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Review

The sacred lotus (*Nelumbo nucifera*) – phytochemical and therapeutic profile

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Abstract

Objectives *Nelumbo nucifera* Gaertn. (Nymphaeaceae), also known as sacred lotus, is a well known medicinal plant. This article reviews the traditional uses, phytochemistry and therapeutic reports on different parts of *N. nucifera* viz. the seeds, rhizomes, leaves and flowers. This review also describes various compounds isolated from different parts of this plant and the therapeutic benefits derived from those phytoconstituents.

Key findings There are several therapeutic benefits of this plant for which different parts are used. The extracts of rhizomes, seeds, flowers and leaves have been reported to have varied therapeutic potential. Several bioactive compounds have been derived from these plant parts belonging to different chemical groups, including alkaloids, flavonoids, glycosides, triterpenoid, vitamins etc., which all have their own therapeutic impact. Thus, the pharmacological effects and various active ingredients of different parts of *N. nucifera* are well understood.

Summary In this review we explore the current pharmaceutical, phytochemical and pharmacological knowledge about this well known plant species as well as several emerging aspects for research on *N. nucifera.*

Keywords *N. nucifera*; pharmacological; phytochemical; therapeutic

Introduction

Nelumbo nucifera, now placed in the mono-generic family Nymphaeaceae, has numerous common names (e.g. Indian lotus, Chinese water lily and sacred lotus) and synonyms (*Nelumbium nelumbo, N. speciosa*, *N. speciosum* and *Nymphaea nelumbo*).[1] All parts of *N. nucifera* have many medicinal uses. The leaf, rhizome, seed and flower are traditionally used for the treatment of pharyngopathy, pectoralgia, spermatorrhoea, leucoderma, small pox, dysentery, cough, haematemesis, epistaxis, haemoptysis, haematuria, metrorrhagia, hyperlipidaemia, fever, cholera, hepatopathy and hyperdipsia. In Ayurveda this plant is also used as a diuretic and anthelmintic and in the treatment of strangury, vomiting, leprosy, skin diseases and nervous exhaustion.[1–3] In popular medicine it is used in the treatment of tissue inflammation, cancer, skin diseases, leprosy and as a poison antidote.[4,5] Several pharmacologically active constituents that are responsible for the medicinal values have been isolated from the leaf, rhizome, seed and flower. Different classes of phytoconstituents has been isolated from various parts of *N. nucifera.* The most important classes include alkaloids, steroids, triterpenoids, flavonoids, glycosides and polyphenols.[6–12] Studies on different parts of *N. nucifera* have shown a variety of pharmacological activities. Extracts of different parts have shown anti-ischaemia,[13] antioxidant,[14–19] anticancer,[5,11] antiviral,[20,21] anti-obesity,[22] lipolytic,[23] hypocholesterolaemic,[24] antipyretic,[25] hepatoprotective,[26] hypoglycaemic, antidiarrhoeal, antifungal, antibacterial, anti-inflammatory and diuretic activities.[8,27–34]

This article reviews the traditional uses, phytochemistry and therapeutic reports on different part of *N. nucifera* viz. the seeds, rhizomes, leaves and flowers. This review will also describe various compounds isolated from different parts of the plant and the therapeutic benefits derived from those phytopharmaceuticals.

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History of medical and other uses

Since ancient times, lotus was common along the banks of the river Nile, together with the closely related species 'sacred blue lotus' (*Nymphaea caerulea*). The Pharoic Egyptians worshipped the lotus flowers, fruits and sepals, which were widely depicted as architectural motifs. From Egypt, it was carried to Assyria and widely planted throughout Persia, India and China. It was first brought into horticulture in Western Europe during 1787 as a stove-house water lily under the patronage of Sir Joseph Banks. Nowadays it can be seen almost everywhere in modern botanical garden collections. It is a common plant in Australia, China, India, Iran and Japan. It was introduced from China into Japan and has been cultivated for more than 1000 years. In China, it is an industrial crop grown on over 40 000 hectares. In India, it is widespread and has even be found in Himalayan lakes at altitudes of up to 1400 m.[3]

Traditionally all parts of *N. nucifera* have various medicinal uses. Rhizomes are prescribed as demulcents for haemorrhoids and are beneficial in dysentery, chronic dyspepsia, and have nutritive, diuretic and cholagogue activities.[35,36] The stem is used in indigenous Ayurvedic medicine as a diuretic and anthelmintic and to treat strangury, vomiting, leprosy, skin disease and nervous exhaustion. The leaves are used for the treatment of haematemesis, epistaxis, haemoptysis, haematuria, metrorrhagia and hyperlipidaemia.[24] The flowers are useful in the treatment of diarrhoea, cholera, fever and gastric ulcers.[4]

