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Review

A Cosmetic Perspective on the Antioxidant Flavonoids from *Nymphaea lotus* L.

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Abstract: *Nymphaea lotus* L. or water lily is a well-known traditional medicinal plant in Thailand, Indonesia, Vietnam, India, Sri Lanka, China, Nepal, Egypt and many African countries. This species has been reported as a promising flavonoid-rich raw material that can be used as an active ingredient for the development of cosmetic/cosmeceutical products. This review aims to illustrate the cosmetic potential of this species by providing botanical information, traditional uses, flavonoid accumulation, biological activities and future research challenges in the production of *N. lotus* extracts for cosmetic applications.

Keywords: *Nymphaea lotus*; Bua Sai; flavonoids; traditional use; antioxidants; cosmetic applications



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1. Introduction

Admired by the common people as much as by artists such as Claude Monet, the aquatic plant, water lily, is accordingly called the queen flower. The species belonging to the genus *Nymphaea*, popular as ornamentals, is also a traditional medicinal and cosmetic plant. *Nymphaea lotus* L. is a perennial aquatic flowering plant, also known by its common names, water lily, lotus or Egyptian lotus [1–3] (Figure 1).

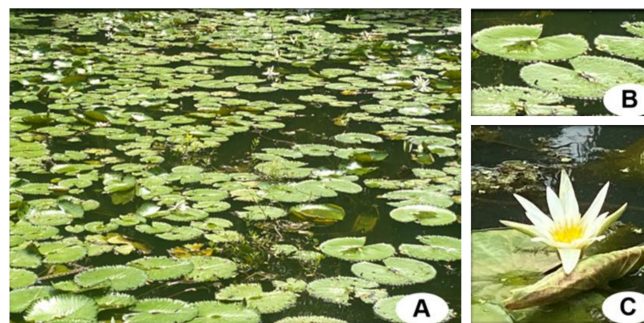


Figure 1. *Nymphaea lotus* L.: (A) Natural habitat; (B) leaves; (C) flower. Pictures from Dr. Duangjai Tungmunnithum on 27 August 2020 in Nakhon Phanom Province, Thailand.

N. lotus can be found throughout the Asian and African regions, regarded as a sacred plant, and has been introduced and naturalized beyond its native habitat [1,3–5]. Bau Sai (Thailand), Sulyeon (Korea), Nettarei (Japan), Bashneen Abiad (Egypt), etc., are some of the different vernacular names of this species across the world.

Almost every part of this plant, such as rhizome, stolon, petiole, young leaves, peduncle and flower, has long been eaten as fresh vegetables or as a cooking ingredient in a

variety of healthy menus [3,5]. In addition, a huge number of studies have reported on its medicinal potential to cure a number of diseases, and its extracts are used in traditional medicines in many countries in Asia and Africa [6–13]. Its cosmetic potential has also been reported [5]. Each of these biological activities has been related to its high flavonoid accumulation capacity [2,3,5].

The purpose of this work is to provide information on the botanical information, traditional uses, accumulation of flavonoids, antioxidant and other relevant potential biological activities and future research challenges in the production of *N. lotus* extracts for cosmetic applications.

2. Botanical Information

2.1. Synonyms

Castalia edulis Salisb.; *Castalia pubescens* Blume; *Castalia lotus* Tratt.; *Castalia mystica* Salisb.; *Castalia pubescens* Wood; *Castalia sacra* Salisb.; *Nymphaea dentata* Schumach. & Thonn.; *Nymphaea lotus* var. *rogeonii* A.Chev.; *Nymphaea liberiensis* A.Chev.

2.2. Species Description

Perennial Herb, Rhizome: erect with many slender stolons. Leaves: suborbicular or ovate-elliptic, 18–50 cm; margin dentate and teeth acute; abaxial dark purple pubescent; adaxial dark green, glabrous; base cordate. Flower: simple, emergent; petiole slender 2–7 m long; Outer perianth oblong, dark green, conspicuously veined, 5–7 cm long; Inner perianth oblong, white, red, or pink, 5–8 cm, Stamens: numerous, filament of inner stamens almost equal to anther in length, connective apically unappendaged. Pistil: 1, many carpels, carpel many and united, ovary half-inferior, parietal placentation. Fruit: ovoid, 3–5 cm. Seeds: ellipsoid, 1–2 mm, many longitudinal ridges [3,5,14].

