

Review

Bioactives of the Freshwater Aquatic Plants, *Nelumbo nucifera* and *Lemna minor*, for Functional Foods, Cosmetics and Pharmaceutical Applications, with Antioxidant, Anti-Inflammatory and Antithrombotic Health Promoting Properties

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Featured Application: Utilization of Freshwater aquatic plants' bioactives for functional foods, cosmetic and pharmaceutical applications with anti-inflammatory, antithrombotic and antioxidant health promoting properties.

Abstract: Despite significant progress, certain inflammation related to chronic disorders, including cardiovascular diseases (CVD) and cancer, still present high mortality rates. Thus, further study is needed to address such pathologies more appropriately. Apart from classic therapeutics, natural bioactives with less toxic side effects have gained attention, including those with potential pharmaceutical properties derived from several plants. Within this article, the potential utilization of freshwater aquatic plants as sources of bioactives with antithrombotic, anti-inflammatory and antioxidant properties is outlined. Emphasis is given to a well-established aquatic plant with known but not fully clarified and overviewed bio-functional and pharmaceutical properties, the Chinese lotus (*Nelumbo nucifera*), as well as to a so far neglected aquatic plant, *Lemna minor*, which has not yet been thoroughly reviewed for such applications. The latent usually grows naturally in large numbers at the surface of stored water basins of retrieved water from the last stages of wastewater treatment facilities. The continuous growth of this aquatic plant in such conditions further suggests that it can be a sustainable source of natural bioactives, if appropriately valorized, with an economic benefit and in a friendly environmental approach. The abundant content of both freshwater aquatic plants in bioactive components with potent antioxidant, anti-inflammatory and antithrombotic activities is thoroughly outlined, while their applications as functional ingredients in several functional products (functional foods, cosmetics and pharmaceuticals) are also discussed. The outlined outcomes urge further study of both aquatic plants and especially of *Lemna* spp. to fully elucidate their potential as alternative sustainable sources of bioactive ingredients for functional foods, supplements, nutraceuticals, nutricosmetics, cosmeceuticals, cosmetics and pharmaceutical products with health-promoting properties against inflammation and thrombosis related manifestations and their associated chronic disorders.

Keywords: aquatic plants; *Nelumbo nucifera*; *Lemna*; chronic diseases; antioxidant; antithrombotic; anti-inflammatory; functional foods; pharmaceuticals; cosmetics

1. Introduction

Several inflammation- and thrombosis-related chronic diseases, including diabetes, various cardiovascular diseases, chronic respiratory diseases and cancer, contribute to the increased mortality rates in developed and developing countries, as well as to the reduced quality of the lives of patients and their families and a heavy burden on the healthcare systems [1,2]. There are several variable and non-variable risk factors associated with these diseases, including the individual's lifestyle (smoking, diet, physical activity, etc.), gender, age, race and genetic profile [2]. The presence or coexistence of such risk factors appears to induce underlying molecular and cellular mechanistic pathways, which cause the continuous triggering of chronic inflammation and, consequently, thrombo-inflammatory complications leading to such chronic diseases. These chronic diseases can develop over time, before cellular dysfunction manifests and causes tissue damage, while if not balanced by our immune system and specific preventative homeostatic processes, they will eventually be established, with specific medical treatment being the needed measures for reducing the increased risk for mortality in such pathological states. On the other hand, the adoption of healthy habits, such as a healthy diet and lifestyle, as well as the utilization of functional products that can reduce chronic thrombo-inflammation and oxidative stress, seem to contribute highly to prevention approaches against these chronic disorders [3].

Research has shown that many natural sources contain important bioactive compounds with anti-inflammatory, antioxidant and antithrombotic activities. A typical category of these sources is several aquatic plants, which are an excellent source of compounds with recognised biological and therapeutic properties against these manifestations. A wealth of active biological metabolites has been isolated from aquatic plants and used as pigments, additives, nutrients, and bio-functional ingredients that have contributed to the discovery of novel products and drug development (Figure 1) [4].

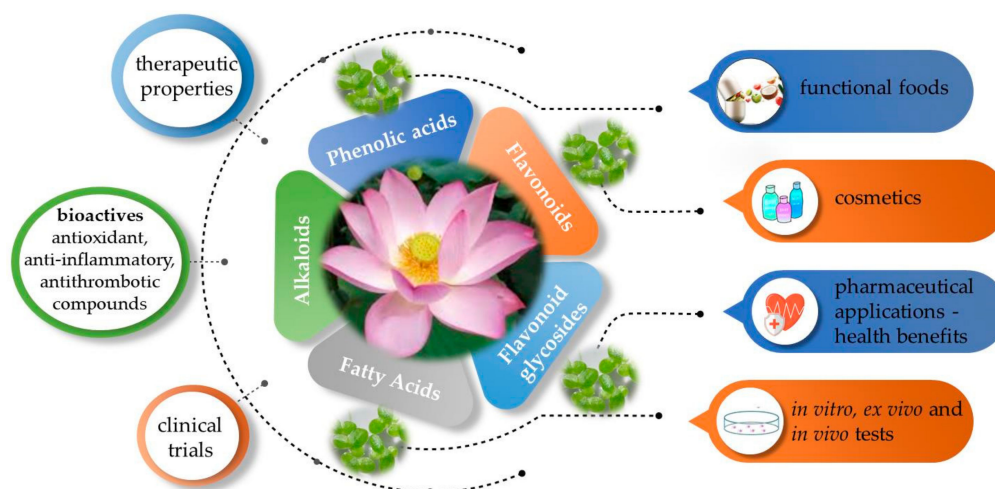


Figure 1. Representative bioactive compounds of freshwater aquatic plants with potential applications in functional foods, cosmetics and pharmaceuticals with health-promoting properties.

The larger family of such aquatic plants includes the *Nelumbo nucifera*, while some studies have also outlined that other less discussed aquatic plants can also contribute to the above approaches, such as representatives from *Araceae* family (e.g., *Lemna* spp). These floating aquatic plants grow in thick layers in still or slow-moving waters and are rich in nutrients [5] and in slow-moving rivers up to 2 m deep.

Lotus (*Nelumbo nucifera* Gaertn.) is an aquatic plant widely distributed in Asia, America and Australia. Lotus flowers, rhizomes and seeds have nutritional and medicinal uses, with their seeds being rich in carbohydrates, proteins, lipids and polyphenols [6]. This plant is often cultivated for its elegant and sweet-scented flowers, constituting the national flower of India [7]. Nevertheless, the plethora of bioactives present in this plant and their

potential applications in several functional products have not yet been fully studied and require further analysis.

Apart from its growth in freshwater rivers and lakes, *Lemna* spp. has recently been observed to grow indigenously on the surface of water basins where water derived from the last stage of urban wastewater treatment facilities is usually stored, in which phosphate and nitrate salts are abundant, indicating that they are beneficial components for the growth of this aquatic plant [5,8]. Therefore, the continuous growth and sustainable availability of *Lemna* spp. in such water facilities seem to serve as rich sources of bioactive constituents, which need further attention for potential applications in products that can contribute to the prevention of chronic diseases, similarly to the well-established aquatic plant *Nelumbo nucifera*. This will ensure a permanent and highly economical source of bioactive compounds with significant antioxidant, anti-inflammatory and antithrombotic activity [9].

Within this study, the rich content in such bioactives observed in both aquatic plants, *Nelumbo nucifera* and *Lemna minor*, is thoroughly outlined, while their potential applications in several applications of functional products with anti-inflammatory and antioxidant health-promoting properties are thoroughly reviewed.

2. Materials and Methods

To carry out the necessary literature review, various databases were explored, such as PubMed, Scholar Google, Science Direct, Scopus and the NIST library. The combinations of keywords used were each one word from the first parenthesis AND one of the words of the second parenthesis: (“*Nelumbo nucifera*” OR Lotus OR *Nelumbo nucifera* Gaertn. OR *Lemna* OR *Lemna minor* OR *Lemna* spp. OR Duckweed) AND (Antioxidant OR Anti-inflammatory OR Inflammation OR Oxidative stress OR Thrombin OR Antithrombotic OR Anti-platelet OR Anti-cancer OR Anti-diabetic OR Antimicrobial OR Antibacterial OR Neuroprotective OR “Functional Food” OR Cosmetic OR Drug OR Pharmaceutical OR Bioactive OR “In vitro” OR “In vivo” OR “Ex vivo”).

Initially, the search was focused on the dates 2014–2024; however, the results were not sufficient, especially for *Lemna* spp., and thus the search time frame was expanded to 2004–2024. In some cases, some older reported studies were also included, which were not previously thoroughly reviewed.

Articles in a language other than English, duplicates and the majority of review articles were excluded, apart from some important recent review articles on general information about these aquatic plants.

3. *Nelumbo nucifera*

3.1. *Nelumbo nucifera*: General Information

The lotus is a water plant that is widespread in Asia, America and Australia fresh waterbodies. Lotus (*Nelumbo nucifera* Gaertn.) is one of two species of the genus *Nelumbo* in the family *Nelumbonaceae* [7]. *N. nucifera* Gaertn. is cultivated in many countries, including China, India, Russia and Australia. The other species, *N. lutea* Willd. is found mainly in the eastern and southern parts of North America [10]. There is diversity in the plant physiology and characteristics of the different lotus species. This diversity has been enhanced by hybridization and mutation [11] among other plants of the same category, holding the largest area under cultivation and the largest production as well. According to certain morphological differences, Chinese lotus is usually divided into three types: rhizomatous lotus, lotus seed and lotus flower [12]. The main characteristics of the rhizomatous lotus are the greater height of the plant, the presence of few or no flowers and the edible rhizome with the highest yield. The lotus seed has the largest number of carpids and seeds, with the nutritional properties of the seed being generally considered better than those of the rhizomatous lotus. The latter type of flowering lotus has the lowest plant height of most flowering types and is only used as an ornamental plant [13]. The seeds and lotus rhizome are commonly used as food, while leaves and flowers are mainly used in herbal products.

In traditional Chinese or Indian medicine, these four edible parts are believed to own medicinal functions [14]. The rhizomatous lotus has the largest area under cultivation, followed by the seed lotus. The Chinese lotus is widespread throughout the country, from southern Hainan province to northern Heilongjiang province and from eastern Taiwan to western Tian Mountain in Xinjiang province. The largest area of lotus cultivation is around the Yangtze River [15].

N. nucifera grows as an emergent aquatic plant in water up to 2 m deep on the edges of stagnant water or lakes and slow-flowing rivers [16]. As an aquatic plant, it needs plenty of space and full sun to thrive. It has sturdy, climbing, yellow rhizomes and green fruits. The leaves are large, both aerial and floating, circular, 20 to 90 cm in diameter, sharply pointed with a short margin, stalked, entire, glaucous, non-wetting, strongly cup-shaped in aerial leaves and flat in floating leaves [17]. The leaves are pinnate on stout stems that emerge from a horizontal rhizome at a height of 1 m or more. The apical meristem consists of two protective sheaths enclosing a single flower, a single leaf and a younger flower/leaf node complex. Internal leaf elongation occurs between the primordial leaf of one cluster and the leaflets of the next, younger cluster. The perianth consists of two sepals and 18 to 28 petals. The corolla is pale yellow in *N. nucifera* lutea and pink or white in *N. nucifera*. The fruits are not fleshy [18]. The fruit is a set of dried nuts. Mature nuts are oval, rounded or elongated, up to 1.0 cm long, 1.5 cm long and 1.5 cm wide, with a hard, smooth, brown or grey-black pericarp, slightly longitudinally striated, with a stalk and a seed. The seeds fill the ripe fruit [17]. Extreme seed longevity has been observed, with seeds germinating after 1000 years or more [19].

Several bioactives have been detected in *N. nucifera* (Table 1), with a plethora of health benefits being reported for these bioactives and *Nelumbo*'s extracts for their potential pharmaceutical and cosmeceutical applications (Table 2), as well as in functional feeds and nutraceutical products (Table 3), with several antioxidant, anti-inflammatory and antithrombotic health-promoting effects against several manifestations of thrombo-inflammation and oxidative stress and their associated chronic disorders, either in vitro and/or ex vitro and/or in vivo.

Table 1. Bioactives of *Nelumbo nucifera*.

Part of the Plant		Bioactive Compound (Composition)		Reference
Phenolic acids (mg/100 g DW)	Stamen	<i>p</i> -Coumaric acid	10.78 ± 0.38	[20]
	Petal	Gallic acid	277.84 ± 6.36	
	Seed embryo	Ferulic acid	24.71 ± 2.03	
		<i>p</i> -Coumaric acid	105.34 ± 2.93	
	Leaf stalk	Gallic acid	163.09 ± 8.58	
	Old leaf	Gallic acid	49.38 ± 4.83	
Flavonoids	Flower Stalk	Myricetin	8.89 ± 0.83	[20]
		Luteolin	4.89 ± 0.35	
		Quercetin	59.91 ± 5.64	
		Naringenin	2213.41 ± 11.35	
		Kaempferol	6.40 ± 0.64	
		Isorhamnetin	3.51 ± 0.28	
		Cyanidin	12.02 ± 0.09	
		Delphinidin	20.70 ± 0.24	
	Stamen	Myricetin	7.63 ± 0.35	[20]
		Quercetin	43.94 ± 2.08	
		Naringenin	2185.84 ± 24.21	
		Kaempferol	160.71 ± 13.66	
		Isorhamnetin	192.09 ± 15.70	
		Cyanidin	115.79 ± 10.21	
		Delphinidin	211.63 ± 17.21	

Table 1. Cont.

Part of the Plant		Bioactive Compound (Composition)	Reference
Flavonoids	Petal	Myricetin	8.55 ± 0.29
		Quercetin	196.34 ± 19.03
		Naringenin	2226.69 ± 13.66
		Kaempferol	197.83 ± 19.81
		Isorhamnetin	237.85 ± 13.86
		Cyanidin	349.98 ± 24.28
		Delphinidin	1837.27 ± 52.67
		Luteolin	37.50 ± 1.87
		Quercetin	81.79 ± 3.57
		Naringenin	2241.51 ± 18.41
		Kaempferol	4.92 ± 0.41
		Isorhamnetin	11.56 ± 0.85
		Cyanidin	1901.52 ± 14.15
		Delphinidin	691.58 ± 9.84
	Seed Embryo	Schaftoside	474.1 mg/100 g DW
		Vicenin-2	72.9 mg/100 g DW
		Luteolin 6-C-glucosyl-8-C-arabinoside	46.0 mg/100 g DW
		Isoorientin	49.5 mg/100 g DW
		Orientin	67.3 mg/100 g DW
		Isochaftoside	91.0 mg/100 g DW
		Apigenin 8-C-glucoside	33.1 mg/100 g DW
		Apigenin 6-C-glucosyl-8-C-rhamnoside	30.5 mg/100 g DW
		Flavonoid O-glycosides	-
		Luteolin 7-O-neohesperidoside	83.7 mg/100 g DW
	Leaf Stalk	Kaempferol 7-O-glucoside	14.5 mg/100 g DW
		Isorhamnetin 3-O-rutinoside	17.5 mg/100 g DW
		Diosmetin 7-O-rutinoside	29.1 mg/100 g DW
		Luteolin	12.43 ± 0.77
		Quercetin	35.95 ± 1.94
		Naringenin	1918.10 ± 37.81
	Old Leaf	Isorhamnetin	6.80 ± 0.35
		Cyanidin	7.15 ± 0.74
		Delphinidin	6.15 ± 1.05
		Quercetin	458.56 ± 33.45
		Naringenin	1064.17 ± 75.38
		Kaempferol	3.87 ± 0.31
Leaves		Isorhamnetin	2.67 ± 0.09
		Cyanidin	184.82 ± 11.38
		Delphinidin	39.46 ± 2.42
		Quercetin 3-O-arabinopyranosyl-(1→2)-galactopyranoside	104.9 mg/100 g DW
		Hyperoside	422.0 mg/100 g DW
		Isoquercitrin	274.6 mg/100 g DW
		Quercetin 3-O-glucuronide	393.4 mg/100 g DW
		Quercetin 3-O-rhamnopyranosyl-(1→2)-glucopyranoside	13.1 mg/100 g DW
		Quercetin 3-O-arabinoside	7.7 mg/100 g DW
		Astragalin	150.8 mg/100 g DW
		Kaempferol 3-O-galactoside	29.5 mg/100 g DW
		Kaempferol 3-O-glucuronide	33.6 mg/100 g DW
		Myricetin 3-O-hexose	26.7 mg/100 g DW
		Diosmetin 7-O-hexose	16.2 mg/100 g DW
		Isorhamnetin 3-O-arabinopyranosyl-(1→2)-glucopyranoside	10.0 mg/100 g DW
		Isorhamnetin 3-O-hexose	9.8 mg/100 g DW
		Isorhamnetin 3-O-glucuronide	8.9 mg/100 g DW
		Catechin	22.3 µg/mg in water and 34.3 µg/mg in ethanol
		Quercetin	3.72 µg/mg in water and 1.80 µg/mg in ethanol

Table 1. Cont.