Botany and phytogeography

Although historically the genus *Nelumbo* was considered to be closely related to Nymphaeales, new systematic work has allied *Nelumbo* with the lower eudicots, particularly *Platanus*.[37]

Worldwide, there are only two species of *Nelumbo: N. lutea* Willd. (synonyms: *N. pentapetala* (Walter) Fernald and *Nelumbium luteum* Willd.) and *N. nucifera* (synonyms: *N. speciosa* Willd, *Nelumbium speciosum* Willd, *Nelumbium N. Druce* and *Nymphaea N. L*).[32,38] *N. nucifera* Gaertn., the Indian or sacred lotus, is found throughout Asia and Australia, whereas *N. lutea,* the American lotus or water chinquapin, occurs in eastern and southern North America.[39] *N. lutea* is considered to be a subspecies of *N. nucifera*.[40] In India, *N. nucifera*, commonly known as lotus, kamala or padma, is an aquatic species, requiring plenty of space and full sun in order to thrive. It has stout, creeping, yellow rhizomes and green fruits. The leaves are enormous, reaching 2 feet in diameter. There are two varieties of 'kamala': one has white flowers and is commonly called 'pundarika' or 'sveta kamala'; the other has pink or reddish-pink flowers and is called 'rakta kamala'.[41] The whole plant with flowers is known as 'padmini', the rhizomes as 'kamalkand', the tender leaves as 'sambartika', the peduncle as 'mrinal' or 'visa', the stamens as 'kirijalaka', the torus as 'padmakosa', the seed as 'karnika' or 'padmaksya', and the honey formed in the flowers by the bees feeding upon padma is known as 'makaranda' or 'padma-Madhu'.[42] The plant is often cultivated for its elegant sweet-scented flowers, which are the national flower of India. Almost all parts of lotus are eaten as a vegetable, consumed all over the world, especially in South-east Asia, Russia and some countries in Africa. It is used not only as an ornamental plant and dietary staple, but also as a medicinal herb in Eastern Asia, particularly in China. *N. nucifera* has been cultivated as a crop in Far-East Asia for more than 3000 years, where it was used for food and medicine and played a significant role in religious and cultural activities.[43] Almost all parts of *N. nucifera* are marketed; the rhizome holds the largest share.

Fruit and seeds

The fruit of this plant is an aggregate of indehiscent nutlets. Ripe nutlets are ovoid, roundish or oblongish, up to 1.0 m long and 1.5 cm broad, with a hard, smooth, brownish or greyish black pericarp which is faintly longitudinally striated, pedunculated and single seeded. Seeds fill in the ripe carpel. The seeds are sold as a vegetable in Indian markets, under the name of 'kamal gatta'.[42] The fruits show remarkable dormancy; indeed the longevity of its seeds exceeds that of any known species of flowering plants. Robert Brown, first keeper of Botany in the British Museum, experimented with fruits of *Nelumbo* at various times between 1843 and 1845 and showed that they retained the power of germination after 150 years of confinement in a glass-top box.[44,45]

Traditional uses

The seeds and fruits are used as a health food in Asia and to treat many ailments, including poor digestion, enteritis, chronic diarrhoea, insomnia, palpitations, spermatorrhoea, leucorrhoea, dermatopathy, halitosis, menorrhagia, leprosy, tissue inflammation, cancer, fever and heart complaints, and as an antiemetic, poisoning antidote, diuretic and refrigerant.[4,32,42,46] Lotus seedpods are sometimes used as a traditional medicine for haemostatic function.[47] The seed powder mixed with honey is useful in treating cough.[2] Embryos of lotus seed are used in traditional Chinese medicine to overcome nervous disorders, insomnia, high fevers (with restlessness) and cardiovascular diseases (e.g. hypertension, arrhythmia).[12]

Phytochemistry

The seeds of *N. nucifera* are rich in asparagin, fat, protein, starch and tannin.[48] The lotus seed is composed of three parts – integuments, plumule and cotyledons, which comprise 3.74%, 3.03% and 93.23% of the mass, respectively. The average weight of 100 seeds is 87.35 g. A large amount of glutathione is contained in the plumule (l3 g per plumule) and cotyledons (164 g per cotyledon) of *N. nucifera*; the amount of total plumule increases gradually in the maturing seed. The reduced form of glutathione is dominant in the early stages, while the amount of oxidised form exceeds that of the reduced form at the end of maturation. The amount of the reduced form of glutathione in the unripe fruit decreases markedly upon storage for l year. In general, the rate of germination of the stored seeds seems to be closely related to the content of reduced glutathione.[32,48]