2.3. Flowering Season

The flowering season ranges from early July to late November/early December according to our preliminary survey in the field during January 2018 to September 2020 (*unpublished results*). The flowering season of this aquatic medicinal plant also varies depending on the location of natural habitats. This may be due the nutrients in each natural ecosystem and/or other environmental influences.

2.4. Distribution

N. lotus is mainly distributed in Asia and Africa, especially in Thailand, Vietnam, Indonesia, India, Sri Lanka, Bangladesh, China, Nepal as well as Egypt [1,3–5,15]. In addition, this aquatic medicinal species is also distributed in some specific areas in Europe such as Romania [4]. *N. lotus* was also introduced and naturalized beyond its native habitat, due to the elegance of its flowers, as observed in America and other continents [1,4].

3. Traditional Uses

In Egypt, Thailand, Indonesia and a large number of Asian countries, almost every part of *N. lotus* has long been consumed as a vegetable [3,5,7–9,13,15–17]. As an important ingredient for formulating Asian traditional medicines, particularly for circulatory system syndrome, several parts of this plant, such as root, leaves and flower parts, have been used since ancient times [1,7,13].

With respect to cosmetic uses, *N. lotus* has been traditionally used by Egyptians as well as Asian people as skincare and perfume [1,3,5]. Nowadays, local people still use water or ethanolic extracts for homemade natural cosmetic products [1,3,5]. We may observe that much of the cosmetic potential of this plant is primarily based on its high accumulation of flavonoids, mostly in its flower parts [5,6,11]. In addition, many studies have confirmed its toxicological safety, which is a good argument for future applications [7,10,13,18].

4. Flavonoids from *N. lotus*

There is no denying today that consumers are highly keen on cosmetic products containing active ingredients of natural origin. Flavonoids are a major group of plant polyphenols, well known for their biological activities, relevant for both cosmeceutical and biomedical applications [19,20]. Seventy-four phenylpropanoids, most of them flavonoids, were previously described in the *Nymphaea* genus [8,17,21]. In terms of phytochemistry, *N. lotus* is the most intensively studied species of the *Nymphaea* genus, and all the various parts of this plant have been investigated for their accumulation of flavonoids [2,3,5–8,11–13,16,17,22]. Flowers, stamens in particular, form the richest source of *N. lotus* flavonoids and therefore constitute an attractive raw material for cosmetic applications [5,6,11,16,22]. For example, *N. lotus* stamen extracts may contain more than 475 mg/g dry weight of total flavonoids, making them one of the most valuable natural resources of flavonoids [5].

The major flavonoids derived from *N. lotus* flowers are the flavonol glycosides, isorhamnetin-7-*O*-galactoside, isorhamnetin-7-*O*-xyloside, isorhamnetin-3-*O*-xyloside, myricetin 3-*O*-galactoside, myricetin 3'-*O*-xyloside, quercetin-3-*O*-rhamnoside, quercetin-3'-*O*-xyloside and kaempferol-3-*O*-galactoside, as well as the chalcone glycoside chalcononaringenin-2''-*O*-galactoside (Figure 2) [6,11,16,22]. Recently, we demonstrated that stamens are an enriched source of these flavonoids, and we proposed a validated green ultrasound-assisted extraction method combined with macroporous resin purification to enhance the flavonoid content in stamen extracts [5]. This proposed method was more efficient than the traditional maceration strategy used to prepare *N. lotus* flower extract [5].