Part of the Plant	Bioactive Compound (Composition)	Reference
Alkaloids	(−)-1(<i>R</i>)- <i>N</i> -methylcoclaurine (−)-Lirinidine (5-demethylnuciferine) (−)-Anonaine (−)-Asimilobine (−)-Caaverine (−)- <i>N</i> -Methylasimilobine (−)-nor-Nuciferine (−)-Nuciferine (−)-Roemerine (6 <i>R</i> ,6 <i>aR</i>)-Roemerine- <i>N</i> β-oxide (<i>R</i>)-Roemerine 2-Hydroxy-1-methoxy-6 <i>a</i> , 7-dehydroaporphine 7-Hydroxydehydronuciferine Anonaine Cepharadione B <i>cis</i> - <i>N</i> -Coumaroyltyramine <i>cis</i> - <i>N</i> -Feruloyltyramine <i>D</i> , 1-Armepavine Dehydronuciferine Dehydroroemerine Isoliensinine Liensinine Liriodenine Lotusine Lysicamine Neferine <i>N</i> -methylasimilobine <i>N</i> -Methylasimilobine <i>N</i> -Nornuciferine Nornuciferine Nuciferine Nuciferine <i>N</i> -oxide Oleracein E Pronuciferine Roemerin Trans- <i>N</i> -Coumaroyltyramine Trans- <i>N</i> -Feruloyltyramine	[22]
	(−)-Lirinidine (5-demethylnuciferine) 2-Hydroxy-1-methoxy-6 <i>a</i> , 7-dehydroaporphine Asimilobine <i>D</i> , 1-Armepavine Dehydronuciferine Lysicamine <i>N</i> -Methylasimilobine <i>N</i> -Nornuciferine Nuciferine Nuciferine <i>N</i> -oxide	[22]
	(−)-Lirinidine (5-demethylnuciferine) Anonaine Armepavine Asimilobine Dihydroergotamine Dehydroemerine Dehydronuciferine Demethylcoclaurine Isoliensinine Liensinine Liriodenine <i>N</i> -Methylasimilobine <i>N</i> -Methylcoclaurine <i>N</i> -Methylisococlaurine <i>N</i> -Norarmepavine Nornuciferine Reserpine Roemerin	[22]

Table 1. Cont.

Part of the Plant	Bioactive Compound (Composition)	Reference
Alkaloids	3-Indoleacetic acid Anisic acid Coclaurine Dauricine Isoliensinine Liensinine Lotusine Neferine Nuciferine Tryptophan	[22]
	4'-Methyl-N-methylcoclaurine Armepavine Higenamine Higenamine 4'-O- β -D-glucoside O-Nornuciferine Roemerin	[22]
	Norjuziphrine	[22]
	Reserpine	[22]
Fatty Acids	14 carbonates Pentadecanoate 14-Methylpentadecanoic acid Maleic-7-hexadecane acid Maleic-9-octadecenoic acid 17 carbonates 18 carbonates Anti-9-octadecenoic acid Anti-8-octadecenoic 8,11-Octadecadienoic acid 9,12,15-Octadecatrienoic acid 20 carbonates Behenic acid 23 carbonates	[10]
	Myristic acid (14:0) Pentadecanoic acid (15:0) Palmitic acid (16:0) Palmitoleic acid (16:1) Margaric acid (17:0) Stearic acid (18:0) Oleic acid (18:1) Linoleic acid (18:2) α -Linolenic acid (18:3) Behenic acid (22:0) Saturated fatty acid (SFA) Monounsaturated fatty acid (MUFA) Polyunsaturated fatty acid (PUFA)	[10]

3.2. Antioxidant Health Promoting Effect of *Nelumbo nucifera* Bioactives and Extracts

3.2.1. In Vitro Antioxidant Effects of *Nelumbo nucifera*'s Bioactives and Extracts

Various studies have explored the antioxidant properties of different parts of the lotus plant, including seed embryos [20,21], leaves [20,23], rhizomes [24], seeds [20,25], stamens [20,26] and flower [27] to investigate their potential health benefits and applications as natural antioxidants. These studies have focused on identifying and isolating bioactive compounds, particularly flavonoids and other phytochemicals, from lotus plant parts using diverse chromatographic and analytical techniques.

M. Zhu et al. [21], isolated and characterised 16 flavonoids from lotus seed embryos, comprising eight flavonoid C-glycosides and eight flavonoid O-glycosides (fractions I and II). The antioxidant effects of these fractions were evaluated in comparison to those in lotus leaves through 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid (ABTS) and ferric reducing antioxidant power (FRAP)

assays, demonstrating that both fractions I and II from lotus seed embryos exhibited antioxidant potential.

Many oxidizing enzymes, including superoxide dismutase, may produce hydrogen peroxide *in vivo*. Hydrogen peroxide is not highly reactive but can sometimes be toxic to cells because it can generate hydroxyl radicals within them. DPPH•, is a nitrogen radical with a long-life expectancy. Many antioxidants that react rapidly with transient radicals, like peroxy, may be slow to react or even be inert in the presence of DPPH• [28]. DPPH• is also widely used to evaluate the radical scavenging efficacy of antioxidants [29]. Antioxidants were able to reduce the stable radical DPPH• to the yellow-colored diphenyl picrylhydrazine in the DPPH• assay. This method is hinged on the reduction of an alcoholic solution of DPPH• in the presence of a hydrogen-donating antioxidant [8]. This reaction produces the non-radical form of DPPH-H. DPPH•, is commonly used as a reagent for the evaluation of the free radical scavenging activity of antioxidants [30].

The generation of the ABTS⁺ radical cation forms the basis of one of the spectrophotometric methods used to measure the total antioxidant activity of solutions of pure substances, aqueous mixtures and drinks [31,32]. A decolorization technique, in which the radical is directly generated in a stable form prior to reaction with putative antioxidants, is a more suitable format for the assay. The improved technique for generating ABTS described here involves the direct generation of the blue/green ABTS^{•+} chromophore by reacting ABTS with potassium persulphate. Moreover, superoxide anion radicals are produced by several enzyme systems in autoxidation reactions and by non-enzymatic electron transfer reducing molecular oxygen. These systems are also capable of reducing certain iron complexes, such as cytochrome c [33]. The Ferric Reducing Antioxidant Power (FRAP) assay evaluates the reducing power of antioxidants by measuring their ability to reduce ferric ions (Fe³⁺) to ferrous ions (Fe²⁺). This assay provides information on the electron-donating capacity of antioxidants. While the total flavonoid content in the combined fractions I and II of lotus seed embryos was lower than that in lotus leaves, the C-glycosides in the seed embryos showed higher antioxidant properties compared to the O-glycosides in lotus leaves. This suggests that lotus seed embryos could be valuable sources of natural flavonoids with antioxidant properties, potentially suitable for incorporation into dietary supplements to promote health [21].

Other studies also employed the DPPH assay and yielded similar outcomes. Nguyen Thi Quynh et al. analyzed the methanol extract of lotus seed in six different lotus varieties, identifying 27 phytochemical constituents, with methyl- α -D-galactopyranoside being the predominant compound across all varieties. The study demonstrated notable antioxidant activity of the methanolic extracts of lotus seed varieties, with DPPH radical scavenging ranging from 9.86% to 77.97% and IC₅₀ values lower than those of the control, ascorbic acid [25].

Jung et al. fractionated a methanol extract from lotus stamens using organic solvents (dichloromethane, ethyl acetate, n-butanol), leading to the isolation of seven flavonoids and one sterol glucopyranoside from ethyl acetate fraction, which demonstrated strong antioxidant activity in all model systems [26]. These compounds were evaluated for their antioxidant properties in various model systems (DPPH, reactive oxygen species [ROS], and ONOO⁻) to assess their ability to scavenge free radicals and inhibit ROS generation. ROS test involves measuring ROS levels in biological samples to assess oxidative stress and potential damage to cells. The ROS test typically involves using specific probes or dyes that react with different types of ROS present in the biological sample. These probes undergo a chemical reaction with ROS, leading to a measurable change in fluorescence, absorbance or color. ONOO⁻ test evaluates levels of peroxynitrite, a reactive nitrogen species (peroxynitrite is formed by the rapid reaction between nitric oxide NO and superoxide anion O₂⁻), to understand its role in oxidative and nitrosative stress-related conditions. These tests help assess the impact of these reactive species on cellular function and the effectiveness of antioxidants in combating oxidative damage. The isolated bioactive compounds assessed exhibited antioxidant properties. Kaempferol demonstrated strong antioxidant activity in

all three tests, while 13-sitosterol glucopyranoside did not exhibit any activity in the model systems. Specific flavonoids like Kaempferol 3-O- β -D-glucopyranoside and Kaempferol 3-O- β -D-galactopyranoside showed activity in the ONOO test [26].

Utilizing the DPPH free radical scavenging assay as a standard method for assessing antioxidant capacity, Sranujit et al. demonstrated a correlation between the concentration of bioactive compounds in a plant extract and its antioxidant potential. The extract analyzed in the study contained chlorogenic acid, ferulic acid and coumarin as the primary phytochemicals. Results indicated that the ethyl alcohol (ET) extract derived from lotus petals exhibited superior antioxidant activity compared to the ethyl acetate (EA) extract. This suggests that the bioactive components present in the ET extract possess enhanced antioxidant properties, making it a promising source of antioxidants. Specifically, the antioxidant capacity of the ET extract was found to be 1.5 times higher than that of the EA extract [27].

Finally, Temviriyankul et al. assessed the antioxidant potential of various parts of the Sacred Lotus plant, including the seed embryo, flower stalk, stamen, old leaf, petal and leaf stalk, through hot water extraction. By identifying phenolic compounds and flavonoids in the extracts, the researchers sought to understand how these antioxidants contribute to combating oxidative stress using assays such as DPPH, FRAP and ORAC. Oxygen Radical Absorbance Capacity (ORAC) is a method used to measure the antioxidant capacity of a substance against peroxy radicals. This assay quantifies the ability of antioxidants to scavenge peroxy radicals and inhibit the oxidative damage caused by these radicals. ORAC values provide information on the overall antioxidant potential of a sample and its capacity to protect against oxidative stress. Results indicated that all parts of the Sacred Lotus exhibited antioxidant activities in all three assays. The old leaf showed the highest DPPH radical scavenging and ORAC activities, while the stamen exhibited the highest FRAP activity. The top three parts with the highest overall antioxidant activities were stamen, old leaf, and petal. Subsequently, mixtures of these parts were analyzed to determine the most effective combination with high antioxidant activities using DPPH, FRAP, and ORAC assays. The study revealed that specific mixtures, such as stamen:old leaf:petal (3:0:0) or only stamen, demonstrated the highest antioxidant activities in both DPPH and FRAP assays, while stamen:old leaf:petal (1:3:3) exhibited the highest ORAC activity [20].

In another study, Jiang et al. isolated tryptophan from lotus rhizomes for the first time and identified it as a key active component with antioxidant properties. The evaluation of antioxidant activity included testing erythrocyte hemolysis inhibition by measuring hemolysis percentage after incubating erythrocytes with test samples and assessing lipid peroxidation inhibition by evaluating the malondialdehyde-TBA complex color after incubating kidney homogenates with test samples. The lipid peroxidation assay assesses the ability of antioxidants to inhibit the peroxidation of lipids, which is a key process in oxidative damage to cell membranes. It measures the formation of peroxides as a marker of lipid oxidation. Tryptophan from lotus rhizome extract demonstrated potent antioxidant effects in inhibiting erythrocyte hemolysis, with an IC_{50} value of 156.3 mg/mL. However, in terms of inhibiting lipid peroxidation, tryptophan from lotus rhizome extract, particularly L2c-3, exhibited limited activity. This study marked the successful isolation of tryptophan from lotus rhizome and highlighted its antioxidant properties [24]. According to one in vitro clinical investigation from M.P. Thanushree et al., it was observed that lotus rhizome powder in breadsticks can improve consumers' health because of its antioxidant activity. The breadsticks were made with lotus rhizome powder (LRP), and the nutritional composition was analysed. The results showed that all breadsticks containing LRP had significantly higher amounts of total phenolic content, using the coloured reaction of phenolics with Folin-Ciocalteu reagent and total flavonoid content, with the aluminum chloride colorimetric method when compared with the control. The Folin-Ciocalteu assay is used to determine the total phenolic content in samples. It involves the reduction of the Folin-Ciocalteu reagent by phenolic compounds, leading to a color change that can

be quantified spectrophotometrically. High antioxidant activity by the test of DPPH free radical scavenging activity was also observed [34].

Lotus seeds were roasted until browned, and extract with hot water was consumed by rats for a long period of time to observe the protective effects on skin against photoaging due to UVB irradiation. After 8 months, there was a significant increase in the moisture content in the Lotus Seed Tea (LST) group rather than the water-control group. LST intake has been shown to inhibit the drying of the skin, which is caused by UV radiation to a certain degree [35]. Similar results were presented in the in vitro study of Saeed et al., where Lotus root flour was added to biscuits as a fat mimetic. In this research, different concentrations of lotus root flour (LRF) were added to wheat flour as a fat mimetic to have sample flours of varying wheat-to-LRF ratios. The biscuits had an increase in their total phenolic content, DPPH inhibition and FRAP as compared to the control sample, and it was concluded that wheat flour replacement with LRF in wheat-based food products will improve consumers' health benefits [36].

Several in vitro studies utilised the DPPH radical scavenging activity method to evaluate the antioxidant properties of various *Nelumbo* part extracts. More specifically, various extracts from Lotus Seeds (LS) and Seedpods (LSP) were tested, including fractions obtained using 80% ethanol, hexane, chloroform, ethyl acetate, butanol and water [37]. Additionally, S. Rai et al. examined *N. nucifera* seeds hydroalcoholic extract [38]; J.S. You et al., lotus root ethanol extract [39]; D.-J. Shin et al., 50% and 70% ethanol extracts of lotus root and lotus leaf [40]; Wang et al., methanol extracts of lotus plumule and blossom [41] and Choe et al., ethanol or methanol extracts of lotus leaf [42]. Results consistently demonstrated high antioxidant activity across the different extracts. For instance, You et al. compared the DPPH radical scavenging activity of lotus root extract with ascorbic acid, showing that the lotus root extract exhibited free radical scavenging properties, albeit with slightly lower potency than vitamin C [39].

Rai et al. assessed the nitric oxide radical inhibition assay in addition to the DPPH method, revealing strong antioxidant properties of the extract comparable to rutin, a standard antioxidant [38]. Kim and Shin employed different antioxidant assays, such as ABTS and FRAP, alongside the DPPH method. The findings indicated that the ethyl acetate fraction of LS exhibited the greatest activity in DPPH, ABTS and FRAP assays. On the other hand, water and ethanol fractions of LSP demonstrated the highest activity in DPPH and ABTS assays (water), as well as in the FRAP assay (ethanol) [37].

Furthermore, Shin et al. evaluated various ethanol concentrations and chelating activity. They found that the 50% ethanol extract from lotus roots showed increased scavenging activity against DPPH radicals compared to the 75% ethanol extract. Additionally, the 50% ethanol root extract exhibited higher chelating activities than the leaf extracts; this enhanced activity was attributed to the superior chelating capacity of tannins found in lotus roots [40]. Choe et al. evaluated the total flavonoid content and SOD-like activity in addition to DPPH and concluded that the methanolic extract of lotus leaves exhibited the best activities due to their phenolic content [42]. The Superoxide Dismutase (SOD) activity assay measures the enzyme's ability to catalyze the dismutation of superoxide radicals. It provides information on the antioxidant enzyme's capacity to neutralise superoxide radicals.

In another study, Wang et al. performed various antioxidant assays, including ferrous ion chelating ability, antioxidant activity in a haemoglobin-induced linoleic acid system, scavenging of hydroxyl radicals, and plasmid relaxation assay. The results indicated that both lotus plumule and blossom extracts displayed antioxidant properties. However, lotus plumule exhibited greater antioxidant effects than lotus blossom, particularly in scavenging hydroxyl radicals and ferrous ion chelation. Lotus blossom showed slightly higher scavenging activity of hydroxyl radicals at lower concentrations, while lotus plumule demonstrated higher activity at higher concentrations. Plus, only the methanol extract of lotus plumule exhibited ferrous ion chelating capabilities [41].

These studies also employed methods like the Folin–Ciocalteu assay to assess total phenol content, consistently showing that the presence of phenolics contributes to higher

antioxidant activity [38,39,42] or the reducing power assay [40–42]; these findings align with those of DPPH.

Finally, Mai et al. examined various parts of the lotus plant, such as leaves, flowers, stamens, stems, seedpods, seeds, plumules and rhizomes, using ether as an extraction solvent and evaluated lipid peroxidation, SOD activity, catalase activity, peroxidase activity and glutathione levels. The findings indicated that each part tended to exhibit either the highest or lowest activities in the various methods used, with no single part consistently showing the highest or lowest activity across all assays. This suggests that the choice of method employed plays a crucial role in determining the activity levels observed for each part. Rhizomes were noted for their high vitamin C content [43].

Sohn et al. studied lotus extract for potential hepatoprotective and free radical-scavenging properties using purchased drug-free hepatocytes as a control. More specifically, an ethanol extract from seeds of *N. nucifera* (ENN) was assayed with DPPH for potential antioxidant effects to scavenge free radicals, as well as with carbon tetrachloride (CCl₄) and aflatoxin B1 (AFB1)-induced hepatocyte toxicity models for potential hepatoprotective effect. The reduction of DPPH via spectrophotometric monitoring allowed for the determination of the extract free radical scavenging effect. Hepatocytes treated with ENN reduced CCl₄ cytotoxicity and serum enzyme production. Moreover, ENN suppressed AFB1's cytotoxic and genotoxic effects in a dose-dependent manner [44].

