (FCR) method. The total phenolic contents of ethanol and watery extracts of LGS and LS were determined by the Folin-Ciocalteu Reagent (FCR) method. Total phenolic content was found to be the highest in the ethanol extract of LGS (118.04 ± 0.30 μg/mL) and lowest in the watery extract of LS (39.82 ± 0.37 μg/mL). The antioxidant activity of ethanol and watery extracts of LGS and LS was assessed by the DPPH free radical scavenging assay. Among the crude extracts, the order of RSA was observed as the ethanol extract of LGS (24.57 μg/mL) was higher than the other extracts. The cytotoxicity of the watery and ethanol extracts of LGS and LS was evaluated by a brine shrimp lethality bioassay. From these results, all crude extracts have LD_{50} values greater than 1000 μg/mL. It means that LGS and LS have no cytotoxic activity.

***Keywords**: Dimocarpus longan, Litchi chinensis*, Total phenolic contents, Antioxidant activity, Cytotoxicity

Introduction

Plants are a significant source of medication and are essential to maintaining global health. The medicinal plants are used as potential tools for preserving health and conditions as well as for the treatment of diseases. Two-thirds of the world's population, or many countries, rely on herbal medicine as their primary form of healthcare. Drugs made from plants are becoming more and more popular worldwide. Secondary metabolites or substances found in medicinal plants include tannins, terpenoids, alkaloids, flavonoids, and many others. These compounds have powerful antioxidant, antibacterial, antifungal, anticancer, antidiuretic, anti-inflammatory, and anti-diabetic activities. Due to the lack of even basic health care, numerous people worldwide perish every day from diseases that may be prevented or treated. Malnutrition is frequently linked to diseases in these nations. When compared to medicinal plants, which work in an integrated or probiotic manner with little to no bad effects on the body, chemically manufactured medications may act rapidly, but they have side effects that negatively impact the human body over time. According to the World Health Organization (WHO), traditional medicine is the primary source of care for 80% of the world's population, and the majority of this therapy uses plant extracts and their active ingredients. (Garg *et.al*., 2021).

Fruits, vegetables, plant by-products, spices, and herbs are known to contain a wide variety of phytochemicals, such as polyphenols, carotenoids, and vitamin C, which possesses antioxidant activity (Kumar *et.al*., 2014). Lychee (*L. chinensis* Sonn.) and longan (*D. longan* Lour.) are subtropical evergreen trees belonging to the family Sapindaceae. The two fruits have an indehiscent pericarp surrounding a succulent edible aril with a dark brown seed. The fruits are used to

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Produce liquers, drinks with fresh fruits, fermented beverages, canned syrup, and fruit juices. The pericarp and seed portions of lychee and longan are estimated to contain approximately 30% of the dry weight of the entire fruit and consist of significant amounts of bioactive compounds. The extracts from lychee and longan pericarps and seeds have exhibited excellent antioxidant ability, good anti-tyrosinase, anti-inflammatory, immunomodulatory, and anti-cancer activities, and cytotoxic effects. Thus, the two fruits can be used as an easily accessible source of natural antioxidants from their by-products and/or possible agents in the cosmetic and pharmaceutical industries, functional food ingredients, or natural preservatives. (Xiang *et.al*., 2014).

Botanical Aspect of *Dimocarpus longan* Lour.

Family	-	Sapindaceae
Botanical Name	-	*Dimocarpus longan* Lour.
Genus	-	*Dimocarpus*
Species	-	*longan*
English Name	-	Longan, Dragon's eye
Myanmar Name	-	Thakyar, Nanpyar, laychu, Sagaing Thee, Ta Yote Zee Tee
Part used	-	Seeds

Figure 1 (a) Dried longan plants, (b) Longan fruits, (c) Dried longan seeds

Botanical Aspect of *Litchi chinensis Sonn.*

Family	-	Sapindaceae
Botanical Name	-	*Litchi chinensis* Sonn.
Genus	-	*Litchi*
Species	-	*chinensis*
English Name	-	Lychee
Myanmar Name	-	Lychee
Part used	-	Seeds

Figure 2 (a) Dried lychee plant, (b) Lychee fruits, and (c) Dried lychee seeds

Materials and Methods

Plant materials

Longan and lychee fruits were collected from July to August, 2021 in Sagaing Township, Sagaing Region, and Naungmon, Shan State, Myanmar, respectively. The collected sample was identified as *Dimocarpus longan* Lour. (Longan) and (*Litchi chinensis* Sonn.) fruits at the Botany Department, University of Yangon. The sample was cleaned by washing thoroughly with water and peeled off. Then the fresh seed was cut into small pieces and air-dried at room temperature. The dried samples were ground into powder by a grinding machine, sieved and stored in an airtight container for further use.