Normally, lotus seeds are rich in protein, amino acids, unsaturated fatty acids and minerals.[49] *Nelumbo* seeds have also been found to contain a variety of minerals such as chromium (0.0042%), sodium (1.00%), potassium (28.5%),

calcium (22.10%), magnesium (9.20%), copper (0.0463%), zinc (0.0840%), manganese (0.356%) and iron (0.1990%). Other relevant nutritional elements include total ash (4.50%), moisture (10.50%), crude carbohydrate (1.93%), crude fibre (10.60%), fat (72.17%), and protein (2.70%); its energy value is 348.45 cal per 100 g.[50]

The major secondary metabolites present in the seeds (Figure 1) are alkaloids such as dauricine (**1**), lotusine (**2**), nuci-ferine (**3**), pronuciferine (**4**), liensinine (**5**), isoliensinine (**6**), roemerine (**7**), neferine (**8**) and armepavine (**9**).[6,7,9–12,51,52] Procyanidin (**10**) was isolated form the seedpod of *N. nucifera.*[47] Seeds also contain gallic acid (**11**), D(–)-3′-bromo-O-methyl-armepavine (**12**), D–1,2,3,4-tetrahydro-6-methoxy-1-(*p*-methoxy benzly)-2-methyl-7-isoquinolinol (**13**), saponins and carbohydrates.[19] The seed polysaccharides have also been isolated and characterised. Acid hydrolysis and methylation showed that seed polysaccharides are mainly composed of four types of monosaccharide: D-galactose, L-arabinose, D-mannose and D-glucose.[53] ^{13}C-NMR and in-source pyrolysis–mass spectrometry analysis showed that the fruit wall and seed coat of *N. nucifera* are composed of a complex of polysaccharides, based primarily on galactose and mannose units and insoluble tannins. Curie-point pyrolysis–gas chromatography–mass spectrometry analysis of the fruit wall and seed coat of *Nelumbo* produced some pyrolysis polysaccharide products, including 2-furaldehyde, 2-hydroxymethylfuran, (SH)-furan-2-one, 2,3-dihydro-5-methylfuran-2-one, 2-hydroxy-3-methyl-2-cyclopenten-l-one, 5-hydroxymethyl-2-furaldehyde, anhydrosugar (levogalactosan), anhydrosugar (levomannosan), 1,2-benzenediol, 4-methyl-1,2-benzenediol, 1,6-anhydro-α-D-glucopyranose, 2,6-dimethoxy-4-ethenylphenol and 4-carboxy-2-methoxyphenol.[54]

Pharmacology and toxicology

Anti-ischaemic activity

The seed of *N. nucifera* shows potent anti-ischaemic effects in the isolated rat heart. The effective amount of seed extract against ischaemia induced in the isolated rat heart was assessed by measuring cardiac output; doses of 0.1–30 mg/ml were tested. Maximal recovery was seen at a dose of 10 mg/ml, although cardiac output was similar after treatment with 3 or 10 mg/ml doses (63.5 ± 3.2 and 65.8 ± 4.0 ml/min, respectively). Thus, the 3 mg/ml dose was determined to be the optimum dose for anti-ischaemic effects in the rat.[13]

Antioxidant activity

The ethanol extract of the seed has been evaluated for its antioxidant activity using the 2,2-diphenyl-1picrylhydrazyl (DPPH) free radical assay. Potent free radical scavenging effects were seen, with a median inhibition concentration (IC50) of 6.49 μg/ml.[17] Furthermore, the antioxidant activity of the hydroalcoholic extract of seed has been reported by Rai *et al.*[19] using the DPPH and nitric oxide methods. The hydroalcoholic extract exhibited strong free radical scavenging activity, with IC50 values of 6.12 ± 0.41 μg/ml in the DPPH assay and 84.86 ± 3.56 μg/ml in the nitric oxide assay. These values were lower than those of rutin, a standard free radical scavenger. Administration of the hydroalcoholic extract of seed to Wistar rats at 100 and 200 mg/kg for 4 days before carbon tetrachloride treatment caused significant dose-dependent increases in the levels of superoxide dismutase (SOD) and catalase, and a significant decrease in the level of thiobarbituric acid reactive substances. These changes observed at 100 mg/kg were comparable to those observed with vitamin E at 50 mg/kg.[19]