Antioxidant activity was also observed in dried stamens of *N. nucifera*, which were mixed with ethanol, and the flavonoids were extracted. The lotus stamen extract (LSE) was analyzed by high-performance liquid chromatography (HPLC) and assayed for its potential in vitro cell free antioxidant activity, evaluation of chronological aging, estimation of nicotinamide adenine dinucleotide hydrogen (NADH), NAD⁺, adenosine triphosphate (ATP) contents, etc. The results of LSE analysis revealed a rich antioxidant flavonoid resource with the ability to slow down the aging process of yeast. Interestingly, LSE can be considered an alternative plant source for the development of anti-aging products [45].

The in vitro antioxidant activity of flavonoids from aquatic plants like *N. nucifera* plays a crucial role in combating oxidative stress and safeguarding against various diseases. The structural characteristics of these bioactive compounds justify their antioxidant properties. Phenolic compounds such as chlorogenic acid, ferulic acid and coumarin, along with flavonoids like kaempferol, baicalein, quercetin and rutin, are renowned for their antioxidant effects due to the presence of phenolic hydroxyl groups in their structures. These hydroxyl groups, along with the conjugated double bonds in the molecules, enable them to function as potent free radical scavengers, effectively neutralizing ROS and preventing oxidative damage. The multiple phenolic hydroxyl groups can donate hydrogen atoms or electrons, forming stable phenoxyl radicals through resonance structures, thereby neutralizing free radicals. Additionally, these compounds can chelate metal ions like Fe²⁺ and Cu²⁺, which catalyze free radical formation, further reducing their generation. Moreover, glycosylation of flavonoids enhances their antioxidant activity by improving solubility and stability. C-glycosides, linked to the flavonoid skeleton via carbon-carbon bonds, exhibit greater stability against hydrolysis compared to O-glycosides, attached via oxygen atoms. The position and type of sugar moiety (e.g., glucopyranoside, galactopyranoside) influence solubility and bioavailability, while the flavonoid aglycone retains core antioxidant properties due to its phenolic structure. Lastly, tryptophan, an essential amino acid with an aromatic indole ring structure, acts as a radical scavenger, protecting cells from oxidative stress [46]. The main flavonoids of *N. nucifera* are summarised in Figure 2.

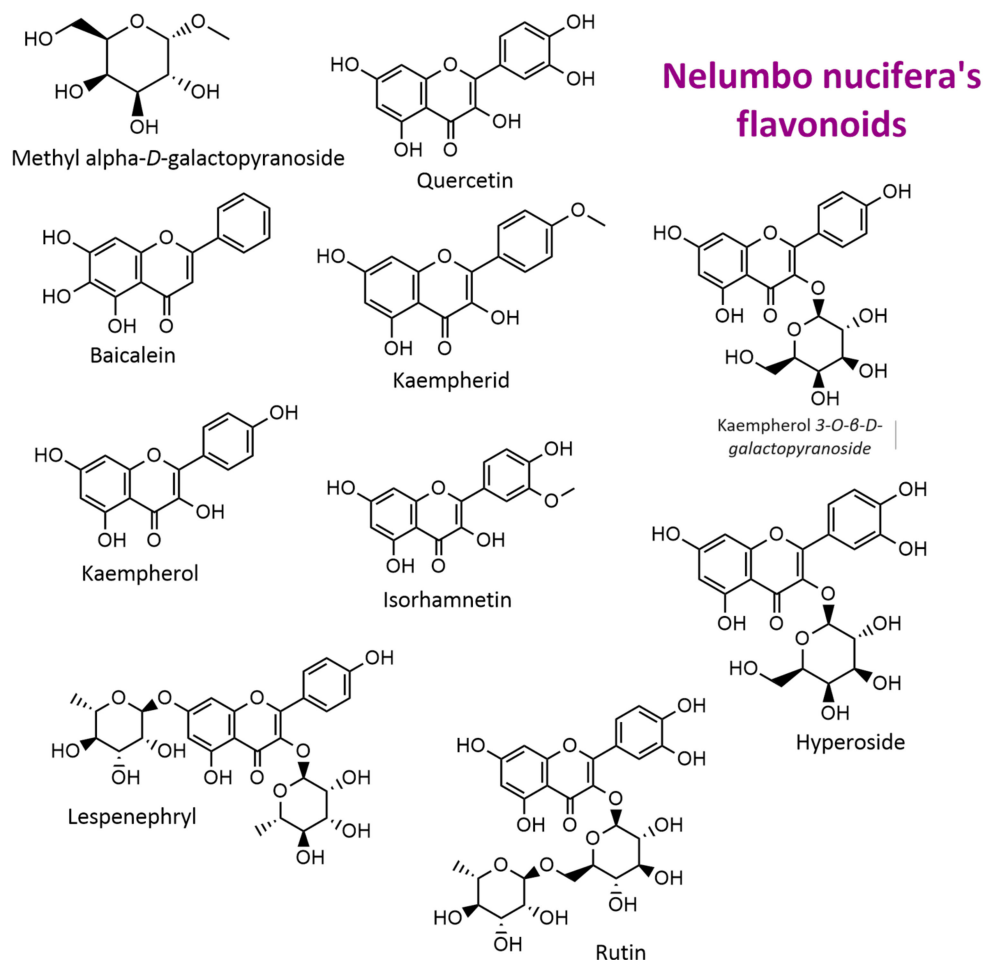


Figure 2. Main Flavonoids of *Nelumbo nucifera*.

3.2.2. In Vivo Antioxidant Effects of *Nelumbo nucifera*'s Bioactives and Extracts

Li et al. evaluated the antioxidant properties of Lotus Leaf Flavonoid Extract (LLFE) in a mouse model of induced oxidative stress using D-Galactose (D-Gal) and Lipopolysaccharide (LPS) injections to simulate aging-related oxidative damage in human embryonic kidney cells. The LLFE was found to contain various flavonoids, such as Baicalein, Kaempferol, Kaempferid, Quercetin, Isorhamnetin, Hyperoside, Lespenephryl and Rutin as identified by Ultra-High Performance Liquid Chromatography tandem Mass Spectrometry (UHPLC-MS/MS) analysis. Mice orally treated with LLFE showed increased levels of antioxidant enzymes, including SOD, Catalase (CAT), Glutathione (GSH) and Glutathione Peroxidase (GSH-Px). These findings suggest that LLFE enhanced the antioxidant defense mechanisms in the mice. Furthermore, LLFE administration led to a decrease in Malondialdehyde (MDA) levels, a marker of lipid oxidation. This reduction in MDA levels indicates that LLFE treatment resulted in reduced oxidative damage, highlighting the potential of *N. nucifera* leaf flavonoids as effective antioxidants in combating oxidative stress-induced damage [23].

According to an in vivo investigation by Kim et al. [35], *N. nucifera* proved to have whitening, anti-wrinkle, and antioxidant cosmetic activities. Root, flower, leaf, seed and stem were extracted three times. Standard procedures were utilised to measure the anti-oxidation, anti-wrinkle and whitening effects as part of the efficacy test. The DPPH free radical-scavenging assay was used to quantify the impact of anti-oxidation. The tyrosinase inhibition assay and the DOPA-oxidase inhibition assay were used to quantify the whitened effect. The elastase inhibition assay was used to quantify the anti-wrinkle effect. Twenty Amaranth Cosmetics applicants underwent a human patch test in the same manner as the

in vivo safety test. Using Haye's test chamber, a patch containing *N. nucifera* extract was applied to an individual's forearm and was left there for a whole day. To determine the differences between samples, the skin condition in the studied area was compared to a blank. *N. nucifera* root extract was combined with water and subjected to room temperature, 40 °C to 50 °C, and refrigeration for a maximum of 30 d as a stability test. Physical characteristics such as phase separation, color and scent were examined throughout storage.

The flower and seed extract of *N. nucifera* exhibited remarkable antioxidation activity. Twenty candidates had their skin irritation evaluated, and the water cream-containing *N. nucifera* 1% root, leaf, flower and stem extract and 4% combined extracts did not cause any irritation. The study's findings indicated that the flower and seed extracts of *N. nucifera* have great promise as a functional cosmetic product for whitening and anti-wrinkles [47].

Three studies collectively aimed to investigate the antioxidant properties of *N. nucifera* extracts, including leaf extract dissolved in distilled water [38,48], seed hydroalcoholic extract [38] and root ethanol extract [39], and their potential to alleviate oxidative stress-related conditions in various experimental models using rats. Mon-Yuan Yang et al. focused on preventing liver injury and early hepatocarcinogenesis induced by 2-acetylaminofluorene (AAF) through antioxidant enhancement [48], while Rai et al. examined the effects on antioxidant enzyme levels in the liver and kidney of rats exposed to carbon tetrachloride, a known oxidative stress inducer [38]. You et al. assessed the impact on obesity and oxidative stress parameters, such as body weight gain, adipose tissue weight, serum lipid levels, insulin and adipokine levels [39].

These studies evaluated oxidative stress markers, antioxidant enzyme activities and liver health indicators, demonstrating a consistent reduction in lipid peroxidation levels and an increase in antioxidant enzyme activities, particularly SOD and CAT, upon treatment with *N. nucifera* extracts. Notably, You et al. highlighted the extract's potential anti-obesity effects by reducing body weight gain and adipose tissue weight, improving serum lipid profiles, and modulating insulin and adipokine levels [39]. Rai found that the changes observed in antioxidant enzyme levels at 100 mg/kg body weight treatment were comparable to those seen with standard vitamin E at 50 mg/kg treatment [38]. Overall, the in vivo methodologies employed in these studies underscore the diverse health benefits of *N. nucifera* in various health contexts.

3.3. Anti-Inflammatory Health Promoting Effect of *Nelumbo nucifera* Bioactives and Extracts

3.3.1. In Vitro and Ex Vivo Ant-Inflammatory Activity of *Nelumbo nucifera*'s Bioactives and Extracts

Two studies investigated the anti-inflammatory effects of plant-derived compounds on RAW 264.7 murine macrophages by utilizing lipopolysaccharide (LPS) as an inflammatory stimulus. Both Kim et al. and Li et al. focused on evaluating the compounds' ability to reduce the expression of pro-inflammatory genes in the macrophages. The linoleic acid study examined the impact of this omega-6 fatty acid on pro-inflammatory gene [inducible nitric oxide synthase (iNOS), cyclooxygenase-2 (COX-2), tumor necrosis factor-alpha (TNF- α), interleukin-1 beta (IL-1 β) and interleukin-6 (IL-6)] expression [49], while the study on the flavonoid compound quercetin-3-O- β -D-glucuronide (Q3GA) from lotus leaves assessed its ability to decrease LPS-induced NO production in RAW 264.7 macrophages [50]. Cell viability assays, such as the cell counting kit-8 (CCK-8) assay in the linoleic acid study and the MTT assay in the Q3GA study, were employed to ensure the safety of the compounds on the macrophages. The results indicated that linoleic acid and Q3GA exhibited anti-inflammatory properties by reducing pro-inflammatory gene expression and nitric oxide (NO) production, respectively, in the LPS-stimulated macrophages. Additionally, Q3GA demonstrated a dose-dependent response and cell safety, suggesting its potential as an anti-inflammatory agent. Those studies, along with previous ex vivo research, used cell-based assays to evaluate the compounds' impact on inflammation in macrophages, although specific outcomes may vary due to different compound characteristics and experimental conditions.

Sranujit et al. utilised lotus petal extract in an ex vivo study in which the phytochemicals chlorogenic acid, ferulic acid and coumarin were identified as the primary compounds through high-performance thin-layer chromatography (HPTLC). This investigation showcased the immunomodulatory impact of the extracts by reducing TNF- α secretion in human macrophages, indicating potential anti-inflammatory properties by inhibiting NF-kB-dependent inflammatory responses. The research suggests that *N. nucifera* flower extracts have the potential to be developed into dietary supplements for individuals with compromised immune functions [27].

Some studies have focused on exploring the anti-inflammatory properties of *Nelumbo* plant extracts, utilizing different parts of the plant and extraction techniques [51]. The studies diverged in terms of the specific cell models employed (LPS-induced murine macrophage RAW 264.7 cells [52] vs. LPS-stimulated HepG2 cells [53] and the plant components investigated, leaf extracts [52] vs. seedpod extract [53]). Methodologies also varied, with Park et al. examining ethanol and water extracts of *Nelumbo* leaves [52] and Tseng et al. focusing on Lotus Seedpod Extract obtained through hot water extraction [53]. Both studies involved analyzing the expression of pro-inflammatory genes like IL-1 β , IL-6, TNF- α , iNOS and COX-2, as well as measuring NO production. However, the RAW 264.7 cells study additionally assessed prostaglandin E2 (PGE2) production and investigated NF- κ B activity [52], while the HepG2 cells research explored the impact of Lotus Seedpod Extract (LSE) and (-)-epigallocatechin (EGC) on NF- κ B activity [53]. Both studies demonstrated anti-inflammatory effects, with the RAW 264.7 cells study showing that *Nelumbo* leaf extracts reduced pro-inflammatory mediators and inhibited NF- κ B activity. Bioactive compounds like catechin and quercetin were identified in both extracts (ethanol and water through LC-MS/MS analysis and HPLC quantification) [52]. In LPS-stimulated HepG2 cells, LSE effectively suppressed pro-inflammatory mediators by disrupting NF- κ B and p38 signaling pathways. Moreover, a diet incorporating LSE exhibited significant anti-inflammatory properties in a mouse model of LPS-induced hepatic inflammation while maintaining cell viability [53].

3.3.2. In Vivo Anti-Inflammatory Health Benefits of *Nelumbo nucifera*'s Bioactives and Extracts

Some other in vivo studies share a common focus on exploring the anti-inflammatory properties of lotus plant extracts, albeit with variations in the specific conditions studied, bioactive compounds identified, methodologies employed and outcome measures assessed. Li et al., Tang et al. and Wang et al. investigated the anti-inflammatory effects of extracts derived from *N. nucifera* leaves, namely lotus leaf flavonoid extract (LLFE) [23], *N. nucifera* leaves extract (NLE) [54] and *N. nucifera* Gaertn. leaves [55], respectively, while Liao and Lin examined the Lotus Plumule Polysaccharide (LPPS) [56]. These studies all highlight the presence of bioactive compounds in lotus plant extracts, such as flavonoids, phenolic acids, and polysaccharides, which are credited for their anti-inflammatory properties. However, the specific bioactive components differ among the studies. For instance, LLFE contains Baicalein, Kaempferol, Kaempferid, Quercetin, Isorhamnetin, Hyperoside, Lespenephryl and Rutin [23]. NLE comprises phenolic acids and flavonoids, with major polyphenols like gallic acid, protocatechuic acid, catechin, gallocatechin gallate, caffeic acid, epicatechin, rutin, quercetin and naringenin [54]. *N. nucifera* Gaertn. leaves extract contains Sitosterol, Pronuciferine, Nuciferine, Nelumboside, Quercetin and Kaempferol [55], and LPPS consists of polysaccharides [56].

All these studies utilised *in vivo* mouse models to evaluate the anti-inflammatory effects of lotus plant extracts in different inflammatory conditions, including aging-induced inflammation [23], alcoholic steatohepatitis [54], atherosclerosis [55] and type 1 diabetes [56]. The methodologies employed vary across the studies, encompassing analyses of mRNA expression levels of pro-inflammatory cytokines [23], immunohistochemistry and Western blot analysis of liver tissue sections [54], molecular docking and network pharmacology [55] and cytokine gene expression analysis in various organs [56]. Despite these

methodological differences, a consistent trend is observed in all studies, indicating a reduction in pro-inflammatory cytokines like TNF- α , IL-6, IL-1 β and interferon- γ (IFN- γ) following treatment with lotus plant extracts, suggesting their potential anti-inflammatory efficacy. Additionally, Li et al. demonstrated an increase in the levels of anti-inflammatory cytokines IL-10 and IL-12, crucial for immune response modulation and inflammation suppression [23], while a study conducted by Liao and Lin showed reduced spleen size and altered cytokine gene expression in the liver, indicating protection against spontaneous inflammation in non-obese diabetic (NOD) mice and the ability to balance pro- and anti-inflammatory responses in type 1 diabetes [56]. Another clinical study by Liu et al. showed that *N. nucifera* extracts using ethanol as a solvent inhibited the production of cytokine genes, slowed cell cycle progression and proliferated human peripheral blood mononuclear cells. Blood from 15 healthy subjects was taken and analyzed with lymphoproliferation test, cell cycle analysis and polymerase chain reaction (PCR). The results showed that the extract suppressed proliferation in peripheral blood mononuclear cells (PBMC) activated by phytohaemagglutinin (PHA). It also reduced tissue inflammation-in part by inhibiting PBMC proliferation [57].