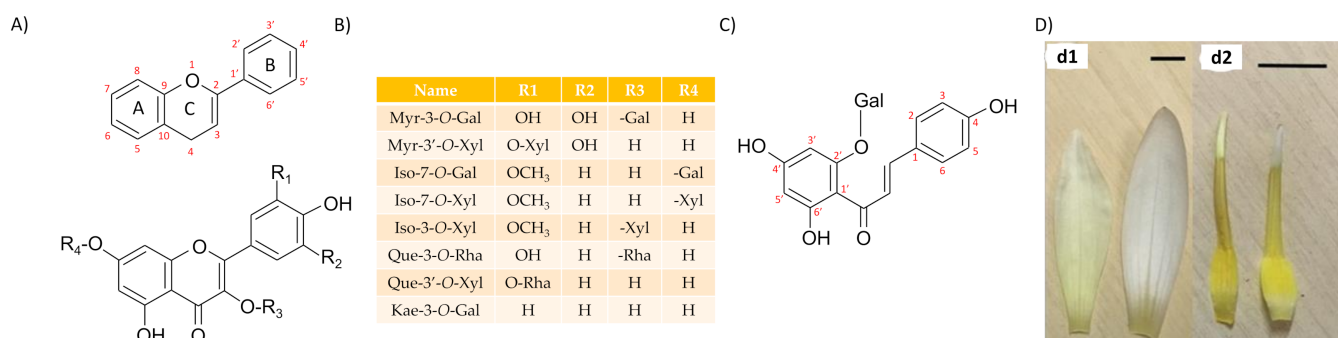


Figure 2. Chemical structures and names of the main antioxidant flavonoids from *N. lotus* stamen extracts. (A) General chemical structure with atom numbering of a flavonoid. (B) Chemical structures of the main flavonol glycosides extracted from *N. lotus* stamen extract. (C) Chemical structure of chalcononaringenin-2''-*O*-galactoside from *N. lotus* stamen extract. Myr: myricetin; Iso: isorhamnetin; Que: quercetin; Kae: kaempferol; Gal: galactoside; Xyl: xyloside; Rha: rhamnoside. (D) d1: *N. lotus* petals (Bar scale = 1 cm); d2: *N. lotus* stamens (Bar scale = 1 cm). Pictures by Duangjai Tungmunnithum.

Relevant to mention is also the presence of significant amounts of flavonoids in the *N. lotus* leaves [2,10,23]. Both *N. lotus* aqueous and acetone leaf samples were analyzed, while both flavanols and proanthocyanidins were significantly higher in the acetone leaf extracts than in the aqueous extracts [23]. The main flavonoids from *N. lotus* leaf extract are derivatives from myricetin such as myricetin 3-*O*-rhamnoside [2] (Figure 3). Two very unusual macrocyclic flavonoids named nympholide A and nympholide B, as well as their probable precursor myricetin-3'-*O*-(6'-*p*-coumaroyl) glucoside, were also isolated from *N. lotus* leaf extracts [2] (Figure 3). This indicates that this plant is not only valuable from a quantitative point of view for its accumulation of flavonoids, but also as a source of very uncommon derivative flavonoids capable of introducing innovation to cosmetics.