Procyanidin and condensed tannin isolated from the seed pod of *N. nucifera* have several pharmacological activities, including lipid auto-oxidation, lipoxygenase inhibition and free radical scavenging comparable to butylated hydroxytoluene (0.1%). At a concentration of 62.5 μg/ml, procyanidin inhibited lipoxygenase activity by more than 90%, with an IC50 value of 21.6 μg/ml.[47]

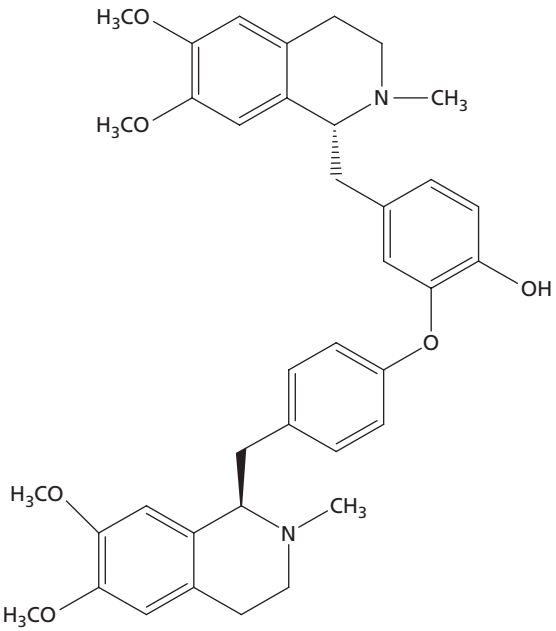
Hepatoprotective activity

An ethanol extract of the seed was studied for hepatoprotective effects in carbon tetrachloride and aflatoxin B1-induced hepatotoxicity models. Cell death caused by carbon tetrachloride was significantly inhibited in a dose-dependent manner by the ethanolic extract at concentrations between 10 and 500 μg/ml. The same extract reduced the genotoxicity of aflatoxin B1, showing complete inhibition at a concentration of 250 μg per plate.[17]

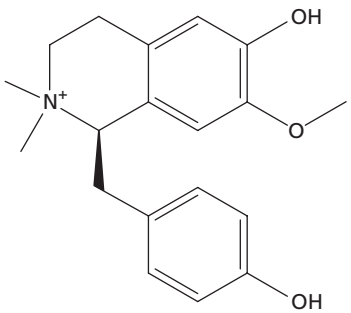
Antiproliferative activity

The ethanolic extract of *N. nucifera* seed suppressed cell cycle progression, cytokine gene expression and cell proliferation in human peripheral blood mononuclear cells (PBMC). To study the effects on PBMC proliferation, resting cells or cells activated with phytohaemaglutinin (PHA) were treated with 100 μg/ml of an ethanolic extract of *N. nucifera* seed.[5] Cell proliferation was determined on the basis of uptake of tritiated thymidine. PBMC proliferation was not affected by DMSO treatment. Ciclosporin blocked PHA-activated PBMC proliferation. Ethanolic extract of *N. nucifera* seed (100 μg/ml) significantly suppressed PBMC proliferation stimulated with PHA. The ethanol extract of *N. nucifera* suppressed proliferation in PHA-activated PBMC. The stimulated cell cycle progression in PHA-activated PBMC was significantly arrested at G_0/G_1 stage, and gene expression and production of interleukin(IL)-2, IL-4, IL-10, interferon gamma (IFN-γ) and cyclin-dependent kinase 4 in activated PBMC were also decreased by *N. nucifera* extract.[5] Liu and co-workers have isolated (S)-armepavine ($C_{19}H_{23}O_3N$; molecular weight 313) from *N. nucifera* seed extract. (S)-Armepavine inhibited the proliferation of human PBMCs activated with PHA and gene expression of IL-2 and IFN-γ without direct cytotoxicity, which leads to the improvement of autoimmune diseases in MRL/MpJ-lpr/lpr mice.[11] The mechanism involved in these inhibitions is blockade of membrane-proximal effectors such as IL-2-inducible T cell kinase and phospholipase C γ in a phosphatidylinositol 3-kinase-dependent manner.[55]

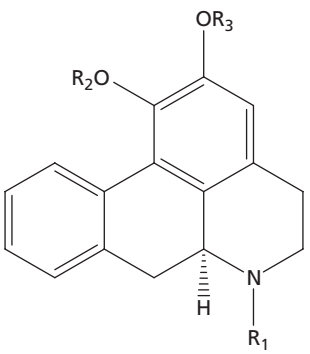
An isoliensinine alkaloid isolated from the seed embryo had inhibitory effects on the proliferation of porcine coronary arterial smooth muscle cells induced by angiotensin II. Its mechanisms were investigated by counting cultured cell number, the MTT assay, immunohistochemistry and Western blotting. Angiotensin II (0.1 μM) significantly evoked cell proliferation by 42%, which could be dose-dependently inhibited by 0.01–3 μM isoliensinine; the



