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Lotus toot extract: An investigation of its antioxidant and anticancer properties for potential application in functional foods

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ABSTRACT

This study investigates the effects of lotus root extract (LRE) on antioxidants and cancer prevention to understand how lotus can be used as a natural ingredient in health-promoting foods. We tested the extract's ability to neutralize harmful molecules using the DPPH assay and found that its effectiveness increases with the amount of extract used. The concentration of 22.21 g/mL was key for the extract to show its protective effects. Additionally, our research showed that LRE is good at removing harmful substances like nitrite (21.14 µg/mL) and hydrogen peroxide (24.91 µg/mL). Importantly, lotus extracts were especially effective against human breast cancer cells. Through MTT analysis at 15.60 g/mL, we found LRE to be very effective in killing these cancer cells. This killing process involves the increase of certain proteins (p53, caspase 3, and 9) and the breaking up of DNA, which are signs of cancer cells being programmed to die. Our findings indicate that LRE could be a strong candidate for cancer prevention. This research sheds light on how lotus root extract could be used in health-promoting foods and suggests it might be worth exploring more as a treatment against cancer.

1. Introduction

As per the most recent cancer data registry records, the year 2020 witnessed an alarming 19.29 million new cases and a staggering 9.95 million deaths worldwide attributed to cancer, thus establishing it as the second leading cause of global mortality and morbidity (Sung et al., 2021). It is worth noting that adopting a healthy lifestyle, inclusive of dietary control, holds the potential to avert a significant proportion of cancer-related fatalities (Islami et al., 2018). Research suggests that at least 20 percent of malignancies could be prevented or their drug regimen efficacy enhanced through a diet rich in fruits, vegetables, and dietary fibers (Tseng, 2009; Mittelman, 2020).

Many plants, including fruits, vegetables, spices, whole grains, and herbs, are rich sources of active substances that are beneficial to health. These plants contain a large number of secondary substances, known as phytochemicals and bioactive compounds, which are important for creating new anticancer drugs. There is increasing scientific evidence showing that these active substances can affect various characteristics of cancer. These include cancer's continuous growth, its ability to avoid cell death, changes in how it uses energy, its capability to escape detection by the immune system, its inflammatory reactions, its ability to spread within the body, grow new blood vessels, and move to other parts of the body. They manage to do this by influencing different cellular pathways that either promote or suppress cancer development (Gutheil et al., 2012; Bhanot et al., 2011; Aggarwal et al., 2006; Margel et al., 2011).

Curcumin, which comes from turmeric; genistein, found in soybeans; tea polyphenols, present in green tea; resveratrol, from grapes; sulforaphane, extracted from broccoli; isothiocyanates, found in cruciferous vegetables like cauliflower and cabbage; silymarin, from milk thistle; diallyl sulfide, obtained from garlic; lycopene, from tomatoes; and rosmarinic acid, found in rosemary, are examples of compounds derived from plants that have been discussed in this

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context (Kaiser et al., 2021; Mondal et al., 2021; Wong et al., 2021; Tuli et al., 2019; De Greef et al., 2021).

Nelumbo nucifera Gaertn., known by various names such as lotus, holy lotus, Indian lotus, and sometimes referred to as the Chinese water lily, is a plant that is widely used for food and medicinal purposes. This plant, which is a large, perennial aquatic species, has a long history of being used to treat conditions like cystitis (bladder infections), nephropathy (kidney diseases), enteritis (inflammation of the intestine), fevers, and insomnia. It is part of the *Nelumbonaceae* family. For over 7,000 years, the lotus has been grown not only as a food source and for its medicinal properties but also for its ornamental value. For more than 2,000 years, virtually every part of the lotus plant has been consumed and used as a remedy throughout Asia (Chen et al., 2019; 2021; Tungmunnithum et al., 2018).