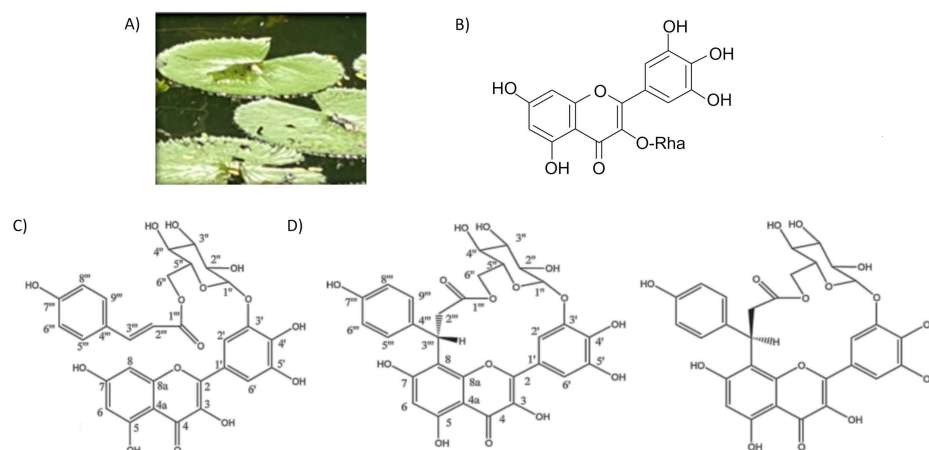


Figure 3. Chemical structures and names of the main antioxidant flavonoids from *N. lotus* leaf extracts. (A) *N. lotus* L. leaf morphology. (B) Chemical structure of myricetin-3-O-rhamnoside. (C) Chemical structure of myricetin-3'-O-(6'-p-coumaroyl) glucoside. (D) Chemical structure of the two macrocyclic flavonoids nympholide A and nympholide B. Picture by Duangjai Tungmunnithum.

5. Antioxidant and Other Biological Activities for Cosmetic Applications

A careful review of the literature data revealed that most of the publications on *N. lotus* extracts were primarily concerned with its medicinal potential to cure many diseases [6–8,10–13]. However, several publications have specifically shown the high cosmetic and/or cosmeceutical potential of water lily. The most relevant activities for cosmetic applications are related in this paragraph.

5.1. Antioxidant Activity

As natural antioxidant molecules, flavonoids play a vital function in the natural defense mechanism to detoxify free radicals or reactive oxygen products (e.g., hydroxyl radicals, superoxide or singlet oxygen), which are extremely reactive agents that pass across the human/animal body and cause detrimental effects on cells [19,20,24,25]. The antioxidant potential of *N. lotus* extracts has been intensively investigated, revealing their important role as free radical scavengers to prevent and reduce the damage caused by reactive oxygen species.

5.1.1. In Vitro Evaluation of the Antioxidant Activity

An understanding of the chemistry and the reaction mechanisms behind the antioxidant behavior of a plant extract can be provided by in vitro cell-free chemical-based assays. The in vitro cell-free antioxidant assays can be essentially categorized into different subgroups based on the mechanism of chemical reaction involved, with ABTS (2,2-azinobis (3-ethylbenzthiazoline-6-sulphonic acid)) based on a hydrogen atom transfer reaction (HAT), FRAP (ferric reducing antioxidant power) based on an electron transfer reaction (ET), while DPPH (1,1-diphenyl-2-picryl-hydrazyl) can be considered a mixed assay [5,26–28]. The capacity to scavenge cellular radicals such as hydrogen peroxide or nitrite oxide radical can also be evaluated [23].

In this context, extracts from different parts of *N. lotus* were evaluated for their in vitro antioxidant activity (Table 1). In line with its high accumulation of flavonoids, the type of extract that has gained the most attention is the flower extract [5,16,29]. DPPH, ABTS and FRAP assays were used to investigate the in vitro antioxidant activity of *N. lotus* flower hot water extracts [16]. The antioxidant activities that resulted were similar to the synthetic antioxidant BHT (butylated hydroxytoluene) [16]. In line with these results, stamen ethanolic extracts displayed a similar antioxidant capacity as BHT, as determined by DPPH, ABTS and FRAP assays [5], and petal ethanolic extracts demonstrated a similar antioxidant capacity as ascorbic acid, determined using DPPH assay [29]. A strong correlation between this antioxidant activity and the flavonoid content was observed [5,16,29]. From a

mechanistic point of view, this antioxidant activity, correlated with the various flavonoid components found in these *N. lotus* extracts, was particularly linked with the capacity of electron donation, which has been previously associated with the degree and position of hydroxylation and methoxylation of the flavonoid ring B [30].

The in vitro antioxidant activities of *N. lotus* aqueous and acetone leaf extracts were examined by the DPPH, ABTS and FRAP assays as well as by hydrogen peroxide and nitric oxide radical scavenging activity [23]. The presence of flavonoids was evidenced in both aqueous and acetone extracts. DPPH and nitric oxide radical scavenging activity were highest in acetone extract, while ABTS radical cation scavenging capacity was higher in aqueous extract. However, the hydrogen peroxide scavenging activity was not different between the two extracts [23].