Dauricine (**1**)



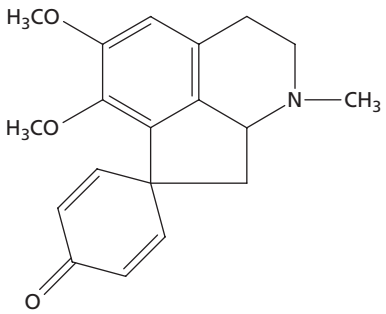
Lotusine (**2**)



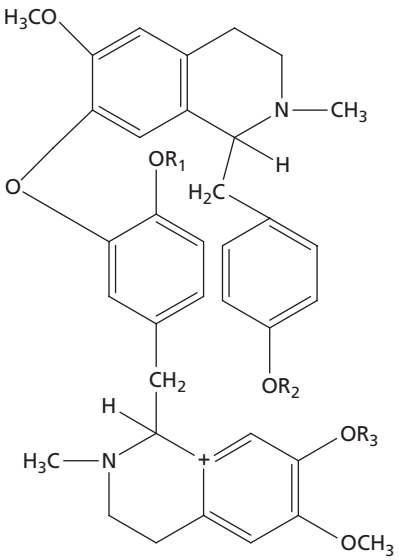
Nuciferine (**3**) – $R_1 = R_2 = R_3 = CH_3$

N-Nornuciferine (**15**) – $R_1 = R_2 = CH_3$; $R_3 = H$

O-Nornuciferine (**24**) – $R_1 = H$; $R_2 = R_3 = CH_3$



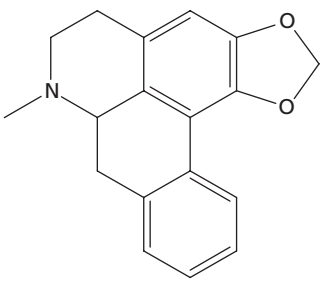
Pronuciferine (**4**)



Liensinine (**5**): $R_1 = R_2 = H$; $R_3 = CH_3$

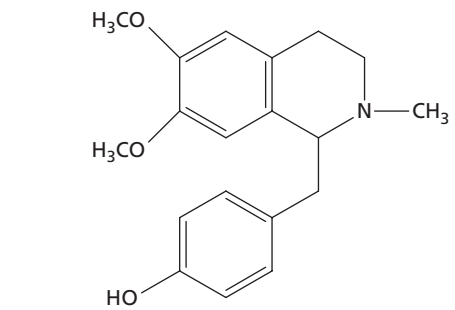
Isoliensinine (**6**): $R_1 = R_3 = H$; $R_2 = CH_3$

Neferine (**8**): $R_1 = H$; $R_2 = R_3 = CH_3$

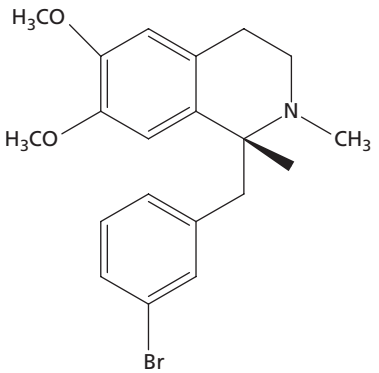


Roemerine (**7**)

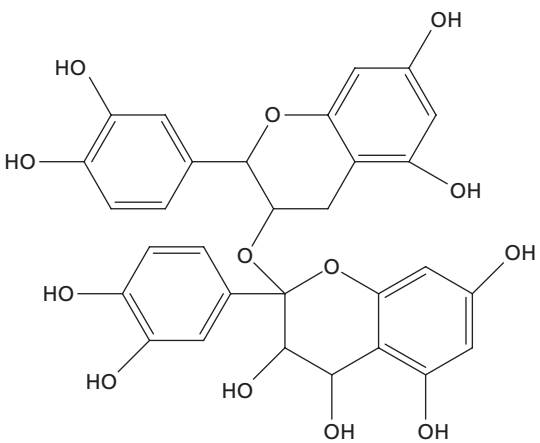
Figure 1 *(Continued)*



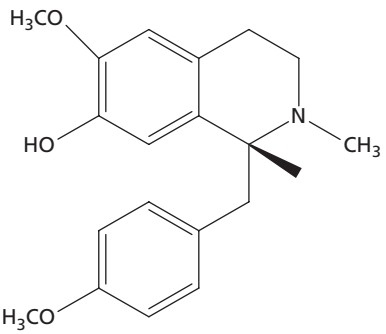
Armepavine (**9**)