Mukherjee et al. explored the anti-inflammatory effects of betulinic acid, a steroidal triterpenoid compound derived from the *N. nucifera* rhizomes, in male albino Wistar rat models with paw edema induced by carrageenin and serotonin. It aimed to assess the anti-inflammatory potential of this bioactive compound and compare its effectiveness with standard drugs like phenylbutazone and dexamethasone. Betulinic acid was administered orally at specified doses to evaluate its impact on inflammation by measuring paw volumes and inflammation rates at different time points. The findings indicated significant anti-inflammatory activity at doses of 50 and 100 mg/kg, showing effects similar to phenylbutazone and dexamethasone. The study suggested that betulinic acid may operate through a delayed mechanism akin to glucocorticoids, hinting at a potential interaction with the glucocorticoid receptor [58].

These and other in vivo studies aimed to evaluate and validate the anti-inflammatory effects of *N. nucifera* plant extracts using animal models (rats [48,59,60] or mice [53]) with various plant extract formulations. In two of these studies, rat models were employed to facilitate the in vivo evaluation of the anti-inflammatory activity of plant extracts from different parts of the *N. nucifera* plant. They utilised the Carrageenin-induced paw edema model to induce inflammation and assess the anti-inflammatory properties of methanol-extracted lotus seeds (white and red) [59] and ethanol-extracted lotus fruit [60]. Paw volume measurements were taken at various time points post-inflammation induction to evaluate the extent of edema and the effects of the extracts. Periyasamy and Gopakumar also included a COX-2 enzyme inhibition assay to investigate how lotus seeds inhibit the COX-2 enzyme [59]. Both studies reported a significant reduction in paw volume, indicating the anti-inflammatory effects of lotus seed and fruit extracts in response to carrageenin-induced inflammation. However, the time point at which the maximum reduction in paw edema occurred differed, with Periyasamy and Gopakumar observing it at the third hour after carrageenin injection [59], and Rajput et al., at the fifth hour [60]. Furthermore, the study on lotus seed extracts demonstrated anti-inflammatory properties and significant inhibition in the enzyme assay, with white lotus seed extracts at higher doses showing more pronounced inhibition [59]. While both studies attributed their results to the presence of phytochemical constituents in the plant extracts, specific compounds like flavonoids, saponins and tannins were specifically highlighted in one of the studies [59].

In two additional studies, the anti-inflammatory properties of *N. nucifera* extracts were assessed in liver inflammation and damage using animal models, with one study involving rats and the other involving mice [53]. The *N. nucifera* Leaf Extract (NLE) was obtained using distilled water in one study [48], while Lotus Seedpod Extract (LSE) was prepared using hot water in the other [53]. The targeted inflammatory pathways differed between the studies, with the former investigating the impact of NLE on AAF-induced hepatocarcinogenesis [48], while the latter examined the effects of LSE on LPS-induced

hepatic inflammation [53]. In terms of liver damage assessment, Mon-Yuan Yang et al. aimed to alleviate 2-acetylaminofluorene (AAF)-induced liver damage, inflammation, and oxidative stress [48], while Tseng et al. focused on observing changes in hepatic lobular architecture and liver damage induced by LPS [53]. Both studies reported a decrease in pro-inflammatory mediators (IL-6, TNF- α) following treatment with *N. nucifera* extracts, indicating their potential anti-inflammatory effects. Furthermore, the results on LPS-induced hepatic inflammation demonstrated a dose-dependent reduction in serum markers of liver damage, improvement in hepatic lobular architecture, reduced secretion of pro-inflammatory mediators and increased levels of antioxidant enzymes [53]. HPLC analysis was conducted in both studies to identify specific phenolic compounds present in the extracts. The compounds identified included gallic acid and catechin in one study [48] and catechin, procyanidin B2, *p*-coumaric acid and (-)-epigallocatechin (EGC) in the other [53]. These compounds are believed to contribute to the anti-inflammatory effects of the extracts and their ability to modulate liver damage, inflammation and antioxidant enzyme activity.

Mukherjee et al. aimed to explore the anti-inflammatory effects of *N. nucifera* rhizomes in male albino Wistar rat models with paw edema induced by carrageenin and serotonin. It aimed to assess the anti-inflammatory potential of the methanol extract and compare its effectiveness with standard drugs like phenylbutazone and dexamethasone. The extract was administered orally at specified doses to evaluate its impact on inflammation by measuring paw volumes and inflammation rates at different time points. The findings indicated significant anti-inflammatory activity at doses of 200 and 400 mg/kg, showing effects similar to phenylbutazone and dexamethasone. The study suggested that the methanol extract may operate through a delayed mechanism akin to glucocorticoids, hinting at a potential interaction with the glucocorticoid receptor [58].

3.4. Antithrombotic Effects of *Nelumbo nucifera*'s Bioactives and Extracts

3.4.1. Anti-Coagulant Effects of *Nelumbo nucifera*'s Bioactives and Extracts

Rajput et al. conducted an in vivo study to assess the impact of orally administered *N. nucifera* fruit ethanol extract on coagulation parameters in male Wistar rats. By analyzing markers like Activated Partial Thromboplastin Time, Prothrombin Time, Thrombin Time and fibrinogen levels, the study sought to investigate the extract's influence on the rats' coagulation processes. The results demonstrated a considerable lengthening of prothrombin and thrombin time, as well as a significant decrease in fibrinogen levels, in comparison to the control group and test groups that received varying doses of the extract. These data imply that *N. nucifera* fruit extract exhibits considerable anti-coagulant properties, possibly related to its flavonoid-rich composition and the presence of alkaloid (i.e., Neferine [61]).

Zhou et al. investigated the effects of Neferine on platelet function using the pure compound, although it is typically extracted from *N. nucifera*. The study investigated Neferine's effects on platelet aggregation, adhesion, and thrombosis using washed mice platelets and Platelet Rich Plasma (PRP) in in vitro assays, as well as in a collagen-epinephrine-induced acute pulmonary thrombus mouse model in vivo. Results indicated that Neferine inhibits platelet activation, adhesion and aggregation while promoting the disassembly of pre-formed platelet aggregates and was also dose-dependent, suggesting its potential as an antiplatelet and antithrombotic agent [62].

3.4.2. Anti-Platelet Effects of *Nelumbo nucifera*'s Bioactives and Extracts

Further investigations assessed the antiplatelet properties of *N. nucifera*. Two studies focused on evaluating its antiplatelet effects as follows. Durairaj and Dorai analyzed the antiplatelet activity of white and pink *N. nucifera* flowers through an in vitro assay [63], while Sharma et al. explored the impact of whole plant extract of *N. nucifera Gaertn* using in vitro, in vivo, and ex vivo approaches [64]. Both studies assessed antiplatelet activity through in vitro techniques. The first study evaluated the antiplatelet effects of *N. nucifera* flowers (white and pink) using PRP post exposure to varying plant extract concentrations [63]. The second study employed in vitro clot lysis and ex vivo platelet aggregation assays, utilizing

PRP obtained from blood samples collected after administering substances to experimental groups orally [64]. The outcomes of both studies aligned with those of the Neferine study previously analyzed, which also utilised PRP. It is noteworthy that the white flower tested in the initial study demonstrated higher efficacy, potentially attributed to its elevated levels of alkaloids, flavonoids, phenols, tannins, steroids, glycosides and saponins, consistent with the findings of the anticoagulant study. The findings were also consistent with in vivo administration of varying doses of the hydroalcoholic extract to experimental Albino Wistar rat groups, followed by measurement of tail bleeding time, tested in the second study [64].

The aforementioned facts are supported by in vivo clinical trials conducted with this objective. Sharma et al. exhibited that lotus had anti-thrombotic properties. Extracts of *N. nucifera* were prepared and analyzed with HPTLC and GC-MS. Three doses of extract were prepared and consumed by albino rats, and Platelet Poor Plasma (PPP) was prepared. The results suggest that hydroalcoholic extract flavonoids could be turned into medication for treating patients' thrombosis [64]. Durairaj and Dorai showed that *N. nucifera* Gaertn flowers, both white and pink, have antiplatelet properties, using classic antiplatelet treatment (aspirin) as positive controls. The antiplatelet activities of air-dried and powdered flowers macerated with ethanol were examined in PRP platelet aggregation models. Pink and white, the floral extracts of *N. nucifera*, exhibited significant antiplatelet action in a dose-dependent manner, peaking at 500 µg/mL [63].

3.5. Other Health Benefits of *Nelumbo nucifera*'s Bioactives-Extracts and Relative Functional Food Products

3.5.1. Anti-Obesity Health Benefits

Several studies reported that the *N. nucifera* plant has many potential pharmacological properties to treat ailments like obesity [48,63,64]. Certain research articles discuss the entirety of the lotus plant, encompassing the root, stem, leaf, flower and seed, along with their extracts that harbor a variety of phytochemicals like phenolic acids, alkaloids, flavonoids and steroids. These components are known to support anti-obesity effects [27,51]. Other studies focus on specific parts of the lotus plant that demonstrate anti-obesity properties, such as lotus root [39], lotus seeds [25] and lotus leaves [49,54,55]. In particular, the latter studies highlight that *N. nucifera* leaves, commonly dried and referred to as *Folium nelumbinis*, contain essential components like alkaloids and flavonoids, that are closely linked to their pharmacological activities, including anti-obesity effects [55] and that the beneficial effects of plant-based foods, including anti-obesity effects, can be amplified through fermentation [49]. Furthermore, certain studies have indicated a correlation between the anti-obesity effect and the antioxidant properties of the plant [52].

Two different studies showed the same anti-obesity effect of lotus. The first one dealt with rats and mice, which consumed ethanol extract. A-amylase and lipase measures were taken, and plasma triacylglycerol level was measured (rats were given the lipid emulsion and *N. nucifera*'s bioactive extracts [NNE] orally). Based on the IC₅₀ value, NNE was found to inhibit the activities of lipase and a-amylase in vitro. At 1 h following oral administration of a lipid emulsion to rats, the plasma triacylglycerol level was markedly raised in the NNE-treated group and drastically decreased in the other group. NNE dramatically reduced the mice's weight increase due to high-fat diet-induced obesity. In summary, findings imply that NNE is an effective treatment for obesity since it promotes thermogenesis, upregulates adipocyte lipolysis and restricts absorption [65]. The second study, referring to human preadipocytes, shows the ethanolic extract of lotus root (*N. nucifera*) has anti-lipogenic properties. In rats fed a high-fat diet, it was also found to have anti-obesity and antioxidant properties. The ethanol extract was analyzed for total phenolic content using the Folin-Ciocalteu assay, and total flavonoid content was determined by the colorimetric method. Antioxidant activity was determined by DPPH free radical scavenging, and the extract was found to have free radical scavenging activity. According to these findings, lotus root may have antioxidant and anti-obesity properties, making it a viable functional and nutritional ingredient to combat obesity-related disorders [39].

3.5.2. Neuroprotective Health Benefits

Temviriyankul et al. concluded that the Sacred Lotus exhibits therapeutic potential for preventing or managing Alzheimer's disease due to its antioxidant properties and ability to inhibit enzymes [20]. Tang et al. mention that lotus seedpod is abundant in flavonoids and serves as a significant natural reservoir of catechin oligomers and polymers, known as procyanidins. Recent research has demonstrated that procyanidins derived from lotus seedpod have anti-memory impairment properties [54].

Proof of the above is an experiment based on lotus and drugs that showed *N. nucifera* fruit's impact on scopolamine-induced impairments in motor coordination and memory. The fruits of lotus were soaked in ethanol, and once achieved, the solid Lyophilized material was kept at -20°C until it was needed again and then taken orally at doses of 50, 100 and 200 mg/kg. Gum tragacanth was given orally to the control group at a dose of 10 mL/kg as a placebo. Piracetam tablets were given to animals as a reference drug. The rats were also administered scopolamine for the induction of amnesia. The tests that were run were Morris's water maze task, Rota rod test and statistical analysis. Amnesic rats' escape latency was significantly reduced by lotus extract at 200 mg/kg when compared to the control group. In terms of riding time, the effects of *N. Nucifera*'s Fruit (NNF) extract at all three doses, 50, 100 and 200 mg/kg, were nearly identical to the control [66].

Lotus extract has been demonstrated to enhance cognitive impairment resulting from scopolamine. Within the experiment, it was observed that mice injected with scopolamine and on a diet containing lotus extract showed a significant reduction in the impairment of memory caused by scopolamine through Adult Hippocampus Neurogenesis (AHN). The tests that were performed in mice were Y-maze behavior analysis, Enzyme-linked immunosorbent assay and Western blot analysis, among others. Results showed that scopolamine decreased cognitive function, while the extract of lotus coadministration restored learning and memory capacities [67].

Intui et al. [68] conducted an in vivo study to present the neuroprotective effect of lotus petal tea in poisoned rats by mancozeb (Mz). Seventy-two rats consumed dried lotus powder in hot water (tea) and were analysed by Y-maze spontaneous alternation, learning and memory behaviour and hormonal test essays. Results showed that petal tea increased the level of neuroprotective picolinic acid. The white *N. nucifera* petal tea, especially at a high dose, had beneficial health effects, while at a low dose, it possessed serious neuroprotective effects against Mz toxicity [68].

3.5.3. Anti-Tumor, Chemotherapeutic and Cytotoxic Anti-Cancer Health Benefits

Several studies have emphasised the potential of *N. nucifera* in cancer treatment [38,51,52,63,64] or specifically in addressing certain types of cancer, like breast cancer and hepatocarcinogenesis [48,54]. In one study, it was noted that Leaf Extract (NLE) has the potential to inhibit the proliferation and metastasis of human breast cancer cells. The study's findings also suggest that the NLE has the potential to be developed as a natural agent for preventing hepatocarcinogenesis based on the experimental results [54]. Additionally, the second study indicates that nearly all parts of the plant have the ability to suppress the growth of human breast cancer cells both in laboratory settings and in living organisms [54]. Specific studies have highlighted the anti-cancer activity of lotus seeds [25,37] and lotus leaves [55], while some others emphasise the importance of phytochemicals in lotus plants [21,27,43]. One study mentions that lotus extracts contain a variety of phytochemicals such as phenolic acids, alkaloids, flavonoids, and steroids, that contribute to their anti-cancer properties [43]. Another study highlights the multiple pharmacological benefits of Flavonoid C-glycosides, including their anticancer activities [21]. Additionally, a separate study mentions that extracts from the root and leaf of white lotuses demonstrate anticancer activities in laboratory settings [27].

Studies have been conducted to prove anti-cancer activities of lotus. *N. nucifera* stamen extract owns an anticancer action in vitro against human colon cancer HCT-116 cells. Fresh lotus stamen was extracted with ethanol, placed in purchased cells and analysed with

reverse transcription-polymerase chain reaction (RT-PCR) assay, flow cytometry analysis and the cell viability assay. It was discovered that lotus stamen ethanol extract (LSEE) therapy significantly reduced the number of cells proliferating in HCT-116 cancer cells and increased the percentage of cells in the sub-G1 phase. Additionally, it was discovered that LSEE therapy might alter mRNA expression of Bcl-2 family members linked to apoptosis in HCT-116 cells [69].

To fully elucidate the antitumor and chemotherapeutic activity of Lotus, some of its NLEs were tested after being enhanced with flavonoids. Nine polyphenols were used as standards, while extracts of *N. nucifera* were added to cells and analyzed by utilizing immunoprecipitation tests, flow cytometry, and Western blot analysis, where NLE's inhibitory effect on MCF-7 human breast cancer cells was investigated. According to their in vivo investigation, mice implanted with MCF-7 cells showed significant reductions in tumor volume and weight following treatment with NLE (0.5 and 1%) in comparison to control samples. These findings verified that, after NLE treatment, cell-cycle arrest alone was sufficient to cause tumor regression. In summary, our research suggests that NLE exhibits anticancer activity and holds great promise as a future chemotherapeutic drug [70].

A cytotoxic effect was spotted in a test that the extract was introduced in A549 and H460 cells, while plasmid was analysed with DNA polymerase via Western blot analysis, cell viability measurements and colony formation. At the end of this process, it was discovered that the stamen extract was cytotoxic to colon cancer cells, while the lotus extract prevented breast cancer cells from proliferating and spreading. Axl gene expression was transcriptionally downregulated, a fact associated with its Lotus' pro-apoptotic and antiproliferative effects in cells [71].

3.5.4. Hepatoprotective Health Benefits

Several studies have mentioned that lotus seeds [25,37,38] and lotus leaves [55] exhibit hepatoprotective activity. Its specific activity is attributed to various pathways involving bioactive compounds present in the extracts [25]. For instance, Flavonoid C-glycosides are noted to possess multiple pharmacological benefits, including hepatoprotective effects [21].

A clinical trial by Lin et al. utilised *N. nucifera* leaf extract enriched with flavonoids to decrease oxidative stress and liver damage caused by a high-fat diet. In this work, hamsters were fed a high-fat diet to induce hyperlipidemia, hypercholesterolemia, and fatty liver. Lotus extract, enriched with flavonoids, was administered to hamsters, while the drugs used were silymarin and simvastatin (reference). Triglyceride, lipoprotein, total cholesterol and liver lipid extraction assays showed similar effects to silymarin in alleviating liver injury and lipid peroxidation. Compared to silymarin treatment, the level of reduction in hepatic triglycerides and cholesterol was higher with NLE supplementation. Results suggest that NLEs may develop into a potent hepatoprotective drug [72]. Sohn et al. have also observed its hepatoprotective activity [44].