Phytochemical investigation of longan and lychee seeds

Phytochemical tests for the longan and lychee seeds were carried out according to the test tube methods to investigate the presence and absence of alkaloids, α-amino acids, carbohydrates, cyanogenic glycosides, glycosides, organic acids, phenolic compounds, reducing sugars, saponins, starch, steroids, tannins, terpenoids, and flavonoids (Geetha *et al*., 2014 and Harbone, 1984). The observed results are shown in Table 1.

Preparation of crude extracts for biological activities

About 20 g of dried powder sample was extracted three times with 95 % ethanol (each time with 100 mL). Extraction time was allowed for 6 h and then filtered. Then, the solvent was removed by the rotary evaporator, providing the ethanol extract. To obtain watery extract, 20 g of dried powder samples was separately soaked in 100 mL of distilled water. It was then concentrated by evaporating the solvent on a water bath to get a watery extract. In this way, crude extracts were obtained. The crude extracts were dried and kept in a refrigerator for a few weeks.

Determination of total phenol contents (TPC) as gallic acid equivalent

The total phenolic content was determined by the Folin-Ciocalteu method. Each extracted sample solution (0.5 mL) was added into 5 mL of FC reagent (1:10) and incubated for 5 min. To each tube, 4 mL of 1 M sodium carbonate solution was added, the tubes were kept at room temperature for 15 min, and the UV absorbance of reaction mixture was read at λ_{max} 765 nm. The blank solution was prepared as in the above procedure by using distilled water instead of the sample solution. Total phenolic content was estimated as micrograms of gallic acid equivalent per milligram (μg GAE/mg) of crude extract (Hishamuddin *et al*., 2020).

Determination of Antioxidant Activity by DPPH Free Radical Scavenging Assay

Preparation solutions

DPPH (2 mg) was thoroughly dissolved in 95 % ethanol (100 mL). This solution was freshly prepared in the brown-coloured reagent bottle and stored in the fridge for no longer than 24 h. Each of the tested samples (ethanol and watery extracts) (10 mg) was thoroughly mixed with 10 mL of ethanol. The stock solution was obtained. This stock solution was twofold serially diluted with ethanol to obtain the desired concentrations: 3.91, 7.81, 15.63, 31.25, 62.5, and 125, 250 μg/mL.

Determination of Antioxidant Activity

DPPH free radical scavenging activity was determined by a spectrophotometric method. The control solution was prepared by mixing 1.5 mL of DPPH solution and 1.5 mL of ethanol in the brown bottles. The sample solution was also prepared by mixing thoroughly 1.5 mL of DPPH solution and 1.5 mL of test sample solution. These bottles were incubated at room temperature and were shaken on the shaker for 30 min. After 30 min, the absorbance of each solution was measured at 517 nm by using a spectrophotometer (Hishamuddin *et al*., 2020). Absorbance measurements were done in triplicate for each solution, and the percent inhibition of oxidation was calculated by the following equation.

$$\% \text{ RSA} = (A_{control} - A_{sample}) \times 100 / A_{control}$$

where

% RSA = % radical scavenging activity

$A_{Control}$ = Absorbance of control solution

A_{Sample} = Absorbance of sample solution

Determination of Cytotoxicity by Brine Shrimp Lethality Bioassay

Preparation of solutions

Accurately weighed 5 mg of each sample (ethanol extract, watery extract, potassium dichromate and caffeine) was separately dissolved in 5 mL of distilled water to obtain a respective stock solution (1000 µg/mL). Desired concentrations (1000, 100, 10, 1 µg/mL) of each solution were prepared from this stock solution by tenfold serial dilution with distilled water.