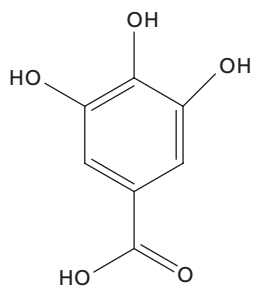
D(–)-3′-bromo-O-methyl-armepavine (**12**)



Procyanidin (**10**)



D-1,2,3,4-tetrahydro-6-methoxy-1-(*p*-methoxybenzly)-2-methyl-7-isoquinolinol (**13**)



Gallic acid (**11**)

Figure 1 Major secondary metabolites present in the seeds of *Nelumbo nucifera.*

percentage of inhibition of isoliensinine was 25% at 0.01 μM. These results suggest that isoliensinine possesses antiproliferative effect, which is related to a decrease in the over-expression of platelet-derived growth factor, basic fibroblast growth factor and proto-oncogenes c-fos, c-myc and hsp70.[56]

The effect of neferine on platelet aggregation, thromboxane A_2/prostaglandin (PG) I_2 and cAMP/cGMP balance were studied using turbidimetry and radioimmunoassay. It significantly inhibits rabbit platelet aggregation induced by ADP, collagen, arachidonic acid and platelet-activating factor with IC50 values of 16, 22, 193 and 103 μM, respectively. Neferine was found to increase vascular 6-keto-$PGF_{1\alpha}$ and platelet cAMP levels in a dose-dependent manner, but inhibited arachidonic acid-stimulated thromboxane A_2 release from platelets.[57]

Anti-inflammatory activity

At at a dose of 10 mg/kg, the seed extract of *N. nucifera* inhibited the production of pro-inflammatory cytokine tumour necrosis factor-α (TNF-α) and increased anti-inflammatory cytokine IL-10 in BALB/c mice with systemic inflammation induced by an intraperitoneal injection of lipopolysaccharide (LPS).[58] Studies in LPS-challenged mice showed that a high dose (20 mg/day) of seed extract significantly decreased TNF-α levels in the serum and significantly increased the levels of IL-10 produced by peritoneal macrophages. This result demonstrated that

administration of the seed extract before systemic inflammation attenuates acute inflammation *in vivo*.[58]

Anti-fertility activity

The petroleum ether extract of the seed has been reported to possess anti-fertility activity in female albino mice – at a dose of 3 mg/kg. It blocked the oestrus cycle at the metoestrus stage compared with ethyl oleate (0.1 ml/20 g). The extract significantly reduced uterine weight and affected the oestrus cycle by blocking biogenesis of ovarian steroids at an intermediate stage.[59]

Anti-arrhythmic activity

Neferine, an alkaloid isolated from the seed embryo of *N. nucifera*, has been reported to have anti-arrhythmic effects on rabbit sinoatrial nodes and clusters of cultured cardiac myocytes from neonatal rats.[60,61] Neferine inhibits the slow transmembrane Na^+ and/or Ca^{2+} current of the myocardium, which leads to its anti-arrhythmic action.[60,61] Neferine causes non-specific inhibition of the Na^+, Ca^{2+} and K^+ cardiac transmembrane currents in guinea-pig papillary muscles and atria, which relates to its anti-arrhythmic activity.[9] Experiments in anaesthetised cats showed that intravenous neferine at concentrations of 1–10 mg/kg dose-dependently decreased the amplitude and prolonged the duration of the monophasic action potential, decreased left ventricular pressure and prolonged the sinus cycle length. These effects demonstrated that neferine has similar electromechanical properties in the heart as quinidine.[62]

Liensinine is another alkaloid isolated from the seed of the lotus which has been reported to have anti-arrhythmic effect; its mechanism may be related to blockade of Ca^{2+} and Na^+ influx. Intravenous liensinine (3 mg/kg) temporarily inhibited all parameters of haemodynamics in anaesthetised and pithed rats. Its effects were slightly stronger than those of quinidine (3 mg/kg); the inhibitory effects of liensinine (12 mg/kg) on all haemodynamic parameters were comparable to those of verapamil (1 mg/kg). The haemodynamic effects of liensinine may be similar to verapamil but different from quinidine.[63] Liensinine at 10–100 μM was shown to concentration-dependently decrease the amplitude of the action potential. The effects of liensinine on slow action potentials and slow inward currents have also have been studied and suggest that liensinine possesses calcium antagonistic effects.[64]