3.5.5. Anti-Pyretic Effects

Different parts of *N. nucifera* mentioned in several trials exhibit pharmacological anti-pyretic activity [48,51,54]. It was mentioned that fruits, which are commonly used as a healthy component of Asian cuisine [60,61] using different extracts of rhizomes [58], seed embryos [49], stamens [26] or as a whole [63], might be used as a traditional cure/remedy for fever. *N. nucifera* has also demonstrated high anti-pyretic potential in a clinical trial when the ethanol extract was injected into rats as a drug. At prearranged intervals, the body temperature of every rat was taken rectally prior to and for 6 h following the oral administration of 200 and 400 mg/kg of stalk extract or saline (control). It was observed that *N. nucifera* Stalk Extract (NNSE) significantly reduced normal body temperature for up to 3 h at a dose of 200 mg/kg and significantly reduced body temperature for up to 6 h at a dose of 400 mg/kg, exhibiting similar action to paracetamols' [73].

3.5.6. Anti-Diabetic Health Benefits

Numerous studies have noted the anti-diabetic activity of the *N. nucifera* plant, focusing on different parts such as some plant parts [40] or leaves [52,55] and even the whole plant [27,38,63]. Sranujit et al. specifically linked plants' activity to the presence of various phytochemicals in lotus extracts, including phenolic acids, alkaloids, flavonoids and steroids [27].

Another study that investigated the anti-diabetic effect of lotus was conducted on rabbits. Powdered flowers were used for oral administration. Alcoholic and aqueous extracts were then studied after being consumed by rabbits in doses. NNEs dramatically reduced the rabbit's weight increase due to high-fat diet-induced obesity. In summary, findings imply that NNEs are an effective treatment for obesity since they promote thermogenesis, upregulate adipocyte lipolysis and restrict absorption [74]. Moreover, Kumari and Arivuchudar followed an in vivo study and specified the nutritional value and the antidiabetic activity of beneficial lotus products. For example, a trial using lotus seed powder to make tarts was tested by semi-trained people for organoleptic analysis (texture, mouthfeel, taste and appearance). Their low sodium and high magnesium content are effective in treating heart disease, diabetes and high blood pressure. These astringent properties aid in the halt of chronic diarrhea [75].

Tseng et al. mentioned that lotus seedpod is rich in flavonoids and acts as an important natural source of oligomers and polymers of catechin, which are also denominated procyanidins. In recent years, procyanidins from lotus seedpod have been shown to possess this anti-glycative activity [53].

For instance, the research work of Mukherjee et al. on the effect of lotus rhizome extract on blood sugar levels in rats was investigated. The rats consumed the extract and were analysed by a blood test for glucose tolerance. Regarding the effect of this extract on streptozotocin-induced diabetic rats, as well as normal and glucose-fed hyperglycemic ones, some important statements were declared. Specifically, it was suggested that the ethanolic extract of *N. nucifera* improved blood glucose tolerance and enhanced the action of exogenously injected insulin in normal rats. Overall, *N. nucifera* enhanced insulin's exogenous injection effects and increased glucose tolerance [76]. Finally, You et al. [39], mentioned that the hypoglycemic activity of *N. Nucifera* may result from lotus root, while Jung et al. [26] supported its derivation from stamens.

3.5.7. Anti-Aging Properties

Tseng et al. mentioned that lotus seedpods are rich in flavonoids and is an important natural source of oligomers and polymers of catechin, which are also denominated procyanidins. Recently, procyanidins from lotus seedpods were found to possess this anti-age activity [53]. Concurrently, Mahmood and Akhtar conducted an in vivo study that indicated the plant's anti-aging ability. Plus, Lotus was able to improve facial skin appearance after being tested synergistically with green tea. Thirty-three subjects (all men) were divided into three groups. The first one was given green tea, the second multiple lotus emulsions and the third a combination of the two products. A special UV light video camera was used to measure the surface evaluation of living skin's (SELS's) characteristics. Reportedly, every day at bedtime, all subjects applied these products on one side of their faces and the placebo on the other side. The treatment effects on skin smoothness (SEsm), scaliness (SEsc), roughness (SEr), and wrinkles (SEw) exhibited intriguing and noteworthy changes. The combination of green tea and lotus in various emulsions provided an exceptional combined anti-aging result [77].

3.5.8. Anti-Bacterial Activities

Several studies mentioned the anti-microbial [38,39,63] and anti-bacterial [58] effects of *N. nucifera*. Tho et al. presented the antibacterial activity of *N. nucifera* seed extract in the synthesis of silver nanoparticles (AgNPs). *N. nucifera* extract was used for the preparation

of nanoparticles and analyzed using UV-Vis spectral analysis. As shown by the AgNPs, they exhibited effective antibacterial activity against gram-negative bacteria [78].

3.5.9. Health Effects against Hypcholesterolemia and Hyperlipidemia

Some previous studies have proposed that *N. nucifera* (NN) has many potential properties to treat ailments like hypocholesterolemia [39,64]. Hyun Ah Jung et al. showed *N. nucifera* embryos' cholinesterase inhibitory and BACE1 actions. Methanol extracts of different lotus parts (dried leaves, stamens, seeds, embryos and rhizomes) were tested in vitro with enzyme assays BACE1 (b-site amyloid precursor protein-cleaving enzyme 1) and ACh (acetylthiocholine iodide)-BCh (butyrylthiocholine chloride). Finally, the significant BACE1 and cholinesterases (ChEs) of NN inhibitory effects, as well as its scavenging properties, were confirmed by the presence of major compounds including neferine, liensinine and vitexin, as well as the minor compounds quercetin 3-O-glucoside and northalifoline, thus indicating their potent use in the treatment of Alzheimer's disease (AD) [79].

Napradit et al.'s clinical trial, on the other hand [80], explained the effects of lotus tea on lowering blood lipid levels in hyperlipidemic subjects. Thirty subjects consumed dry crush lotus with hot water two times per day (morning and evening), and their blood was examined for total cholesterol, high-density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL) cholesterol and triglyceride indices. As a result, new evidence declared the potency of lotus stamen tea to be utilised as an alternative treatment or supplement for dyslipidemia because the total cholesterol level was decreased (blood lipid level), as well as the mean blood LDL cholesterol, which the main risk factor of atherosclerosis level. This study hypothesised that if subjects receive lotus stamen tea for a longer period of time and in larger amounts via the presence of a control group, all awaited results will be significantly greater [80].

3.5.10. Effects against Sleep Disorders

One study noted that Lotus fruits are commonly used as a healthy component of Asian cuisine and as a traditional cure for various ailments (e.g., sleep disorders [60,61]). An additional examination studied the sleep-promoting activity of lotus rhizome water extract (LRWE). Rats were orally administrated with lotus rhizome and were injected intraperitoneally (hypnotic, sedative) to measure sleep latency time. The γ -aminobutyric acid (GABA) receptor content and brain receptor expression in mice were measured. The experiment showed that oral injection of lotus extract (LE) increased the amount of time a person spent sleeping by lengthening their nonrapid eye movement (NREM) sleep duration. It also increased the amount of GABA, a major neurotransmitter, in the brain and the expression of GABAA receptors [81].

3.5.11. Anxiolytic, Antidepressant and Antiepileptic Effects

All these features the plant possesses are highlighted through different studies. Recent research revealed that the LD₅₀ value of NNF was higher than 5 g/kg, whereas its neuropharmacological role was also established to be anxiolytic, antidepressant, and antiepileptic [60]. Additionally, it was reported that lotus seeds or their extracts possess an antidepressant action [25], while lotus roots have their own anti-anxiety activities [40].

A few studies conducted in vivo experiments with rats, mice or people and used further drugs as the control for the necessary comparisons, which displayed anxiolytic, antidepressant and antiepileptic activities. Lotus fruit showed an anti-epileptic activity in an experiment using rats. Thirty-five rats, which consumed 2% gum tragacanth (control), diazepam 1 mg/kg and *N. nucifera* extract (50, 100, 200 mg/kg), were used in this experiment. Forty minutes after the first administration, rats consumed strychnine, and then results were recorded. Rats that escaped convulsions 30 m after strychnine administration were considered protected. At a dosage of 200 mg/kg, *N. nucifera* fruit extract demonstrated strong anti-epileptic efficacy, which significantly delayed the onset of convulsions and

enhanced the animals' survival rate (42.85%). *N. nucifera* may be useful in treating epilepsy and its co-morbidities, which include depression and anxiety [82].

Rajput and Khan found that the fruit (seed and seedpods) of *N. nucifera* had anxiolytic and antidepressant properties. In the experiment, extract was prepared by the seedpods of the fruits after being powdered and macerated in ethanol for 30 d. High doses of extract were administered to the animals; gum tragacanth was used as a control drug, and toxicity behaviour signs were observed for a week. All mice were tested with the elevated plus maze test, the light and dark test and the forced swimming test; all results went through thorough statistical analysis. When given to mice at doses of 100 and 200 mg/kg on the second day of the test session, *N. nucifera* fruit significantly reduced the amount of time mice were immobile compared to the control group. After treatment with this extract, it demonstrated anxiolytic effects at the test of light and dark [83].

Anti-anxiety positive effects were also demonstrated without the intake of any further drugs. Dimond et al. [84] conducted an in vivo similar study, which showed the effect of *N. Nucifera* Extract on symptoms of moderate to severe anxiety in individuals. Participants received lotus extract, and a questionnaire was given to them. After completing the trial, 18 (90%) participants were included for statistical analysis, out of whom none was on any medications, except for one who was under oral contraceptives; none tested positive for COVID-19. This open-label investigation proved that consuming *N. nucifera* leaf extract reduced anxiety symptoms in people with moderate to severe anxiety [84].

3.5.12. Other Health Benefits

Additionally, despite all positive outcomes collected from the aforementioned tests, in some experimental studies, the plant displayed more less-common behaviors. For instance, *N. nucifera* also exhibited antiemetic [37,38], diuretic [37–39], anti-hyperlipidaemia [55], antidiarrheal [37–39,42,54,58,60,63] antifungal [38,40,56], refrigerant [37,38], antifertility [37,38] anti-ischemic [21], antiarrhythmic [21], anti-HIV [21] and antiviral [21,49] abilities. Other studies suggest that Lotus may also be used for the treatment of nervous disorders [37,38], insomnia [37,38], hypoglycemia [37,38,56] and cardiovascular diseases [21,37,38,64], such as hypertension and arrhythmia [25,37,38,62] and rheumatoid arthritis [37,38]. Lotus displayed concurrently the ability to reduce the development of atherosclerosis [40,48] and to protect against radiation [53]. Its utilization as cooling medicine for skin diseases [37,38] and leprosy [47,60,61,64] and as an antidote to poison [37,38] are vital as well. This fruit is, in fact, widely used for treating various health disorders like leukoderma, pharyngopathy, hematemesis, spermatorrhea [64], epistaxis, hemoptysis, hyperlipidaemia, ischemia, metrorrhagia and hematuria [60]. In other experiments, lotus exhibited antihypertensive, negative inotropic effect and relaxation on vascular smooth muscle [62]. Chronic diarrhea, hypertension, palpitation arrhythmia, pain, menorrhagia [63], spermatorrhea, leucorrhoea [64] and bad breath [64] were also claimed to be improved by *N. nucifera*'s action [61]. Other lotus characteristics that must be highlighted are its antiproliferative [25], anti-fibrosis [25], astringent [25] and immunomodulatory activity [25] and its ability to act as an ideal treatment to gastritis [52], bleeding [52], hemorrhoids and enuresis [42].

Moreover, the plant exhibited therapeutic qualities in an in vivo study by Zheng et al. that aimed at demonstrating the possibility of finding proper medication to treat muscle atrophy. Liensinine (LIE), a plant-derived chemical, was examined in this case using drugs' affinity response target stability assay (DARTS) and the cellular thermal shift assay (CETSA). In this experimental procedure, mice were employed and received the prescribed therapy for 3 d in a row. The results of this investigation demonstrate the exceptional effectiveness of LIE in the treatment of muscular atrophy [85].

N. nucifera also promoted the health and growth of Grass Carp fish when the fish consumed an extract made from alcoholic lotus leaves. To evaluate the effects of the alcoholic extract of lotus leaf (AELL) on improving the performance and health status of the grass carp, juvenile fish were fed four different experimental diets for 8 weeks after the AELL was prepared. Tests were conducted on feed intake, final body weight, specific

growth rate and weight gain rate. Grass carps' health status was positively impacted by AELL in a dose-dependent manner by increasing important gene expressions and enzyme activity [86].

Lotus' indisputable nutritional value was also proven in an experiment where Lotus steam cookies were supplemented on the nutrition of children between the ages of 6 and 12 years old who were suffering from anaemia and malnutrition. Cookies were prepared in different ratios of lotus powder, and judges were asked to rate the product based on colour, texture, taste, flavour (aroma) and its final acceptability in general. Lotus cookies supplementation significantly improved the nutritional status of children, while iron-based cookie consumption also increased the haemoglobin levels of participating children. These innovative cookies may be used as supplementary alternatives for iron, calcium, and fiber for daily dietary needs [87].

Highly acceptability, nutritional value and supplementary potency of Lotus biscuits for elderly people were also found since the product was positively evaluated according to its point taste, texture, colour, flavour and mouthfeel [88]. Similar results were obtained in a test of adding leaf powder to chicken patties in refrigerated storage. Specifically, five batches (three with different lotus concentrations, one control and one with 0.05% ascorbic acid) were prepared, and tests including sensory evaluation (by a 12-membered panel), colorimeter (for measuring the colour) and Tarladgis method 2-thiobarbituric acid (TBA) (for lipid oxidation) were performed. Chicken meat patties with added lotus leaf powder scored lower on hardness, gumminess, and chewiness indices than the control sample. Thus, the results of this study proved that adding lotus leaf powder to chicken meat patty formulations might improve shelf life during refrigeration storage. It must be noted that the chicken meat patty with lotus leaf powder displayed sensory properties similar to the control chicken meat patties [89].

Table 2. Studies on *Nelumbo nucifera* extracts' cosmeceutical and pharmaceutical potential in drug discovery.

Bio-Functional Ingredient	Model	Study Design	Outcomes	Conclusions Potential Pharmaceutical Application	References
Lotus's Root, Flower, Leaf, Seed and Stem Combined Extract	In vitro	- Whitening effect was tested with tyrosinase inhibition assay and DOPA-oxidase inhibition assay. - Anti-wrinkle effect was measured with an elastase inhibition assay	- Extracts showed a higher elastase inhibition effect than adenosine at the concentration range tested. - Remarkable antioxidant activity. - Formulated cream showed no significant irritation (Human Patch Test)	Anti-wrinkle, anti-oxidant, whitening cosmetic agent	[47]
Lotus Extract	Placebo-controlled comparative study with a split-face design	Application of green tea and lotus extracts on participants. UV light video camera was used to measure the four SELS characteristics	Green tea plus lotus-treated group showed very significant improvements in the SEr parameter toward baseline values after the 60 d treatment course. Improving Facial Skin Surface Parameters	Functional cosmetic agent for smooth skin	[77]
Lotus's Stalks' Extract	In vivo (Adult albino rats)	Injection of the ethanol extract in rats with normal body temperature and yeast-induced pyrexia as a drug. Body temperature of every rat was taken rectally.	200 mg/kg reduced normal body temperature for up to 3 h, while 400 mg/kg significantly reduced body temperature for up to 6 h. - Comparable dose-dependent effect like paracetamol.	Anti-pyretic agent	[73]

Table 2. Cont.

Bio-Functional Ingredient	Model	Study Design	Outcomes	Conclusions Potential Pharmaceutical Application	References
Lotus's Seeds' Extract	In vivo (Wister Rats)	Rats underwent an elevated plus maze to access anti-anxiety activity, followed by light and dark testing. Rats underwent a forced swimming test to assess antidepressant activity.	Increase of open-arms entries and lightroom weighting time, along with a decline in time spent immobile in each test	Anxiolytic and antidepressant agent	[83]
Lotus's Leaves' Extract	In vivo (Hamsters)	Hamsters were fed a high-fat diet to induce hyperlipidemia. Lotus extract enriched with flavonoids was administered to hamsters. The drugs used were silymarin and simvastatin (reference) and triglyceride, lipoprotein, total cholesterol, and liver lipid extraction assays.	- Similar effects to silymarin in alleviating liver injury and lipid peroxidation. - The level of reduction in hepatic triglycerides and cholesterol was higher with NLE supplementation compared with silymarin treatment.	Improvement in high-fat diet-induced hepatic injuries	[72]
Lotus's Leaves' Extract	In vivo (Male C57BL/6 mice)	Mice injected with scopolamine and used a diet containing lotus extract. Tests performed in mice included Y-maze behavior analysis, enzyme-linked immunosorbent assay, and Western blot analysis, among others.	- Significant reduction in the impairment of memory caused by scopolamine through AHN. - Scopolamine decreased cognitive function, while co-administration with lotus extract restored learning and memory capacities.	Potential protectant of cognitive function	[67]

Table 2. Cont.