Numerous biological and medicinal activities have been discovered in the whole plant, along with crude extracts, fractions, and constituents, along with anti-oxidative, anti-inflammatory, immunomodulatory, antipyretic, disinfectant, sedative, antithrombotic, hypoglycemic, hypolipidemic, and effective in curing amnesia, ulcer, cardiac dysrhythmia and reducing fats and cholesterols (14–16,16–19). The involvement of numerous bioactive phytocompounds, such as polyphenolic compounds, flavones, phenolics, alkaloids, terpenoids, steroids, essential fats, as well as glycosides, has been linked to the lotus plant's remarkable health-promoting and disease-fighting properties (Tungmunnithum et al., 2018; Sharma et al., 2017; Krubha and Vasan, 2016; Agnihotri et al., 2008; Paudel and Panth, 2015; Morikawa et al., 2016; Kashiwada et al., 2005).

As a result, it is critical to perform the current study in order to learn about the beneficial bioactive components of LRE so it can be used in large quantities. As a result, the goal of this study was to conduct a systematic and critical analysis of the antioxidant properties and cancer-preventive prospects of LRE and its biologically active phytocomponents while also understanding the molecular and cellular mechanisms of action.

2. Research method

2.1. Chemicals

All solvents, standards, and reagents are analytical and HPLC grade. 1,1-diphenyl picrylhydrazyl (DPPH) free radical, hydrogen peroxide, and sodium nitroprusside were purchased from Fluka Chemicals. Caspase 3, 9 and p53 kits from MyBioSource (San Diego, California, United States), 3-(4, 5-dimethylthiazolyl-2-yl)-2,5-diphenyl tetrazolium bromide (MTT), ethidium bromide and agarose were obtained from m Sigma Aldrich, St. Louis, MO, USA. Methanolic extraction 100 grams of dried Lotus root were powered and extracted by 1 L of 80% methanol in soxhlet as per the previous protocol (Ranjbarnejad et al., 2017) and dried in rota-vapor and kept at -20°C till further use.

2.2. Free radical scavenging activity assay

The samples were assessed using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay's indicated methodology (Abdel-Hameed et al., 2013). 50 µL of each sample was mixed with 150 µL of 200 µM methanolic solution of DPPH and incubated at RT in the dark for 16 minutes. All samples were examined at the absorbance wavelength (515nm). The DPPH radical scavenging activity was calculated according to the following equation: EQ-1

$$Percent\ scavenging = \left(\frac{Absorbance\ Control - Absorbance\ Sample}{Absorbance\ Control}\right) * 100$$

The reaction was done in triplicate, and the results were shown as % Inhibition DPPH radical.

2.3. Hydrogen peroxide radical scavenging assay

A 20 mM hydrogen peroxide pH 7.4 solution was made using a phosphate buffer. In order to create different concentrations, 2mL hydrogen peroxide solution in PBS was added to 1 mL samples and the standard. After 10 minutes, the absorbance at 230nm was measured (Srinivasan et al., 2007).

2.4. Nitric oxide scavenging activity

The NO-scavenging capacity of extracts was determined using earlier methods with a few minor modifications. The amount of extract needed to quench 50% of the NO radicals produced by sodium nitroprusside was used to express the activity as a % of inhibition and an IC_{50} value.

2.5. MTT assay on MCF7 cells

LRE concentrations were measured and prepared from 0.78 to 200µg/mL, and MCF-7 cells are being cells were grown for 48 hours to check dose-dependent viability (23,24). The data show absolute cell survival after treatment because the MTT assay uses mitochondrial activity to recognize viable cells. As a consequence, this strategy was employed to evaluate LRE activity. The IC_{50} values were calculated using GraphPad Prism V-5.1 and a log (inhibitor) vs normalized response on a variable slope (San Diego, CA, USA).

2.6. Morphological changes on MCF-7 cells

We were able to evaluate the cytotoxic effects of LRE by visualizing the morphological changes in MCF7 cells. The procedure dose, which was assumed to correspond to the IC_{50} value of LRE, was determined using phase-contrast microscopy. (15.00µg/mL). We identified morphological

characteristics such as membrane blebbing, cell shrinkage, and necrosis.