N. lotus seed extracts obtained after n-hexane extraction revealed the presence of natural antioxidants, with DPPH and FRAP radical scavenging activities comparable to those of ascorbic acid and rutin [31]. These results may be of special interest to boost the oxidative stability of emulsions largely used in cosmetic formulations to replace potentially dangerous synthetic antioxidants such as BHT [27,28].

Finally, a particularly significant result showing the high potential of species member from the genus *Nymphaea* in cosmetics was obtained with extract from rhizomes. In order to search for new active cosmetic ingredients of natural origin, the authors of the study examined approximately 60 plants collected from Jeju Island (Korea) [32]. Rhizome extracts of water lily displayed the highest free radical scavenging activity among the 60 plants screened [32].

Table 1. Overview of the antioxidant activity of *N. lotus* extracts.

Extract	Type	Remark	Reference
IN VITRO ASSAYS			
Flowers	DPPH, ABTS, FRAP	antioxidant capacity similar to BHT	[16]
Stamens	DPPH, ABTS, FRAP	antioxidant capacity at least similar to BHT	[5]
Petals	DPPH	antioxidant capacity similar to vitamin C	[29]
Rhizomes	DPPH	showed the highest free radical scavenging activity among 60 plants screened	[32]
Leaves	DPPH, ABTS, FRAP, NO radical and H ₂ O ₂ scavenging	antioxidant capacity at least similar to vitamin C and rutin	[23]
Seeds	DPPH, FRAP	antioxidant capacity similar to vitamin C	[31]
CELLULAR ASSAYS			
Petals	Red blood cells	protection against oxidative stress-induced hemolysis	[29]
Stamens	Yeast cells	inhibition of UV-induced oxidative stress	[5]
Whole plants	B16 melanoma cells	cellular antioxidant action	[33]
ANIMAL STUDIES			
Flowers	Albino male rats	inhibition of oxidative stress markers	[6]
Whole plants	Wistar male rats	inhibition of carbon tetrachloride-induced oxidative stress	[34]

DPPH: 2,2-diphenyl-1-picrylhydrazyl; ABTS: 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid); FRAP: Ferric Reducing Antioxidant Power Assay; BHT: Butylated hydroxytoluene.

5.1.2. Cellular Antioxidant Activity and Animal Model Studies

From a purely predictive point of view, in vitro cell-free assays are interesting on the basis of chemical reactions but do not always depict in vivo conditions. The relevance of these in vitro assays should be limited to the chemical reactivity toward the considered radicals, but in vivo validation is imperative. Various cellular and/or animal models can be considered for this purpose.

The strong antioxidant potential of water lily extracts has been confirmed across various cellular models [5,29,33]. Whole plant ethanolic extracts of water lily have been

reported as a chemopreventive agent in the modulation of cellular oxidative stress-induced apoptosis in B16 melanoma cells, acting by a complex oxidant vs. antioxidant action depending on the concentration applied [33]. Both aqueous and ethanolic extracts of *N. lotus* petals significantly decreased AAPH (2,2'-Azobis(2-amidinopropane) dihydrochloride)-induced hemolysis, revealing their protective action on cell membranes against free radicals [29]. This protective activity on cell membranes was also observed with flavonoid-enriched extracts from *N. lotus* stamen, which significantly reduced the production of reactive oxygen and nitrogen species as well as lipid membrane peroxidation following UV-induced oxidative stress in yeast cells [5]. Interestingly, this action implied the activation of several genes, such as the mitochondrial *SOD2* (*superoxide dismutase 2*) gene, involved in the antioxidant reaction [5].

In animal models, inhibition of oxidative stress markers by flower aqueous extract in albino male rats [6], as well as inhibition of carbon tetrachloride-induced oxidative stress by whole plant methanolic extract in Wistar male rats [34], was reported.

Table 1 summarizes the results of the antioxidant activity of different water lily extracts obtained with various in vitro assays as well as different cellular and animal models.