Anti-fibrosis activity

The inhibitory effect of isoliensinine isolated from the seeds was studied on bleomycin-induced pulmonary fibrosis in mice.[65] Administration of isoliensinine remarkably suppressed the increase in hydroxyproline content and abated the lung tissue injury induced by bleomycin. It enhanced SOD activity and decreased the malondialdehyde level in a concentration-dependent manner. Moreover, isoliensinine significantly inhibited the over-expression of TNF-α and transforming growth factor-β (TGF-β) induced by bleomycin. These results indicated that isoliensinine possesses significant inhibitory activity against bleomycin-induced pulmonary fibrosis, probably due to its antioxidant and/or anti-inflammatory activities and inhibitory effect on TNF-α and TGF-β induced by bleomycin.[65]

Antiviral activity

Ethanol extract of the seed (100 μg/ml) significantly suppressed replication of herpes simplex virus-1 (HSV-1), with an IC50 of 50 μg/ml. Furthermore, a sub-fraction of *N. nucifera* (NNFR) has an inhibitory effect on HSV-1. NNFR at a concentration of 50 μg/ml inhibited HSV-1 replication in HeLa cells by up to 85.9%, attenuating aciclovir-resistant HSV-1 propagation.[20] In a bioassay-guided fractionation, NNFR significantly blocked HSV-1 multiplication in HeLa cells without apparent cytotoxicity. The production and mRNA transcription of infected cell protein was found to be decreased in NNFR-treated HeLa cells. The antiviral actions of NNFR is therefore likely to be mediated through inhibition of immediate early transcripts, such as infected cell protein (ICP) 0 and ICP4 mRNA and then blocking the downstream accumulation of all viral products.[20]

Leaves

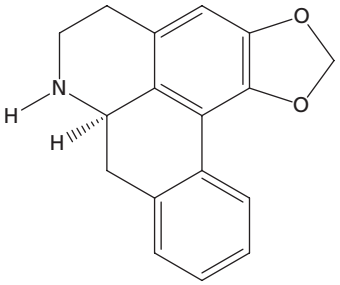
The leaves are large and orbicular, 20–90 cm in diameter and non-wettable. Leaves are of two types: aerial and floating, and are petiolated and entirely glaucous. The aerial leaves are cup-shaped whereas the floating leaves are flat. The petioles of the aerial leaves are erect, smooth, greenish or greenish brown in colour with small brown dots and are sometimes rough. The aerial leaves are usually 24–33 cm in length, and the floating leaves 23–30 cm. Odour is distinct; fractures are fibrous.[42] The young leaves are eaten as vegetables and used in traditional medicine.[66]

Traditional uses

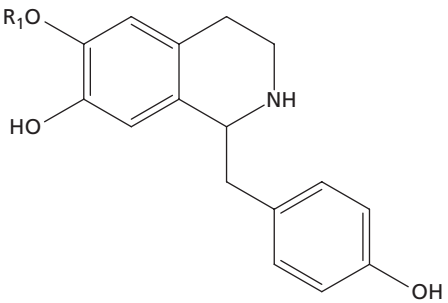
The leaf juice is used for the treatment of diarrhoea, and *Glycyrrhiza* spp leaf decoction is used for the treatment of sunstroke. The dried leaf is used in summer heat, to invigorate the function of the spleen and to arrest bleeding by reducing heat in the blood. The leaf extract has diuretic and astringent properties, and is used to treat fever, sweating and as a styptic.[67] The leaves are used in the treatment of haematemesis, epistaxis, haemoptysis, haematuria, metrorrhagia, hyperlipidaemia and obesity. Primarily they are used for clearing heat, removing heatstroke, cooling the blood and to stop bleeding.[22,68] The stem is used in Ayurvedic medicine as a diuretic, anthelmintic and to treat strangury, vomiting, leprosy, skin diseases, nervous exhaustion and diarrhoea.[3]

Phytochemistry

Combined gas/liquid chromatography–mass spectroscopy has shown that the leaves are rich in a number of alkaloids. In the analysis of non-phenolic fractions of the leaf extract (Figure 2), the major components had retention data and mass spectra identical to those of nuciferine, roemerine, anonaine (**14**), pronuciferine and N-nornuciferine (**15**). Two benzylisoquinoline alkaloids, (+)-1(R)-coclaurine (**16**) and (–)-1(S)-norcoclaurine (**17**), were also found in leaf extract of *N. nucifera*.[21] Six non-phenolic bases were identified: roemerine, nuciferine, anonaine, pronuciferine, *N*-nornuciferine and liriodenine (**18**) and two phenolic bases, armepavine and N-methyl-coclaurine