Procedure

Brine shrimp cysts (0.5 g) were put into 1 L of artificial sea water in a bottle. This bottle was placed near a lamp. Light is essential for the cysts to hatch. Brine shrimp cysts required to hatch constant supplied oxygen and 24 h incubation at room temperature. 9 mL of artificial seawater and 1 mL of different concentrations of samples and standard solutions were added to each chamber. Alive brine shrimp (10 nauplii) were placed into each chamber. They were incubated at room temperature for about
24 h. After 24 h, the number of dead or survival brine shrimps were counted and the estimation of cytotoxicity was done by 50 % lethality dose (LD_{50}) (Singh *et al*., 2015). The control solution was prepared as in the above procedure by using distilled water instead of the sample solution.

Results and Discussion

Phytochemical Investigation of Longan and Lychee Seeds

The preliminary phytochemical investigation was carried out to determine the different types of phytoconstituents present in the samples. From this investigation by the test tube methods, the test revealed the presence of alkaloids, α-amino acids, carbohydrates, glycosides, phenolic compounds, reducing sugars, saponins, starch, steroids, tannins, terpenoids, and flavonoids in both samples, whereas cyanogenic glycoside was found to be absent. These samples can be employed as antioxidant,

antibacterial, anticancer, and anti-tumor drugs due to the presence of phenolic compounds, steroids, tannins, terpenoids, and flavonoids.

Table 1 Results of Preliminary Phytochemical Investigation of the Longan and Lychee Seeds

No.	Test	Extracts	Test reagents	Observation	Results	
					LGS	LS
1	Alkaloids	1% HCl	(i) Dragendorff's reagent	Orange ppt	+	+
			(ii)Mayer's regent	White ppt	+	+
			(iii) Sodium picrate	Yellow ppt	+	+
			(iiii) Wagner's reagent	Brown ppt	+	+
2	α-Amino acids	H_2O	Ninhydrin reagent	Purple spot	+	+
3	Carbohydrates	H_2O	10 % α-naphthol and H_2SO_4	Red ring	+	+
4	Cyanogenic Glycosides	H_2O	H_2SO_4+sodium picrate solution	No brick red	-	-
5	Glycosides	H_2O	10 % lead acetate	White ppt.	+	+
6	Organic acids	H_2O	Bromocresol green	Yellow ppt	+	+
7	Phenolic Compounds	H_2O	$K_3Fe(CN)_6$+$FeCl_3$	Deep blue colour	+	+
8	Reducing Sugars	H_2O	Benedict's solution	Yellow ppt.	+	+
9	Saponins	H_2O	Distilled water	Frothing	+	+
10	Starch	H_2O	Iodine solution	Deep blue colour	+	+
11	Steroids	PE	Acetic anhydride+ conc: H_2SO_4	Greenish blue color	+	+
12	Tannins	H_2O	5% $FeCl_3$ solution	Bluish black ppt	+	+
13	Terpenoids	$CHCl_3$	Acetic anhydride+ Conc: H_2SO_4	Red colour	+	+
14	Flavonoids	EtOH	Conc: HCl+Mg turning	Pink colour	+	+

(+) = presence (-) = absence ppt = precipitate

Total Phenolic Content of Crude extracts of Longan and Lychee Seeds

The total phenolic content of ethanol and watery extracts of longan and lychee seeds was evaluated with a spectroscopic method using a Folin-Ciocalteu reagent. Phenolic contents have gained considerable interest because of their potential beneficial effects on human health. Phenolic contents possess antiviral, anti-inflammatory, anticancer, and antioxidant activities, and so on. The total phenolic content values were given in Table 3 and Figure 4. As the results of the total phenolic content showed, ethanol and watery extracts of longan seeds had significantly higher phenolic content than those of lychee seeds (118.04 ± 0.30) µg GAE/mg, 92.08 ± 0.04 µg GAE/mg, (59.96 ± 0.12) µg GAE/mg, and (39.82 ± 0.37) µg GAE/mg,

respectively. From these results, both samples considered may be used to produce natural antioxidant supplements in the food and pharmaceutical industries.

Moreover, it is well known that plant phenolics are highly effective free radical scavengers and antioxidants, and the activity is derived largely from the phenolic and polyphenolic compounds. Therefore, the investigation of the antioxidant activity of longan and lychee seeds was of great importance.