5.2. Other Relevant Biological Activities for Cosmetic Applications

The anti-aging action of water lily extracts has been proposed [5,32]. In human fibroblast cells, water lily extract from rhizomes showed a significant inhibition of the activity of the elastase enzyme and the expression of the *MMP-1* (*matrix metalloproteinase-1*) gene [32], both involved in the breakdown of the extracellular matrix and associated with skin aging [35,36]. In yeast, stamen extracts significantly activated *SIR2* (*Silent Information Regulator 2*, ortholog of the *sirtuin-1* human gene) gene expression, which maintains cell longevity, and has been reported to be crucial in the control of oxidative stress and in the regulation of the aging process [37].

Other water lily species also revealed biological activities relevant for cosmetic applications, and these could be important to explore. Ethanolic flower extract of *N. alba* showed highly significant dose-independent anti-inflammatory activity compared with the standard drug diclofenac sodium [38]. Ethanolic extracts of *N. nouchali* and *N. stellata* flowers exhibited antimicrobial activity [39] that could be valuable for the development of natural preservatives. Phytosomes (i.e., lipid-based vesicular delivery systems) were formulated from methanolic extracts of *N. nouchali* [40] and can be used for the encapsulation of plant-derived cosmeceuticals such as polyphenolic compounds.

6. Future Research Challenges

It is evident from this literature survey that *N. lotus* and its flavonoids have tremendous potential for many cosmetic applications, but there are still many remaining challenges in rationalizing its traditional uses in cosmetics for future applications:

To seek specific *N. lotus* populations in each country or area that contains either the highest amount or an original accumulation of flavonoids. This would help to minimize the costs of supplying raw plant material to the cosmetic/cosmeceutical industries and to obtain the bioactive molecules from the best local populations of *N. lotus*.

To investigate both flavonoid profiles and biological activities of rhizome, leaves, perianth and stamen in a single comparative study, in order to be able to choose the best part of *N. lotus* for cosmetic/cosmeceutical product development for the industrial sector.

To investigate in detail the new pharmacological and biological activities of this aquatic medicinal plant, such as anti-inflammatory, anti-aging, anti-wrinkle, anti-melasma properties. A small number of previous studies have shown a possible interest in these biological activities related to cosmetic use for *Nymphaea* species.

To develop innovative green chemistry to enhance the quality and quantity of flavonoid enriched extracts from *N. lotus*. There is no denying that today, consumers pay more attention to cosmetic and cosmeceutical products that are environmentally friendly.

To authenticate the raw plant material and ensure that it is *N. lotus* before proceeding further in the next step of research experiments. According to our literature search and intense review, some publications aimed to work on *N. lotus*, but the plants which were used in these studies were other species in the same genus or another lotus plant species (*Nelumbo nucifera* Gaertn.) that shared the same common name “lotus”. Note that in herbal medicines, *N. lotus* (Bau Sai) stamens are sometimes used to adulterate *N. nucifera* (Bau Luang) stamens. The price of *N. lotus* stamens is, indeed, much lower and their supply is much higher than that of *N. nucifera*. This makes stamens of *N. lotus* an attractive starting material for industrial cosmetic applications which could contribute to the development of a new market, thus restricting these adulteration issues.

7. Conclusions

N. lotus is widely distributed and it is used as an essential ingredient for traditional medicines in many countries. Almost all parts of this plant have long been consumed as food and used as traditional cosmetics. It is evident from this literature survey that *N. lotus* has tremendous potential for many cosmetic applications. *N. lotus* is one of the most valuable natural resources of bioactive flavonoids, which is obviously related to the cosmetic potential of this plant. *N. lotus* is abundant, widely distributed, easy to grow and the fact that it can be cultivated makes it a perfect raw material for industrial applications. Further research on the cosmetic value of this medicinal plant will need to validate its potency and explore its various biological activities, in order to scientifically rationalize its traditional usages and to promote its future use in the cosmetics industry. However, it is evident from this literature survey that *N. lotus* and its flavonoids have tremendous potential for many future cosmetic applications.

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