2.7. Caspase-3, caspase-9 and p53 assay by ELISA

Caspase activity was determined using caspase-3, caspase-9, and p53 ELISA kits (Ahmed et al., 2022). Each well in a 96-well plate received 50,000 MCF-7 cells. In a humid incubator, the cells were maintained for 24H at 37°C with 5% CO2. The 96 well plate plates that included the LRE-treated, as well as control cells, were then left at room temperature to equilibrate. Caspase-3 and 9 reagents have been added to every well of a plate containing 100µL of culture media (LRE-treated and controlled). The plate was completely covered, and thus the mix was agitated at 500rpm for 30 seconds. After 30 minutes of room temperature incubation, the transmittance at 405 nm was measured using an ELISA reader.

2.8. DNA fragmentation and apoptosis induced by LRE

MCF-7 cell line cells were treated with LRE at IC_{50} and $2XIC_{50}$ to induce apoptosis in order to study DNA fragmentation. In accordance with the manufacturer's recommendations, DNA purification kits were used to extract the DNA (Thermo Fisher Scientific, CA, USA). Following quantification, 2µg of each DNA sample was loaded for electrophoresis on a 1.5% agarose gel, and the gel was stained with ethidium bromide before being photographed under ultraviolet light.

3. Results and discussion

3.1. Antioxidant and free radical scavenging activity

Oxidative stress and reactive oxygen species have been implicated in several human diseases, including carcinoma, arteriosclerosis, and inflammatory disorders, as well as aging processes (Pham-Huy et al., 2008). Recently, interest has increased considerably in finding naturally occurring antioxidants for use in foods, cosmetics, or medicinal materials to replace synthetic antioxidants, which are being restricted due to their carcinogenicity (Engwa, 2018). Dietary and herbal formulations that have free radical scavenging potential have gained importance in treating such chronic diseases. Many studies have linked the antioxidant activities of fruits and vegetables and medicinal herbs to their phenolic compound content (Pham-Huy et al., 2008; Sharifi-Rad et al., 2020; Lobo et al., 2010; Young and Woodside, 2001). The antioxidant properties of 80% methanol extracts for LRE were chemically estimated using the DPPH radical scavenging activity IC_{50} values of 22.21µg/mL (Fig. 1 and Table 1).