Table 2 The Absorbance of Standard Gallic Acid Solution at λ_{max} 765 nm

No.	Concentration (µg/mL)	Absorbance at λ_{max} 765 nm
1	3.125	0.603
2	6.25	0.373
3	12.5	0.252
4	25	0.194
5	50	0.164
6	100	0.147

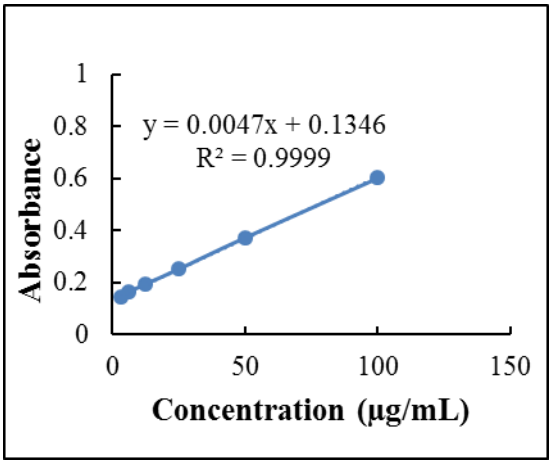

Figure 3 A calibration curve of gallic acid standard curve

Table 3 Total Phenol Contents of Crude Extracts of Longan and Lychee Seeds

No.	Samples	Extracts	TPC (µg GAE/mg ± SD)
1	LGS	Watery	92.08 ± 0.04
2		Ethanol	118.04 ± 0.30
3	LS	Watery	39.82 ± 0.37
4		Ethanol	59.96 ± 0.12

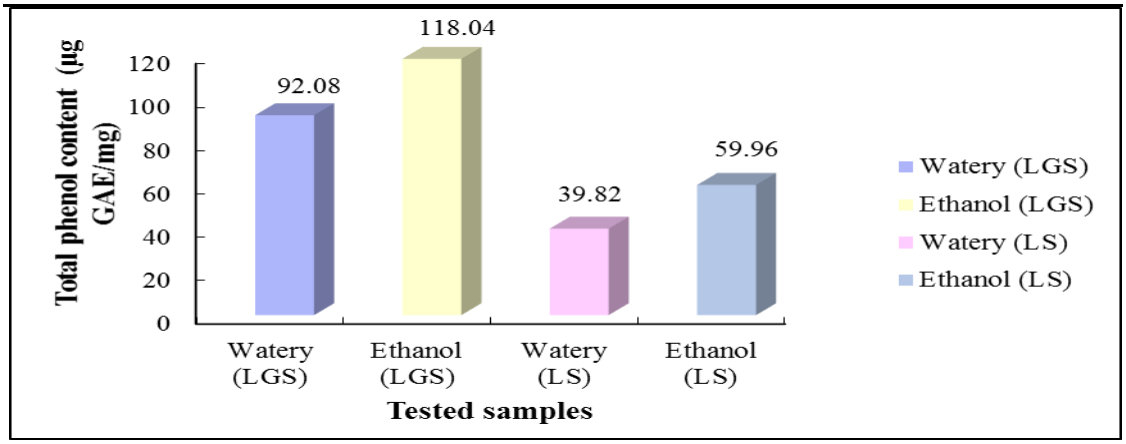

Figure 4 Histogram of total phenolic contents of ethanol and watery extracts of longan and lychee seeds

Antioxidant Activity of Crude Extracts of Longan and Lychee Seeds by DPPH Free Radical Scavenging Assay

Oxidation plays an essential role in living organism's ability to produce the energy demanded for natural processes. Still, the unbridled production of oxygen-derived free radicals may bring about cancer, atherosclerosis, and other conditions. Synthetic antioxidants are suspected to cause liver damage and cancer. Thus, it's necessary to develop and use natural, effective antioxidants to protect the body from free radicals and delay the development of numerous habitual conditions. DPPH is one of the most popular methods employed for the evaluation of antioxidant capability. In the radical form, the molecule of DPPH has an absorbance at 517 nm, which will vanish after the acceptance of an electron or hydrogen radical from an antioxidant, resulting in a stable diamagnetic molecule. Furthermore, DPPH has the advantage of being unaffected by certain side reactions of polyphenols, such as metal ion chelation and enzyme inhibition (Zhao *et al*., 2020). In this study, ascorbic acid was used as a standard, and different concentrations of extracts were used. According to the observations, the ethanol extract of longan seeds (24.57 µg/mL) was lowest IC_{50} value than the other extracts in both samples as shown in Table 4 and Figure 5 and 6. Since the lower IC_{50} values showed the higher antioxidant activities, the ethanol extract of longan seeds was found to have the most effective free radical scavenging activity.