Bio-Functional Ingredient	Model	Study Design	Outcomes	Conclusions Potential Pharmaceutical Application	References
Lotus Fruit Extract	In vivo (Wister rats)	Administration of 2% gum tragacanth (control), diazepam 1 mg/kg and <i>N. nucifera</i> extract (50, 100, 200 mg/kg). Forty minutes after the first administration, the rats consumed strychnine and recorded.	- Rats that escaped convulsions 30 m after strychnine administration were considered protected. - Extract demonstrated strong antiepileptic efficacy, which significantly delayed the onset of convulsions and enhanced the animals' survival rate (42.85%).	Anti-epileptic agent	[82]
Lotus's Rhizomes' Extract	In vivo (Charles-Foster rats)	Administration of lotus extract in rats, whose blood was then tested for glucose tolerance. Observation of streptozotocin-induced diabetic rats and glucose-fed hyperglycemic as well.	Extract of <i>N. nucifera</i> rhizome effectively reduced the blood glucose level in normal as well as in glucose-fed hyperglycemic rats.	Blood sugar level control agent	[76]
Lotus's Leaves' Extract	In vivo (ICR mice and Wistar rats)	Rats and mice that consumed ethanol extract, a-amylase and lipase, and expression of UCP3 mRNA in C2C12 myotubes were evaluated.	- In 1 h, the plasma triacylglycerol level was markedly raised in the extract-treated group and drastically decreased in the other group. - Extract dramatically reduced the mice's weight increase due to high-fat diet-induced obesity.	Anti-obesity agent	[65]

Table 2. Cont.

Bio-Functional Ingredient	Model	Study Design	Outcomes	Conclusions Potential Pharmaceutical Application	References
Lotus's Root's Extract	In vitro (Human pre-adipocytes)	Treatment with an ethanol extract of Lotus (<i>N. nucifera</i>) root (ELR) in human pre-adipocytes	Inhibition of lipid accumulation and lesser expression of adipogenic transcription factors	Anti-adipogenic, anti-oxidant, anti-obesity agent	[39]
	In vivo (Male Sprague–Dawley rats)	Administration of ELR in rats fed a high-fat diet	Significant decrease in cholesterol, triglyceride and serum leptin and insulin		
Lotus's Seeds' Extract	In vitro (Human peripheral blood mononuclear cells)	Blood of 15 healthy subjects was taken and analysed with lymphoproliferation testing, cell cycle analysis and PCR	- The extract suppressed proliferation in PBMC cells activated by PHA - It also reduced tissue inflammation, in part by inhibiting PBMC proliferation	Suppressor of cell cycle in human peripheral blood mononuclear cells	[57]
Lotus's Rhizomes' Extract	In vivo (Male Institute of Cancer Research mice and Sprague-Dawley rats)	Oral administration of lotus rhizome was followed by injection of sedative. Mice examined GABA (receptor) content and brain receptor expression.	Oral injection of LE increased the amount of time that a person spent sleeping by lengthening their NREM sleep duration.	Sleep-promoting agent	[81]
Lotus Extract	In vivo (Albino rats)	Three doses of prepared extract were consumed by albino rats, and platelet-poor plasma (PPP) was prepared.	Hydroalcoholic extract flavonoids could be turned into a medication for use in treating patients' thrombosis.	Thrombolysis agent	[64]

Abbreviations: Skin Roughness (SEr), Surface Evaluation of Living Skin (SELS), Adult Hippocampus Neurogenesis (AHN).

Table 3. Studies on *Nelumbo nucifera*’s bio-functional ingredients and extracts in functional foods and nutraceutical applications with health promoting properties.

Bio-Functional Food	Model	Study Design	Outcomes	Conclusions Potential Nutraceutical Application	References
Lotus Stamen Tea	Randomised, controlled interventional study (30 participants with hyperlipidemia)	Lotus tea was consumed, and participants’ blood was examined (total cholesterol, HDL-cholesterol, LDL-cholesterol and triglyceride)	- The total cholesterol level was decreased (blood lipid level), mean blood LDL cholesterol. - The main risk factor of atherosclerosis level was decreased.	Adjunct to dyslipidemia therapy	[80]
White Lotus Petal Tea	In vivo (Wistar rats’)	Rats were administered lotus tea and/or mancozeb. They then underwent Y-maze spontaneous alternation test, learning, memory behaviour test and hormonal assay.	Increased the level of neuroprotective picolinic acid	Neuroprotective agent	[68]
Lotus Seed Tea	In vivo (Hairless rats’)	Water group and tea group were exposed to radiation after 6 months of administration and examined for moisture content, protein oxidation and abnormal keratinocyte formation.	Tea group exhibited increased moisture content, epidermal and horny layer thickness and protein carbonyl values, which supports the observed enhanced protection against UVB exposure.	Functional photoprotective and antioxidant agent	[35]
Bio-Functional Food		Study Design	Outcomes	Conclusions Potential Nutraceutical Application	References
Tarts with Lotus Seed Powder		Organoleptic analysis (texture, mouthfeel, taste, appearance) by semi-trained people	Low sodium and high magnesium content were effective in treating heart disease, diabetes and high blood pressure.	Heart health and diabetes treatment agent	[75]

Table 3. Cont.

Bio-Functional Food	Model	Study Design	Outcomes	Conclusions Potential Nutraceutical Application	References
Biscuits with Lotus Seed flour		Organoleptic analysis (texture, mouthfeel, taste, appearance)	Highly acceptability of elderly people	Healthy flour alternative with high acceptability amongst elders	[88]
Lotus Root Flour (LRF) as a fat mimetic in biscuits		- Physicochemical, nutritional and stability evaluation - Sensory evaluation	The formulation with 15% LRF exhibited higher total phenolic content and DPPH inhibition with the same FRAP as the control.	Functional and nutritional flour substitute	[36]
Chicken Patties with Lotus Leaf Powder		Shelf-life assessment. Sensory evaluation (by 12-member panel), colorimeter (to measure the colour), Tarladgis method 2-thiobarbituric acid (TBA; for lipid oxidation)	Can improve shelf-life during refrigerated storage; it showed sensory properties similar to control	Bioactive agent that increases shelf-life	[89]
Breadsticks with Lotus Rhizome Powder		Coloured reaction of phenolics with Folin–Ciocalteu reagent, aluminum chloride colorimetric method, DPPH for free radical scanning	Higher amounts of total phenolic content and total flavonoid content, high antioxidant activity	Bioactive agent with antioxidant activity	[34]

4. *Lemna minor*

4.1. *Lemna minor*: General Information

Lemna spp. is an aquatic plant known in the literature as duckweed. The common name duckweed is derived from its worldwide distribution with waterfowl, such as ducks, and its rapid growth rates [5]. Duckweeds are small floating aquatic plants that grow in dense beds in still or slow-moving waters rich in nutrients. The water in which this type of aquatic plant grows can be either fresh or brackish. They are found in a wide range of geographical and climatic zones, except arid deserts and permafrost regions. However, they grow best in tropical and temperate zones, and many species can survive in extreme temperatures [90,91].

The natural habitat of *Lemna* spp. is the surface of fresh or brackish water protected from the action of wind and waves. It grows in water temperatures from 6 to 33 °C and pH values from 5 to 9. Optimum growth conditions for high biomass production are water temperatures between 20 and 28 °C and pH between 6.5 and 7.5 [92,93]. Unlike most plants, duckweed can survive at relatively high salinity levels (up to 4000 mg/L total dissolved solids) [90]. Nutrients are absorbed from all leaf surfaces. *Lemna* spp. can utilise many nitrogen compounds found in the water in which they grow. The most important of these is the ammonium ion (NH_4^+), which is the main precursor for its growth [92]. The leaf of this aquatic plant is flat and oval. Many species have adventitious roots that act as an organ of stability and tend to elongate as the mineral nutrients in the water become depleted. Compared to most plants, *Lemna* spp. leaves have few fibers as they do not need to support upright structures. However, roots appear to be more fibrous [94].

Lemna spp. is used as a general term to refer to various species of the genus *Lemna*, when the specific species is not identified. Of course, it should be noted that there are many more species in the genus *Lemna*, and the classification of duckweeds is constantly evolving with research. The differences in size, shape and reproductive strategies between these species contribute to their adaptability to different aquatic environments. Some of the best-known species of the genus *Lemna* are listed below:

1. *Lemna minor* is one of the most common and well-known species of duckweed. It has a single, small, flat fringe and reproduces rapidly, forming dense colonies on the water surface [8].
2. *Lemna gibba* is characterised by its oval or swollen leaves. It is slightly larger than *Lemna minor* and often forms floating mats in the water [95].
3. *Lemna trisulca* is unique in appearance, with leaves that are elongated and divided, resembling tiny green ivy leaves. Unlike the previous species, *Lemna trisulca* does not float on the surface of the water but is partially submerged [96].
4. *Lemna japonica* is another species of duckweed commonly found in aquatic environments. It is similar in appearance to *Lemna minor* but may have some distinctive features [97].
5. *Lemna perpusilla* is a small species of duckweed with tiny leaves often found in calm, nutritious waters and is characterised by rapid vegetative reproduction [98].

Several bioactives have been detected in *Lemna* (Table 4), with health benefits also being reported for the antioxidant, anti-inflammatory and antithrombotic health promoting effects of its bioactives, extracts and relative functional products (foods, nutraceuticals, cosmetics and pharmaceuticals) against several manifestations of thrombo-inflammation, oxidative stress and associated chronic disorders (Table 5), either in vitro and/or ex vitro and/or in vivo.

Table 4. Bioactives of *Lemna species*.

Bioactive Compound			References
Fatty acids (%) of total)	(14:0) Myristic Acid	0.55 ± 0.0	[99]
	(16:0) Palmitic Acid	21.74 ± 0.5	[99]
	(16:1) Palmitoleic Acid	2.76 ± 0.0	[99]
	(18:0) Stearic Acid	2.1 ± 0.4	[99]
	(18:1) Oleic Acid	1.77 ± 0.1	[99]
	(18:2) Linoleic Acid	15.89 ± 0.3	[99]
	[18:3 (GLA)] γ-Linolenic Acid	n.d.	[99]
	[18:3 (ALA)] α-Linolenic Acid	54.42 ± 0.7	[99]
	(18:4) Stearidonic Acid	n.d.	[99]
	(20:0) Arachidic Acid	0.33 ± 0.0	[99]
	(22:0) Behemic Acid	0.44 ± 0.1	[99]
Total FA/Dry Weight			10.6 ± 0.8 [99]
Total phenolic content	WELM	22.0 ± 0.8 µg/mg extract	[8]
	EELM	16.7 ± 0.0 µg/mg extract	[8]
	MELM	20.44 mg GAE/100 g	[100]
Other bioactives	β-carotene	0.116 mg/100 mL	[100]
	Lycopene	0.091 mg/100 mL	[100]
	Saponin	23.25 mg/g	[9]
	Flavonoids	0.83 mg/g	[9]
	Alkaloids	6.40 mg/g	[9]

Abbreviations: G.A.E. = Gallic Acid Equivalents; n.d. = not detected.

4.2. Health Promoting Effect of *Lemna minor* Bioactives, Extracts and Related Functional Products

4.2.1. Antioxidant Effects of *Lemna minor*'s Bioactives and Extracts

Several studies have investigated the antioxidant properties of *L. minor* to explore its potential health benefits and applications as a natural antioxidant. These studies have mainly focused on identifying compounds with beneficial health effects through standard antioxidant tests utilizing standard solutions.

In an experimental mouse model, the protective activity of *L. minor* was observed in chronic bleomycin-induced lung inflammation. Ex vivo electron paramagnetic resonance (EPR) evaluation was used for the assessment of the ascorbate (Asc) and ROS production and the protective effect of *L. minor* extract (LME) against Bleomycin (BLM)-induced oxidative changes and lung inflammation. Reduced ratios of SOD to CAT and Asc to ROS may act as cues to complete the regulation of protective antioxidant mechanisms after LMEs pretreatment. Recent results have demonstrated that the LME-dependent reduction of oxidative dysfunction was induced by chronic administration of BLM and was associated with restoring homeostatic balance and activating antioxidant enzymes that influence lung inflammation. Interestingly, by limiting direct intracellular ROS damage, *L. minor* is a highly efficient ROS-scavenging antioxidant defense mechanism. In addition, *L. minor* compounds have the potential to scavenge oxyl- and peroxy-radicals involved in post-gamma irradiation lipid peroxidation, probably in a model of bleomycin-induced lung injury. Furthermore, exogenous antioxidant pretreatment with *L. minor* may stimulate endogenous enzyme synthesis and radical scavenging enhancement via H₂O₂, O₂ and OH radicals' formation, in addition to its activity towards directly reducing lung inflammation. In a similar in vivo study, the aim was to investigate the protective effect of *L. minor* extract on the oxidation of lung proteins and the modulation of oxidative stress by BLM-induced lung fibrosis in specific Balb/c mice [101].

The use of BLM led to increased ROS production, undesired protein oxidation and oxidative stress induction. The presence of an iron-dependent mechanism during the inflammatory process caused by BLM accumulation increased hydrogen peroxide (H₂O₂), hydroxyl (-OH) and lipid radicals, respectively [102,103]. Based on available data, *L. minor* is a suitable phyto-remediator, which regulates the activity of antioxidants (CAT, ascorbate

peroxidase [APX] and substrate ascorbate) and keeps the concentration of H_2O_2 and $-OH$, lower than phytotoxic levels. Thus, it does not negatively interfere with electron transport activity and cellular metabolism [104,105]. *L. minor*, therefore, could be used as a protective agent since it directly inhibits the increase of oxidative stress (OS), regulates protein oxidation and collagen deposition, reduces lung lesions caused by BLMs and improves the response to idiopathic pulmonary fibrosis (IPF). The extravascular accumulation of pulmonary fibrin in acute and chronic lung diseases, induced in animals, is thought to be caused by the disruption of the $-OH$ linkage leading to the modification of fibrinogen. On the basis of these findings, it is hypothesised that the therapeutic effect of *L. minor* is due to a modulated cellular response resulting from the high polyphenol content [106,107], leading to a reduction of membrane albumin protein and $-OH$, as well as the reduction and neutralization of amyloid protein aggregates. The intracellular ROS generation and OS damage were indirectly reflected by the depletion of antioxidant enzymes SOD, CAT and GSH activity in lung tissue. In addition, there was a statistically significant increase in the activity of SOD, CAT and GSH and a decrease in the level of lipid peroxidation and the residual ROS under the combination of BLM + *L. minor*. Therefore, *L. minor* could reduce any damage in the lung tissue and influence the stimulation of fibroblasts by reducing the O_2 concentration in the extracellular space and balancing the H_2O_2 content and all products after lipid peroxidation [108].

L. minor, otherwise known as common duckweed, may also be supplemented in the diet of some animals, such as cows, to improve some health parameters. The ethanolic extract of 70% duckweed leaves has a high total phenolic content leading to the potential of phenolic compounds as antioxidants due to the presence of hydroxyl groups. The formation of phenolic ions is increased because of the high concentration of phenolic compounds [109]. N-hexane and ethyl acetate extracts have a very low total phenolic content, in contrast to data in the literature showing high antioxidant activity when phenolic content is high. It is then suggested that as the total phenolic content increases, the antioxidant activity is higher and greater than with low phenolic content. A significant effect ($p < 0.05$) on all antioxidant parameters was observed when the dietary patterns of cows were introduced to duckweed, either solely or in combination with their initial feed plus duckweed [110].

Duckweed extracts were evaluated for their in vitro antioxidant activity and their flavonoid and total phenolic content. The DPPH method (2,2-diphenyl-1-picryl-hydrazyl-hydrate free radical method) has been widely used for the estimation of free radical scavenging activity. DPPH is unaffected by side reactions such as metal ion chelation and enzyme inhibition [111]. The ethanolic fraction of duckweed showed 70.4% antioxidant activity, while the acetone fraction showed the least antioxidant activity at only 21.0%. In fact, antioxidant compounds have the ability to remove or neutralise free ROS radicals from the body before they oxidise lipid, DNA and protein molecules. Thus, they contribute to the protection against various diseases, such as cardiovascular disease and cancer [112]. Among all the different phenolic compounds, flavonoids were mostly found in the ethyl acetate fraction at a concentration of 0.84%, and the total phenolic content in *L. minor* was found to be 2.1% in the ethanol fraction. Due to the presence of hydroxyl groups [113], phenolic compounds are highly soluble in polar organic solvents. Consequently, the ethanolic extract of duckweed showed excellent antioxidant potential and high phenolic content. Because of their ability to donate hydrogen atoms to free radicals and their ideal structural characteristics, phenolic and flavonoid molecules are important antioxidant components responsible for deactivating free radicals [112] and scavenging free radicals, respectively [114]. The presence of high concentrations of phenolic components in the extract may effectively scavenge radicals and, hence, directly contribute to the systems' antioxidant activity [115]. Consequently, the high phenolic content of duckweed plants has undoubtedly confirmed its great antioxidant activity [111]. It must be noted that flavonoids are secondary metabolites of plants that possess high antioxidant activity, which is completely dependent on their structure and the exchange pattern of hydroxyl groups. Ethyl acetate and hexane fractions of duckweed reportedly showed high concentrations

of flavonoids that have anti-inflammatory effects and protect the body from oxidative damage [112].