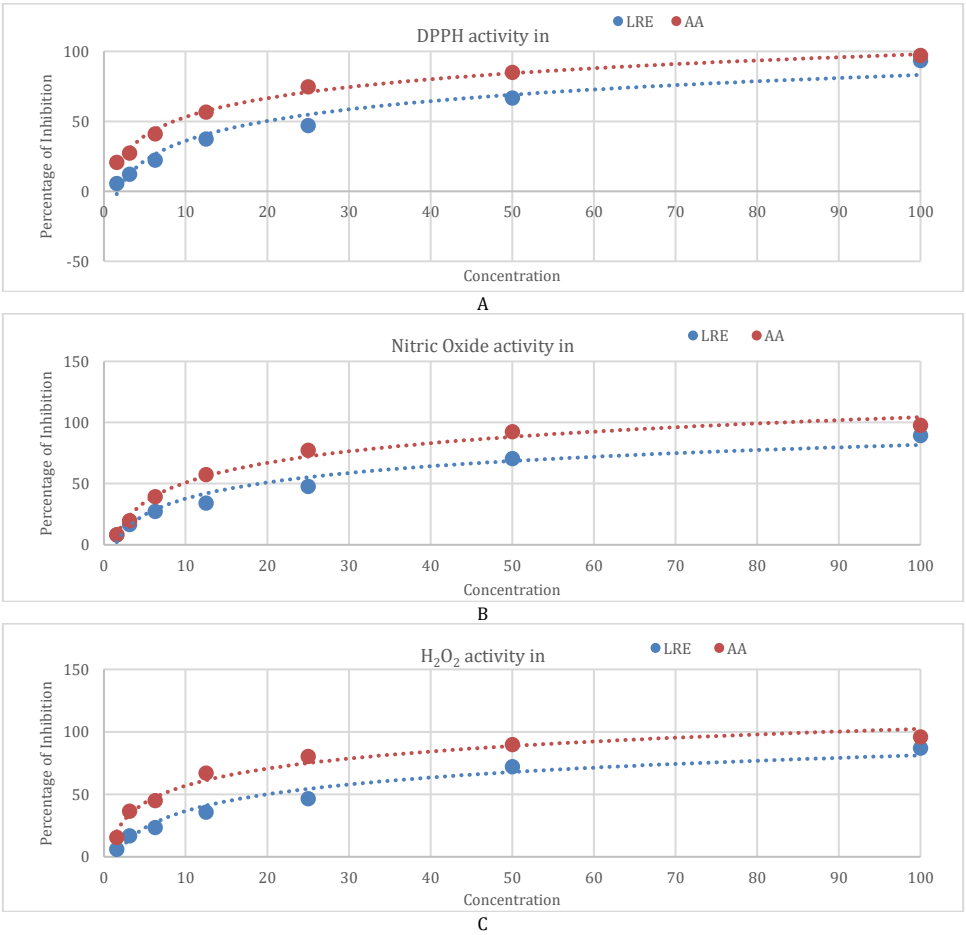

Fig. 1: Dose-response curve for LRE and standard Ascorbic acid for antioxidant properties. A: DPPH, B: Nitric Oxide, and C: Hydrogen peroxide; Values expressed as a mean ±SD (n=3)

Table 1: IC_{50} values for LRE and ascorbic acid for antioxidant properties

	DDPH	Nitric oxide	H_2O_2
LRE IC_{50} (μg/mL)	22.21±2.88	21.14±3.21	24.91±2.19
R^2	0.9647	0.9630	0.9863
Ascorbic acid	8.477±0.772	9.244±0.688	6.613±0.543
R^2	0.9831	0.9892	0.9859

The IC_{50} values nearly equal one-third of standard ascorbic acid. The determination of nitric oxide activity using the sodium nitroprusside method showed that LRE had a high antioxidant capacity with an IC_{50} value of 21.14±3.21μg/mL (Fig. 1 and Table 1). The findings of this study were consistent with previous research on the antioxidant properties of some Lotus species (Morikawa et al., 2016; Paudel and Panth, 2015).

3.2. Cytotoxic effect of LRE on MCF7 Cells by cell viability assay

When particularly in comparison to MCF-7 cell lines, the MTT assays showed a concentration-dependent reduction in viability for LRE (Fig. 2).

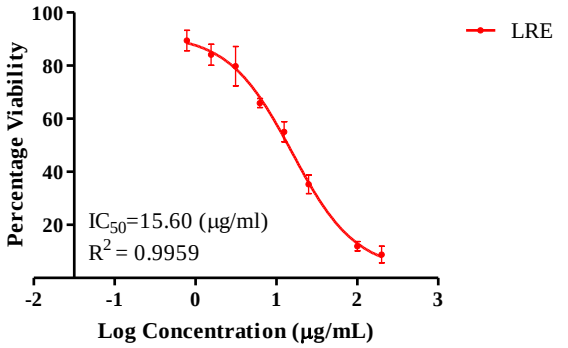

Fig. 2: Dose-response cure for cytotoxicity of LRE after 48H incubation with MCF-7 cells at concentrations 0.78 to 200μg/mL

The IC_{50} value for LRE in MCF-7 cells was determined to be 15.60μg/mL. According to the MTT assay results, LRE had encouraging anticancer activity against breast cancer cell lines, most probably due to the existence of antioxidant phytoconstituents. Previous research has suggested that LRE may be used as an effective drug booster in the treatment of breast cancer (18,32).

3.3. Activation of apoptotic regulatory genes (Caspase-3, caspase-9, and p53) by LRE

Apoptosis is a sort of induction of apoptosis in which intracellular components are disassembled while nearby cells are not injured or inflammatory (30,31). Caspase-3, -9, and p53 activation are primarily responsible for tumor cell apoptosis. To confirm apoptosis, a rise in caspase 3, 9, and p53 production in LRE-treated MCF-7 cells was especially in comparison to the untreated group in this study. Once MCF-7 cells were subjected to LRE, their caspase 3, 9, and p53 activity increased up to fourfold compared to control (untreated) cells (Fig. 3 and Table 2). The increased effectiveness of LRE indicates a possible cause of caspase activation in cancer cells.