Table 4 Average % Inhibition and IC_{50} Values of Crude Extracts of Longan and Lychee Seeds

Samples	**Tested extracts**	**% RSA ± SD of different concentrations (µg/mL)**							**IC_{50} (µg/mL)**
		3.91	**7.81**	**15.63**	**31.25**	**62.5**	**125**	**250**	
LGS	Watery	2.04 ±0.06	5.65 ±0.31	13.33 ±0.35	20.68 ±0.25	45.99 ±0.00	77.24 ±0.51	79.11 ±0.33	105.43
	Ethanol	7.09 ±0.00	15.50 ±0.29	30.79 ±0.21	64.33 ±0.33	87.39 ±0.00	87.56 ±0.27	87.60 ±0.07	24.57
LS	Watery	8.90 ±0.42	11.29 ±0.35	16.81 ±0.25	20.29 ±0.24	32.72 ±0.31	45.35 ±0.06	69.21 ±0.27	146.28
	Ethanol	10.24 ±0.42	14.74 ±0.35	16.92 ±0.25	20.08 ±0.38	50.76 ±0.00	68.12 ±0.12	83.04 ±0.25	61.72
	Std. Ascorbic Acid	46.15 ±0.03	75.81 ±0.28	79.12 ±0.16	85.93 ±0.32	87.37 ±0.37	87.62 ±0.00	87.75 ±0.07	4.41

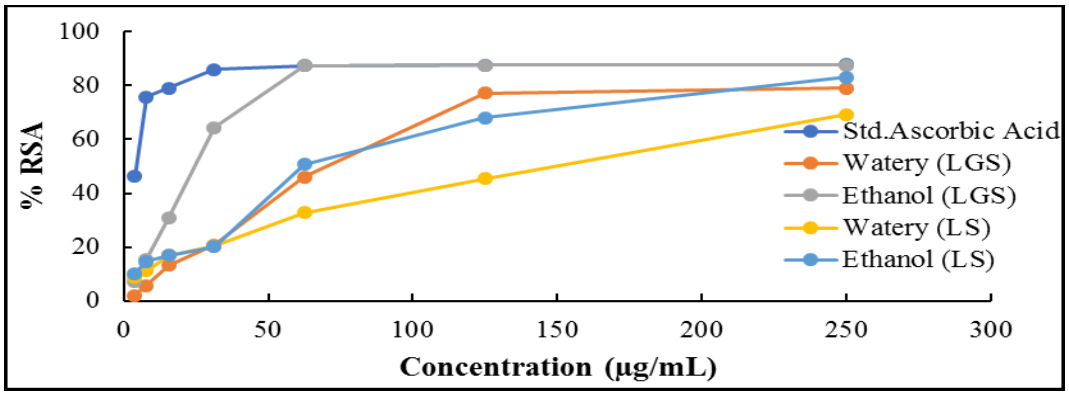

Figure 5 % RSA versus concentration of different extracts of longan and lychee seeds