The ferric thiocyanate method was used to compare the total antioxidant activity of *L. minor* water extract (WELM), ethanol extract (EELM) and some standards. The reference antioxidant solutions were α -tocopherol and trolox (a water-soluble analogue of tocopherol), butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) [8]. This method is used to measure the amount of peroxide, the primary product of lipid oxidation that is produced during the initial stages of oxidation [29]. This technique was used to determine the total antioxidant activity of WELM, EELM, α -tocopherol and trolox in the linoleic acid system. WELM, EELM and the standard compounds showed effective antioxidant activity in this system [8]. In parallel, using the method of Oyaizu [30], the Fe^{3+} - Fe^{2+} transformation was studied to determine the reducing power measurements of WELM and EELM. All experimental observations on the reductive power demonstrated the electron donor properties of WELM and EELM, thereby reducing their ability to neutralise free radicals by forming stable products. The result of the reducing reaction is the termination of radical chain reactions, which could otherwise be highly damaging [8].

Chelation of iron II ions (Fe^{2+}) may provide vital antioxidant effects by retarding metal-catalysed oxidation. Due to its high reactivity, iron is known to be the most important pro-oxidant of the transition metals in lipid oxidation. Effective iron ion chelators can also protect against OS damage by removing iron (Fe^{2+}), which might otherwise participate in an HO-generating Fenton reaction. Thus, antioxidants that are capable of chelating Fe^{2+} will, in fact, minimise the concentration of the ion and inhibit its ability to catalyze the formation of free radicals, which will result in protection against oxidative damage. Ferrozine is able to form complexes with Fe^{2+} in a quantitative manner, but in the presence of chelating agents, complex formations are disrupted. Consequently, color measurement shows the complexes' red color is reduced, therefore providing an estimate of the metal chelating activity of the co-existing chelator. In this assay, WELM and EELM, are inhibited by the formation of ferrous and ferrozine complexes, indicating their chelating activity and scavenge ability of ferrous ions before ferrozine [8].

The riboflavin-methionine light system was utilised to generate superoxide anion from dissolved oxygen. In this procedure, superoxide anion reduces the yellow dye (NBT^{2+}) to produce the blue formazan, which is measured by spectrophotometry at a wavelength of 560 nm. Antioxidants, though, are able to inhibit the formation of blue nitroblue tetrazolium (NBT). The consumption of superoxide anion in the reaction mixture is indicated by the decrease in absorbance at 560 nm, with the presence of antioxidants [8].

A further test of the antioxidant activity of the plant examined gold nanoparticles synthesised from *L. minor*. The samples were found to be free of radical scavengers by the positive DPPH test. These results indicate that *L. minor* and *L. minor* synthesised gold nanoparticles have the ability to quench DPPH radicals. It was also suggested that gold nanoparticles synthesised using *L. minor* were good antioxidants with DPPH free radical scavenging activity, are more active than *L. minor* alone and are comparable to the reference drug [116]. In fact, *L. minor* gold nanoparticles had significant superoxide radical scavenging activity. These results indicate that *L. minor* gold nanoparticles (Lm-GNPs) have superoxide radical scavenging ability. The outcomes of this survey suggest that as good antioxidants with superoxide radical scavenging activity, gold nanoparticles are more active than *L. minor* alone and are comparable to the reference drug. It has been reported that flavonoids are especially effective antioxidants due to their scavenging superoxide anions [116].

In addition, gold nanoparticles can inhibit nitrite formation via direct competition with oxygen and nitrogen oxides in the reaction mixture, bringing their nitric oxide scavenging activity to the forefront. Due to their ability, such nanoparticles significantly decreased the concentration of nitric oxide radicals, comparably to that of a standard drug. A potent scavenger of nitric oxide was demonstrated by the percentage inhibition exhibited by the extract. The *L. minor* and gold nanoparticles had a crucial activity in scavenging nitric oxide

radicals, which indicated that *L. minor* and *L. minor*-synthesised gold nanoparticles (Lm-GNPs), could scavenge nitric oxide radicals. It was also suggested that *L. minor* synthesised gold nanoparticles are more active than *L. minor* alone and, in comparison to the reference drug, as antioxidants with nitric oxide synthase (NOS) scavenging activity. Prolonged production of this radical is instantly toxic. It contributes to vascular collapse associated with septic shock. In contrast, chronic expression of the radical is ascribed to several cancers and inflammatory conditions, including juvenile diabetes, multiple sclerosis, arthritis and ulcerative colitis [116].

Presently, the most reactive oxygen radical is the hydroxyl radical. Nanoparticles removed hydroxyl radicals formed from deoxyribose and prevented the reaction when added to the reaction mixture. The hydroxyl radical scavenging activity of *L. minor* and gold nanoparticles was, as proved, rather significant. All of the above observations indicated that *L. minor* and *L. minor* synthesised gold nanoparticles (Lm-GNPs) have hydroxyl radical scavenging ability. It was also proposed that gold nanoparticles synthesised with *L. minor* are more active than *L. minor* alone and are comparable to reference drugs as good antioxidative agents with hydroxyl radical scavengers. Hydroxyl radicals are major active oxygen species since they cause lipid peroxidation and enormous biological damage. These radicals play an important, direct or indirect role in several pathological conditions, such as cerebral ischemia, Parkinson's disease, hepatitis and carcinogenesis. Thus, *L. minor* and the newly synthesised gold nanoparticles (Lm-GNPs) exhibited antioxidant activity in a concentration-dependent manner by scavenging hydroxyl radicals. The IC₅₀ values obtained for *L. minor* gold nanoparticles are almost equal to those obtained for the standards, implying that *L. minor*-synthesised gold nanoparticles are superb antioxidants when compared to the standard. *L. minor* presents high antioxidant activity, implied by the higher values obtained, especially in the form of a mixture with gold nanoparticles, where its activity increases. Finally, gold nanoparticles have better antioxidative properties than binding substances; hence, in conjunction with *L. minor*, excellent outcomes are expected [116].

4.2.2. Anti-Inflammatory Effects of *Lemna minor*'s Bioactives and Extracts

A plethora of *L. minor* constituents have exhibited significant anti-inflammatory activity. Based on this fact, a number of studies have investigated the anti-inflammatory behavior of the plant, most of which derives from in vivo experimental procedures and techniques.

Karamalakova et al. [101] clearly demonstrated that the use of LME effectively inhibited severe cases of pulmonary inflammation after chronic administration of BLM (bleomycin) in the experimental mouse model. In addition, they observed the protective capacity of LME, both as a regulator of the endogenous antioxidant system and as a means of reducing the level of oxidation in pulmonary cells, especially when combined with BLM. In the groups treated with LME and the LME + BLM combination, no significant positive differences were observed in mortality, daily food consumption and behavior of the mice. LME-treated BLM mice showed no signs of respiratory distress, increased survival rate or managed body weight loss. LME was suggested to have a protective effect against BLM-induced chronic chemotoxicity and lethality [101]. As a continuation to all the above knowledge, this study aimed to determine the possible inhibitory and protective effects of freshwater *L. minor* extract in reducing pulmonary inflammation, idiopathic lesions and modulating pulmonary oxidised proteins and oxidative disorders in BALB/c mice exposed to progressive BLM-induced IPF. Additionally, this research team investigated *L. minor*'s potential regulatory mechanism of inflammatory cytokine expression in lung and plasma after 16 and 33 d of BLM.

To better interpret the experimental results of this study, it is important to be aware that pulmonary fibrosis is characterised by infiltration of the parenchyma by inflammatory cells, followed by an increase in extracellular matrix production due to proliferation and activation of fibroblasts, a case correlated with an increase in interalveolar septa thickness, together with an increased number of mast cells (MCs) in the fibrotic area of BLM-treated animals. Initial results displayed a notable decrease in body weight, appetite, dyspnea, in-

creased collagen accumulation and lung histopathological changes associated with typical IPF clinical features after BLM administration. Prior literature claims that BLM administration increases pulmonary inflammatory influx, promotes collagen deposition in lung cells and alters alveolar capillary, membrane integrity and efficacy. Furthermore, the presented conclusions showed that 33 d of combined *L. minor* therapy statistically significantly prevented the fibrotic process, decreased the inflammatory response, reduced the extracellular matrix and fibroblast proliferation and thus generally improved this process. In addition, daily administration of *L. minor* significantly reduced the density of metachromatic MCs, especially in the interalveolar septa and in the large bronchial wall. BLM + *L. minor* values were close to the control ones. The extract of *L. minor*, specifically, is a possible, potent and modern mucosal adjuvant that restores the proteins' antigenicity. In contrast, the intraperitoneal administration of lemnian, the major apiogalacturonan pectin in *L. minor*, improves the serum titer of specific IgG antibodies. Thus, *L. minor* supplementation has a beneficial effect on BLM-induced inflammation and fibrotic processes. This supplementation may be involved in the modulation of the Th1/Th2 immune response and the subsequent decrease in MCs and eosinophils. This study suggests that *L. minor* aqueous extract stimulates an antigen-specific immune response, restores protein oxidation and determines inflammation and cell-mediated cytotoxicity due to the high content of active proteins and amino acids in its structure. Therefore, *L. minor* modulates BLM-induced inflammation by reducing carbonyl stress. This result is consistent with the fact that *L. minor*, as a non-toxic natural anti-inflammatory product, reduces the area of colorectal lesions and provides lung protection from Mycobacterium tuberculosis in humans [108].

For the next inflammatory response test, male Swiss mice were used. The inflammatory response was assessed with a micrometer by measuring the swelling of the paw pads after 24 h. The effect of *L. minor* on the inflammatory response was determined as an integral indicator to further investigate the leukocyte-modulating capacity of *L. minor*. Duckweeds were found to increase paw pad swelling in mice, which indicated an increase in the non-specific inflammatory response. Such data are very consistent with its ability to stimulate neutrophils and macrophages. In addition, *L. minor* was found to increase the inflammatory response in ovalbumin (OVA)-sensitised mice. All obtained data suggested a direct or indirect activation of murine Th1 lymphocytes, which are well known to mediate the hypersensitivity response of the tissue in sensitised mice [117]. Other studies have noted that plant polysaccharides increase the production of Th1 cytokines [118,119].

L. minor's effect on the level of the inflammatory response to agOVA in OVA-sensitised mice appeared to be related to duckweed's effect on antigen processing upon oral administration. It was found that duckweed was able to increase the duration of the OVA-specific response. Following this pattern, the fragmentation of *L. minor* was carried out to determine the structural regions of the macromolecule that were important for the stimulatory effect. The apiogalacturonan low methoxy pectin (LMP), which is similar to the crude lemnian, was declared as an enhancer of the inflammatory response. The linear galacturonan part of lemnian that was digested with a pectinase was not able to mediate the effect of lemnian on the swell of the foot pads. In addition, apple pectin (AP), which consists mainly of the galacturonan region, also failed to influence inflammatory response. These data suggest that the specific carbohydrate chains in its 'ramified' region play an important role in the pro-inflammatory effect of lemnian. The active region of the lemnian macromolecule that enhances inflammatory response was, in fact, the branched fragment LMP. In summary, all obtained data showed an enhancement of the inflammatory response by *L. minor* [120].

Similar research patterns were followed in the next study, where acute gamma irradiation was used to induce gastrointestinal syndrome in mice. Acute symptoms develop after radiation exposure, resulting in morphological changes in the intestinal villus. Experimental analysis showed that degenerative and regenerative phases in the small intestine are shorter than in the bone marrow. During and after damage, the integrity of the epithelial layer has to be restored as quickly as possible to prevent infection. In mice, this period varies between 24 and 55 h. All experiments were performed to determine morphological

changes in the acute period after irradiation (48 h) and to reduce such changes using *L. minor*. Meanwhile, after irradiation (5 Gy), a thickening and shortening of the intestinal microvilli and villi was observed, as well as a disruption of the tight junction structure. In addition, the villi were broken or dissolved to varying degrees while their arrangement was loosened; epithelial desquamation and disintegration were also seen. A third experimental group that consumed lemnan underwent a regenerative process in the small intestinal mucosa after irradiation [121]. Previous studies show that the outcome of the intestinal regeneration process is determined by the nature of the pectin. The physico-chemical properties of pectins provide them with several advantages in the wound healing process; in particular, their hydrophilicity allows the removal of exudates and the maintenance of an acidic pH, which is expected to act as a barrier against bacteria or fungi. Pectins have the potential to bind to active molecules and protect them against degradation [122,123] while they reduce the secretion of pro-inflammatory factors and immunoglobulins during radiation enteritis [124].

Recovery after irradiation is a multi-component process. Hence, reducing mortality in irradiated mice is a universal parameter of the recovery process since all animals die within 2 to 3 weeks. Therefore, 5 Gy irradiation was chosen for the use of herbal supplements, which causes a medium injury rate. The survival rate of an experimental group (IR + *L. minor*) increased, compared to a second experimental group of irradiated mice. The survival rate increased by $15 \pm 5\%$ [121]. Following, in an ex vivo experiment, isolated mononuclear cells from four subjects were pulsed with carboxyfluorescein-succinimidyl ester (CFSE) and cultured in triplicate in RPMI medium + human serum + gentamicin, without (control) and with increasing concentrations of *Lemna* extract. The total number of mononuclear cells and the CD4⁺, CD8⁺ and B-cell populations were counted by flow cytometry after 24, 48 and 72 h. Cell death (necrosis, early and late apoptosis) and proliferation (number of CFSE-low cells) were assessed for each population, and conclusions were drawn. *L. minor* extracts obtained by decoction appeared to affect immune cells. Cell necrosis did not appear to be induced by these extracts. Between 24 and 48 h, some spontaneous apoptosis was observed in CD4⁺ cells. At very high concentrations of duckweed extracts with no significant cytotoxic effects on other cell populations, apoptosis was unaffected or partially prevented. At low concentrations, these extracts caused a limited expansion of CD4⁺ cells within 48 h. CD8⁺ cells and B lymphocytes were induced to proliferate within 48 h at high concentrations [125].

4.2.3. Hematological Responses of *Lemna minor*'s Bioactives and Extracts and Associated Benefits

Sharma et al. [126] used *L. minor* (duckweed), extracted secondary metabolites (i.e., flavonoids) and determined their immunological activity against specific protein antigens. The results showed that these flavonoids had an immunosuppressive effect at higher doses in human whole blood samples infected with viruses. According to previous literature, the main factor related to a virus-infected blood profile is high plasma haemoglobin concentrations. In lysed virally infected human whole blood samples, exposure to variable doses of flavonoids purified from duckweed caused a reduction in free haemoglobin content at higher doses. The immunosuppressive effect of the flavonoids was clearly demonstrated by the sudden decrease in free haemoglobin in virus-infected and virally infected blood and the reduction in the production of antibodies against a specific protein antigen [126].

The haematological influence of *L. minor* as part of the feed for dairy cows to improve the biosynthesis of their milk has also been investigated. Based on the results of the present study, it appears that adding duckweed to dairy cow rations can improve the overall haematological condition of dairy cows. The effect of the bioactive content of duckweed on increasing the rate of erythropoiesis and preventing damage to blood cells, as well as reducing the risk of oxidative stress, may explain the improvement in haematological values with the administration of duckweed. There was a significant effect on RBC levels when the diet was supplemented with duckweed, either alone (fresh duckweed only) or

in combination with dried duckweed. Through this trial, it was elucidated that a combination of duckweed may improve blood values, while the concentration of haemoglobin (Hb), packed cell volume (PCV), and red (RBC) and white blood cells (WBC) increased significantly with the dietary combination of fresh and dried duckweed. This outcome showed that the effect on erythropoiesis by specific signaling factors was enhanced by the interaction of chemical duckweed compounds with the molecules inside the cells [110].

Subsequently, other researchers investigated the anticoagulant properties of sulphated pectins using *L. minor*. Researchers constructed pectin sulphates by sulphation, with chlorosulphonic acid. As observed, pectin sulphates LM-SO₃ increased the clotting time of human plasma in the prothrombin time test (PT). Original pectins *L. minor* (LM), on the other hand, do not increase the time of human plasma coagulation in the thrombin time test (TT). In the activated partial thromboplastin time (APTT) test, pectin sulphates increase the clotting time of human plasma. However, LM-SO₃ only increases specific antithrombin (aIIa) activity 1.7-fold by inhibiting the rate of chromogenic substrate hydrolysis by thrombin in the presence of antithrombin with the most active pectin sulphates. It is likely that LM-SO₃ requires only antithrombin to comprehend antithrombin activity. It is noteworthy that the anti-factor Xa (aXa) activity of LM-SO₃ in the Rea Clot coagulation test is twice that of BC-SO₃-3 (Bergenan pectin sulphate), although it would be logical to expect otherwise. Most likely, the amounts of Xyl and Api play a role in the manifestation of aXa activity. At the same time, the decrease (almost 40 times) of the aXa activity, calculated in the coagulation test, when compared to the aXa activity of pectin sulphates during the inhibition of the rate of chromogenic substrate hydrolysis, implies the necessity of antithrombin for factor Xa inhibition. Thus, the sulphation of lemnian LM leads to the manifestation of anticoagulant properties, which are associated with the inhibition of the fibrinogen coagulation and many amidolytic activities of thrombin and factor Xa through antithrombin and probably also via other serpins. The specific anticoagulant activity of sulphated pectins is based on the plants' species, the pectin monosaccharide composition and the sulphation degree [127].

4.2.4. Anti-Microbial and Antibacterial Activity of *Lemna minor*'s Bioactives and Extracts

In recent decades, it seems that there has been a great interest in the antimicrobial action of *L. minor*, a fact that is being investigated by conducting in vivo and in vitro experiments. In the present in vivo study, LM was assessed for its potential mucosal adjuvant properties. We used the well-characterised protein OVA as the antigen in the explained mouse model [128].