3.4. DNA fragmentation analysis

Apoptosis is distinguished by DNA internucleosomal rupture, cell membrane blebbing, nuclear chromatin condensation there in the nuclear periphery, and the formation of apoptotic, condensed nuclear bodies. MCF-7 cells have been treated with different concentration levels of LRE to verify the apoptosis induction, and DNA has been isolated and analyzed using agarose gel electrophoresis. MCF-7 cells treated for 24 hours with LRE (15 and 30μg/mL; IC50 and 2X IC50) and standard Doxorubicin (5μg/mL) showed significant internucleosomal fragmentation (Fig. 4).

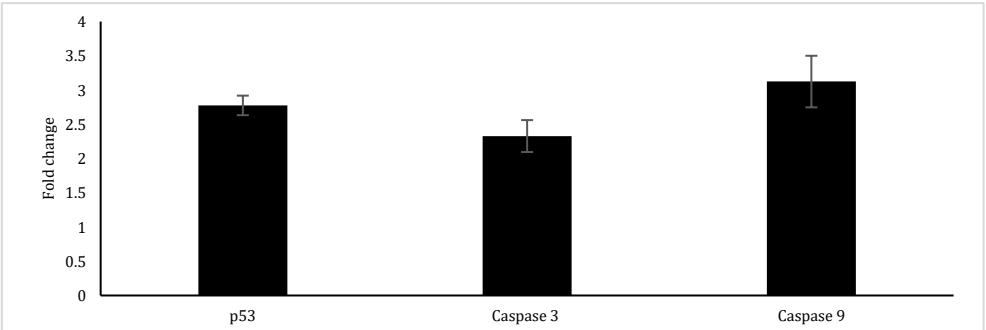

Fig. 3: Activation of caspase 3, 9, and p53 in LRE-treated MCF7 cells was compared to the untreated control. Data presented here is folded change from the untreated control group

Table 2: Expression of apoptotic regulatory genes induced by LRE (n=3)

	P53 (pg/ml)	Caspase 3 (pg/ml)	Caspase 9 (ng/ml)
LRE	1065.67±54.82	310.32±31.08	494.18±59.57
Control	384.00±49.00	133.33±13.52	158.20±33.04

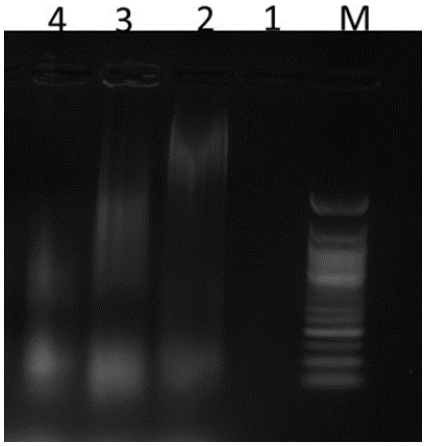

Fig. 4: DNA fragmentation of MCF-7 cells exposed to LRE

In Fig. 4, fragmentations of genomic DNA in MCF-7 cells were treated for 24 h with 15 and 30µg/mL LRE. DNA laddering formation was viewed on ethidium bromide-stained Agarose gel (1.5%). Marker, marker 100 bp; 1=Control, 2=LRE 15µg/mL; 2=LRE 30µg/mL and 4= Doxorubicin 5µg/mL. DNA strand breaks are present during apoptosis, and scratches in DNA molecules could be detected using a DNA fragmentation assay. Apoptosis is well known to be engaged in the stimulation of endonucleases, which ultimately results in DNA fragmentation; this can be seen via electrophoretic investigation (Eastman and Barry, 1992). In this study, agarose gel electrophoretic findings show that inter-nucleosomal DNA fragmentation aided the steady progress of apoptosis in LRE extract-treated MCF-7 cells.

4. Conclusion

Furthermore, the bioavailability, pharmacokinetics, and potential adverse effects of phytochemicals derived from *N. nucifera* are discussed, as are the limitations of the current research and its challenges and future research directions. The lotus roots have strong antioxidant properties, suggesting that they could be used as a promising natural antioxidant source. Lotus needs to be developed further for the treatment of cancer patients, as well as for use as value-added components in the food and pharmaceutical industries rather than for ornamental and culinary purposes.

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Compliance with ethical standards

Conflict of interest

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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