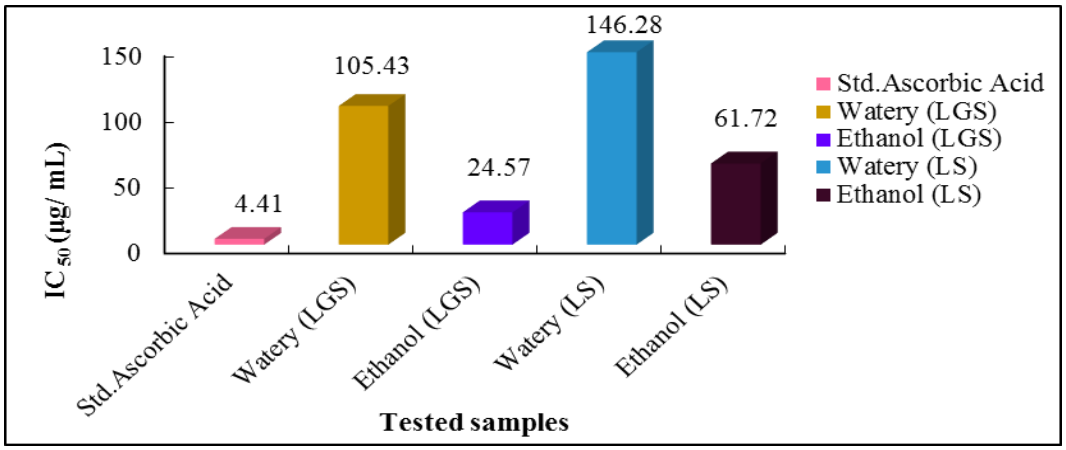

Figure 6 IC_{50} values of standard ascorbic acid and crude extracts of longan and lychee seeds

Cytotoxicity of Crude Extracts of Longan and Lychee Seeds

The brine shrimp test represents a rapid, inexpensive and simple bioassay for testing the plant extract lethality which in most cases correlates reasonably well with cytotoxic and antitumor properties. Most often, a desired biological response is not due to one component but rather due to a mixture of bioactive plant components. Therefore, crude extracts must be screened for biological activity. The brine shrimp lethality bioassay has been proved to be a convenient system for monitoring

biological activities of natural products. From the table 5, it was observed that the LD_{50} value of crude extracts of both samples were found to be > 1000 µg/mL. LD_{50} values of crude extracts, less than 1000 µg/mL was toxic (active) and greater than 1000 µg/mL was non-toxic (inactive). Therefore, water and ethanol extracts of both samples have no cytotoxic effect. Standard caffeine did not showed cytotoxicity until 1000 µg/mL concentrations whereas LD_{50} value of cytotoxic standard $K_2Cr_2O_7$ was 19.99 µg/mL.

Table 5 Cytotoxicity of Different Concentrations of Crude Extracts of Longan and Lychee Seeds against *Artemia salina* (Brine Shrimp)

Samples	Tested Extracts	% of Dead Brine Shrimp in Different Concentrations of Sample (μg/mL)				LD_{50} (μg/mL)
		1	10	100	1000	
LGS	Water	13.33 ±0.33	23.33 ±0.58	26.67 ±0.33	36.67 ±0.00	> 1000
	Ethanol	30.00 ±0.00	33.33 ±0.00	40.00 ±0.58	43.33 ±0.33	> 1000
LS	Water	16.67 ±0.33	20.00 ±0.00	30.00 ±0.58	43.33 ±0.58	> 1000
	Ethanol	30.00 ±0.00	40.00 ±0.33	40.00 ±0.00	46.67 ±0.00	> 1000
Std.	$K_2Cr_2O_7$	43.33 ±0.00	46.67 ±0.58	76.67 ±0.58	100 ±0.00	19.99
	Caffeine	0.00 ±0.00	13.33 ±0.33	23.33 ±0.58	33.33 ±0.58	>1000

Conclusion

In the present study, the result of phytochemical analysis revealed that the extract of *D. longan* Lour. (Longan) and *L. chinensis* Sonn. (Lychee) seeds contained phytochemical constituents such as alkaloids, α-amino acids, carbohydrates, glycosides, phenolic compounds, reducing sugars, saponins, starch, steroids, tannins, terpenoids, and flavonoids, except cyanogenic glycosides. The phenolic content and the total antioxidant activity of the ethanol extract of longan seeds were higher than those of lychee seeds. The results of the total phenolic content of each extract in this study are associated with antioxidant activity, which helps establish a positive relationship between the number of phenolic compounds and the antioxidant activity of the extracts. *In vitro* cytotoxic evaluation indicated that the LD_{50} values of watery and ethanol extracts were found to be >1000 mg/mL in all samples.

In conclusion, food waste is substantially reduced when fruits and vegetable seeds are cost-effective and contain generous amounts of natural phytochemicals needed for better human health. The present investigation shows that the longan and lychee seeds have no cytotoxic effect, high phenolic content, and antioxidant activity according to *in vitro* tests and have considerable implicit benefits for the food, pharmaceutical, cosmetic, and chemical industries.

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