Oral administration of OVA in three oral doses given at weekly intervals gave rise to low endpoint titers of OVA-specific IgG in sera and failed to prime systemic immunity to delayed type hypersensitivity (DTH); hence, similarities to previous studies with protein antigens were found. LM was observed to enhance serum antibody titer up to that induced by *Cholera enterotoxin* (CT), which is claimed to be the most powerful modern mucosal adjuvant used as a positive control. Enhanced DTH reaction in mice in response to OVA as a thymus-dependent antigen revealed the stimulatory effect of LM on Th1 lymphocytes and accessory cell types needed for the expression of the reaction. At the same time, LM appeared to increase the ability of the intestine to take up associated materials, as confirmed by the higher levels of circulating OVA, after feeding in a mixture with LM. Lipid peroxidation has also been shown to provoke the increase of paracellular permeability in Caco-2 cell monolayers. Enhancement by LM of lipid peroxidation in the intestinal tissue is suggested to result in disruption of the epithelial barrier function. Therefore, mucosal adjuvanticity of LM may result from an alteration of the epithelial lipid barrier. It may be suggested that LM facilitates a higher uptake of antigen across the intestinal mucosa by maintaining the contact of antigen with a mucosa for a greater length of time. LM was found to increase amounts of intestinal mucus and was being pectin-proved to form gels in the presence of mono- or divalent cations. Bacterial toxins, such as CT, are potentially too toxic for use in humans. In contrast, pectins have been administered to humans by a variety of routes without toxic effects. The advantages offered by LM-based adjuvants are connected

with their induction, both cellular and humoral immunity, and the potential for excellent safety, tolerability and ease of formulation. The mixed profile (Th1 plus Th2 response types) induced by LM will be of value in vaccination against several infections [128].

Mane et al. [106] tested a whole fresh plant of *L. minor* for its antimicrobial activity against four bacterial and one fungal strain. It was then demonstrated that at a higher concentration, the aqueous extract produced a dose-dependent zone of inhibition after 24 h of incubation against both gram-positive and gram-negative bacteria, including a fungal strain, in lysed human whole blood. These studies confirmed that aqueous extracts have been reported to possess superior antimicrobial activity against various bacterial pathogens and fungal strains, inhibiting their growth significantly [106].

To evaluate the in vitro antimicrobial effect in this study, the *P. fluorescens* inoculum was used to evaluate the antibacterial capacity of LM extracts in six concentrations. Methanol extract showed the highest antibacterial activities, indicating that available active compounds are mainly polar. In *Lemna* species, the presence of phenolic compounds such as gallic acid, tannins, flavonoids, anthocyanins, quercetin and other compounds such as thiol and terpene, mostly steroids, have been reported. These compounds are known to have serious antimicrobial properties [8,129,130]. In the hexane extract, on the other hand, the main antimicrobial activity should be exhibited by terpene compounds introduced as steroids [131].

Over the years, the antimicrobial activity of the plant has been studied against various pathogens. The present study focuses on the phytochemical analysis of duckweed along with its antibacterial potential. For this purpose, compounds were extracted from duckweed using a solvent extraction method, and the efficacy of different solvent extracts of LM was evaluated against selected known pathogens. Results claimed that the ethyl acetate and hexane fractions of the duckweed are more potent antibacterial agents, while the ethanol extract did not show any inhibition of the pathogens. The active compounds were found to be moderately to less polar in nature. Thus, they exhibited an inhibitory effect against all tested organisms, but *P. aeruginosa* and *S. aureus* were found to be more sensitive and inhibited by all three fractions except the ethanol one [112].

The antifungal activity of silver nanoparticles using LM (Lm-AgNPs) against *Aspergillus flavus* was also investigated in an environmentally friendly in vitro study. Fresh Lm was mixed with silver nitrate (AgNO₃) solution, with the mixture being kept at room temperature. Notably, there was a pronounced activity with increasing volume of Lm-AgNPs, where it may be concluded that the synthesised Lm-AgNPs possess remarkable potential as an antifungal agent in the treatment of fungal diseases. After treatment with AgNPs, cells were usually ruptured by the action of silver particles. The Lm-mediated synthesis of AgNPs had greater potential against the fungus *A. flavus* and may serve as a great candidate for the treatment of fungal diseases [132].

4.2.5. Safety Evaluation of *Lemna minor*'s Bioactives and Extracts

After examining the antimicrobial activity of *L. minor* extracts in vitro, zebrafish were used to evaluate the safety of tested extracts for use in the control and treatment of bacterial sepsis in vivo. The median lethal concentration (LC₅₀) of the extracts in *Danio rerio* embryos and larvae was determined by a static type of bioassay without renewal of the test solution. In this test, fish were exposed to the extracts, and mortality and immobility of the test organisms were monitored and recorded at 4, 8, 12, 24, 48, 72 and 96 h. For the embryo tests, only fertilised eggs with no external abnormalities (asymmetries, vesicles) or membrane damage were utilised. Either alive or dead embryos were counted throughout the test using four apical criteria (occulated, segmented, detailed tail and presence of heartbeat). Eggs that did not have these characteristics were considered to be dead at the time of counting. The same procedure was carried out for larvae, except that only alive or dead organisms were observed with respect to treatments.

The embryos and larvae of *D. rerio* were prepared for the experiment in 10% neutral buffered formalin (NBF) and processed in a tissue histoquinete. Tissue evaluation was

qualitative, searching for alterations such as pericardial oedema, yolk sac oedema, skeletal deformities and defined eye and axial malformations (abnormal notochord), which are among the most sensitive acute embryonic and larval sublethal endpoints.

At the macroscopic level, the correct formation of the vertebral column, the eyes, the yolk sac with an abundance of reserve lipid and the heartbeat were assessed. Based on the calculated LC_{50} , the methanol extract showed the highest larval and embryonic tolerance in *D. rerio* at high concentrations, and the chloroform extract showed the highest embryonic and larval mortality at lower concentrations during the 96 h of the experiment. The reference also implies a significant increase in toxicity with moderately lipophilic substances. At the end of this procedure, tissue analysis of *D. rerio* larvae and embryos exposed to the three extracts showed that hexane-exposed and chloroform-exposed extracts of different levels arrested development, failed to develop somites and died within the first 24 h. Embryos exposed to concentrations less than the LC_{50} continued to develop, showing no evidence of abnormality in the morphogenesis of vital structures and differentiation of the central nervous and cardiovascular systems, with no obvious tail malformation or changes in the antero-posterior axis or death.

Danio rerio larvae showed no damage at the epithelial level and no changes at the bone level, such as lordosis or kyphosis, after yolk sac ingestion of the calculated LC_{50} . At 96 hpf, some organs, such as the kidneys, liver and gill system, were not yet fully developed, as was the mouth, which the embryos naturally open once the yolk sac is fully absorbed 3 d after hatch. However, various factors, such as exposure to substances, have been reported to affect the timing of organ development and functionality [131]. Some of the most important effects in response to a toxic substance are the disturbance to the cardiovascular system development, skeletal deformities, osmoregulatory dysfunction, neural defects, growth reduction, and decreased survival [133], where according to this study, no such effects occurred at concentrations below the calculated LC_{50} . In the case of mortality, this could be due to changes in cardiac tissue.

Overall, this study showed that embryos developed adequately at concentrations below the calculated LC_{50} , and larval tissues did not show any abnormalities affecting vital body functions [131].

4.2.6. *Lemna minor*'s Bioactives and Extracts for Functional Foods' Applications in Animals' Feeds

L. minor was proved to be a good source of bioactive components that have the potential to replace soybean meal without compromising the growth performance of some marine animals.

Building on that hypothesis, a recent study evaluated the possibility of including fermented duckweed flour (FDF) in the diets of Pacific white shrimp by assessing the growth performance and the expression of genes related to digestion, stress and immune functional systems. In this work, fishmeal (FM) was replaced by FDF up to 35% (D35 diet) in diets for juvenile Pacific white shrimp without affecting survival. Experimental observations indicated that replacing 35% FM with FDF poses a positive effect on shrimp growth, as the animals grew better than those fed by 100% FM. Trypsin and chymotrypsin are the major digestive enzymes (endopeptidases) synthesised in the Pacific white shrimp midgut gland; however, cathepsin B appears to be the major digestive enzyme when replacing FM with 35% FDF.

Finally, it should be noted that the expression of digestion genes was correlated with the highest growth performance in the D35 diet. In the present study, the expression patterns of heat shock proteins 70 and 90 (Lvhs70 and Lvhs90, respectively) were then compared in the hepatopancreas of shrimp fed with different partial replacements of FM with FDF. Results showed that shrimp fed with FDF were more stressed than those fed with 100% FM, as they showed an apparent up-regulation at the mRNA level, although there is a decreasing trend with diet D35. In shrimp, there are no reports on the effect of plant proteins on the gene expression of HSPs. When comparing the expression of digestion and

stress genes, it is important to note that there is a positive correlation between them from D5 to D35; however, with a D35 diet, the expression of cathepsin B and stress genes has a negative correlation. By introducing FDF into the diet of white shrimp, the expression of all the above-mentioned genes is regulated. Both microbial attack and immunostimulants affect the immune system of these shrimps [134].

In another trial, to evaluate an alternative protein source that is less expensive and more sustainable than conventional ones, Fiordelmondo et al. [135] included duckweed meals in three experimental diets for rainbow trout as a partial replacement for the two main protein feeds, fish meal and soybean meal. Survival rates were very high in all experimental groups. As far as somatic indices were concerned, condition index (KI) and perivisceral fat index (PFI) were influenced by the highest percentage of duckweed inclusion and followed the trend of increasing growth. However, the viscerosomatic index (VSI) and hepatosomatic index (HIS) also displayed differences among the three experimental diets, as well as with the control diet. These variations in VSI and HIS, could be related to the non-energetic carbs, which are accumulated in the liver and transformed into lipids and glycogen, resulting in an increase in these indices, as has been documented in trout-fed diets containing alternative vegetable ingredients rich in non-digestible carbs, both in the form of oligo- and polysaccharides. To overcome this drawback, other trials have proposed the use of duckweed after a fermentation process. This could significantly reduce the antinutritional factors and the crude fibre content [135].

4.2.7. Neuropharmacological Application of *Lemna minor*'s Bioactives and Extracts

The promising extracts of *L. minor* open up the possibility of the discovery of new clinically effective bioactive compounds with potential applications in various pharmaceutical fields, such as neuropharmacology. Neuropharmacology is the study of the effects of drugs on the nervous system, which aims to develop compounds that offer many therapeutic benefits to humans suffering from psychiatric and neurological diseases [136]. There are four types of neuropharmacology tests: open field test, hole-cross test, hole-board test and prolongation effect on phenobarbital-induced sleep time in mice.

In the case of *L. minor*'s neurological effect in vivo, the study of a methanolic extract of the whole plant of *L. minor* proved mild sedative and anxiolytic properties compared to the standard drug Diazepam. This extract did not cause any significant agitation effect from its initial value during the experimental time, which is comparable to the reference drug Diazepam. In the hole-board test, it is significant that an increase in dose has no apparent locomotor activity in mice compared to Diazepam.

Finally, the methanolic extract of *L. minor* in mice shows no significant activity on phenobarbital-induced sleep time in a dose-dependent manner. Observation of the behavior of test animals is the most important step in the evaluation of drug effects on the CNS. Another important step in evaluating drug action on the CNS is to observe its effect on the locomotor activity of the animal. A decrease in locomotor activity may be closely related to sedation resulting from central nervous system depression. The locomotor activity of albino mice is not reduced after the application of *L. minor* methanolic extracts. Finally, the overall summary of this study suggests that *L. minor*'s methanolic extract has no CNS depressant activity in experimental animal models [137].

Table 5. Studies on *Lemna minor*'s bio-functional ingredients potential in pharmaceutical drug discovery.

Bio-Functional Ingredient	Model	Study Design	Outcomes	Conclusions Potential Pharmaceutical Application	References
<i>L. minor</i> Extract	In vivo (Swiss Albino mice model)	Open field test, hole-cross test, hole board test, Prolongation Effect on Phenobarbital Induced Sleeping Time in Mice	The observed neurological effect from the study of <i>L. minor</i>'s methanolic extract of the whole plant included minor sedative and anxiolytic properties compared to the standard drug diazepam.	Mild anxiolytic agent	[137]
<i>L. minor</i> Extract	In vivo (Zebrafish embryos and larvae)	Antimicrobial action against <i>Pseudomonas fluorescens</i>. Determination of the median lethal concentration LC₅₀ of <i>L. minor</i> extracts. Qualitative tissue evaluation on alterations such as pericardial edema, yolk sac edema, skeletal deformations, defined eye, and axial malformations	- The methanolic extract showed the highest tolerance in <i>D. rerio</i> larvae and embryos at high concentrations. - Meanwhile, the chloroform extract exhibited the highest mortality in embryos and larvae at lower concentrations during the 96-h experiment.	Anti-septicemic agent in fish	[131]
<i>L. minor</i> Extract	In vivo (Balb/c male mice)	Mice underwent a normal diet, a bleomycin treatment and/or an extract administration. Lung/alveolar cell experiments were conducted	- LMEs effectively inhibited serious cases of lung inflammations after chronic Bleomycin administration. - LME antioxidant action regulated pulmonary lipid accumulation and oxidative stress at chronic chemo-toxicity.	Oxidative stress therapeutic agent	[101]

Table 5. Cont.

Bio-Functional Ingredient	Model	Study Design	Outcomes	Conclusions Potential Pharmaceutical Application	References
<i>L. minor</i> Extract	In vivo (Balb/c male mice)	Mice will undergo bleomycin-induced IPF, and its potential protection against it when administered <i>L. minor</i> <i>L.</i> extract will be analysed via histological exams	<i>L. minor</i>'s antioxidant activity improved the protective response during the inflammatory process and the IPF initiation. <i>L. minor</i> impairs mast cells' survival and activation, constituting a novel way in which <i>L. minor</i> exerts its anti-fibrotic effects in patients with IPF.	Idiopathic Pulmonary Fibrosis (IPF) modulator	[108]
<i>L. minor</i> <i>L.</i> Powder	In vivo (Male Mus musculus mice)	The mice group underwent gamma irradiation-induced gastrointestinal syndrome.	<i>L. minor</i> reduces typical morphological injury developed after exposure to ionizing radiation. Anti-inflammatory and antibacterial activity	Anti-irradiation agent for increased survival post-exposure	[121]
Lemnan LM (An apiogalacturonan pectin of duckweed)	In vivo (Male Swiss mice)	The mice group was orally immunised thrice at weekly intervals with free OVA or OVA with Lemnan.	Substantial systemic and local mucosal immune responses were attained at oral immunization with the mixture of OVA and lemnan. <i>Lemnan</i> appeared to elicit adjuvant activity via induction of both Th1- and Th2-type responses.	Immunostimulant agent	[128]
Raw matter <i>L. minor</i>	In vivo (5–7th-lactation-old or 7–8th years-old dairy cows)	<i>L. minor</i> feed was given to cows, and biochemical examinations were conducted on their blood after some time	Improvement in haematologic levels with duckweed administration could be due to it increasing the rate of erythropoiesis protecting against oxidative stress.	Functional haematologic agent for dairy cows' feed	[110]

Table 5. Cont.

Bio-Functional Ingredient	Model	Study Design	Outcomes	Conclusions Potential Pharmaceutical Application	References
<i>L. minor</i> Flavonoid Extract	Ex vivo (Virally infected human whole blood samples against OVA)	Proliferation assay, antibody production and other immunochemical examinations	Exposure of virally infected human whole blood samples to flavonoid-rich plant extract reduced free haemoglobin content, exhibiting immunosuppressive effects against ovalbumin.	Immunosuppressive agent against OVA	[126]
Sulfonated pectin of <i>L. minor</i> .	In vitro	Anticoagulant properties of sulphated pectins using <i>L. minor</i>	The sulfation of <i>L. minor</i> leads to anticoagulant properties associated with the inhibition of fibrinogen and potentially other serpins.	Anti-coagulant pectin agent	[127]

5. Conclusions

The present study examines the bioactive properties of two freshwater aquatic plants, *N. nucifera* and *Lemna* spp., known for their antioxidant, anti-inflammatory and antithrombotic compounds. Both plants contain well-established but not fully elucidated bioactives, including flavonoids, alkaloids, phenolic acids and phytochemicals. These compounds exhibit significant antioxidant, anti-inflammatory and antithrombotic properties. They effectively neutralise free radicals, inhibit inflammation and enhance fibrinolytic activity, which may protect against cardiovascular diseases such as hypertension and atherosclerosis. These activities have been extensively studied in clinical trials, particularly in cosmetics, pharmaceuticals and functional foods.

It is interesting to note that *Lemna* spp. stands out due to its distinctive morphology and accelerated growth rate due to its growth sustainability in water treatment tanks, making it susceptible to heavy metal absorption. Consequently, it serves as an efficient water purifier. Overall, this study highlights the potential of *N. nucifera* and *Lemna* spp. as valuable natural resources with therapeutic properties, offering innovative approaches to combat inflammation, thrombosis and related chronic diseases. However, further research is needed to validate these findings through experiments and clinical trials, especially in the case of *Lemna* spp. due to its ability to absorb harmful substances found in its growth environment.

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