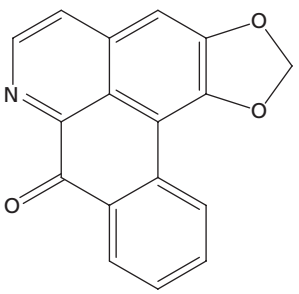


Anonaine (**14**)

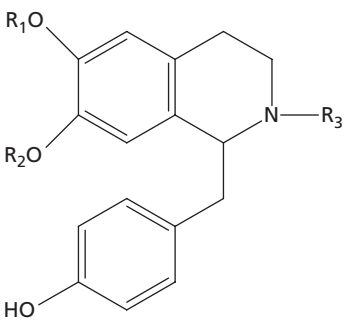


Coclaurine (**16**): $R_1 = CH_3$

Norcoclaurine (**17**): $R_1 = H$

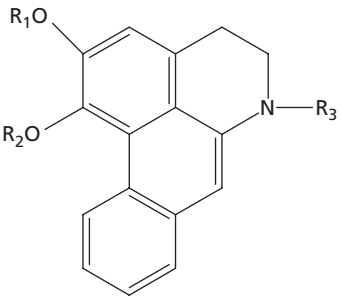


Liriodenine (**18**)



N-methyl-coclaurine (**19**): $R_1 = R_3 = CH_3$; $R_2 = H$

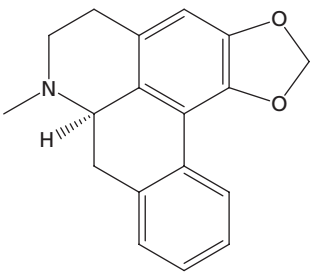
N-methylisococlaurine (**23**): $R_1 = H$; $R_2 = R_3 = CH_3$



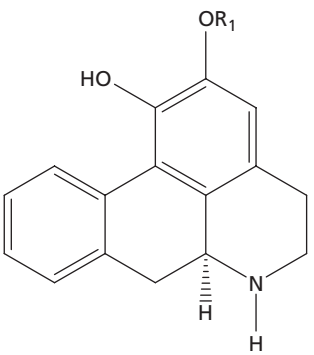
Dehydroemerine (**20**): $R_1 = R_2 = CH_2$; $R_3 = CH_3$

Dehydronuciferine (**21**): $R_1 = R_2 = R_3 = CH_3$

Dehydroanonaine (**22**): $R_1 + R_2 = CH_2$; $R_3 = H$



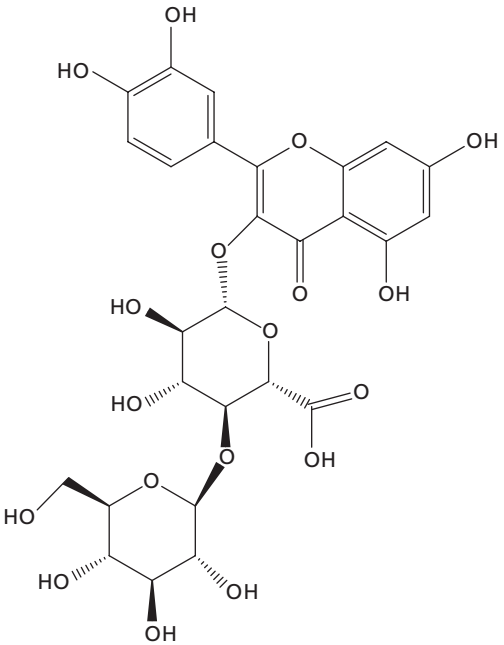
Remerine (**25**)



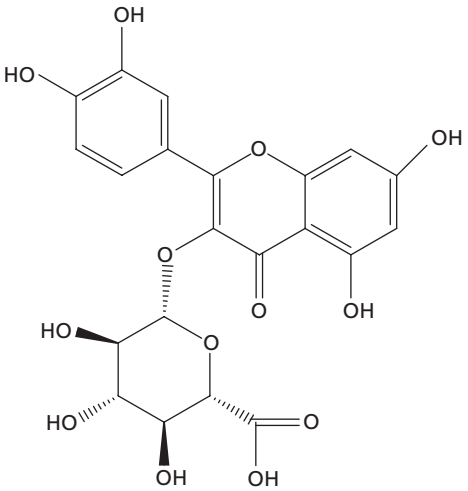
Asimilobine (**26**): $R_1 = H$

Lirinidine (**27**): $R_1 = CH_3$

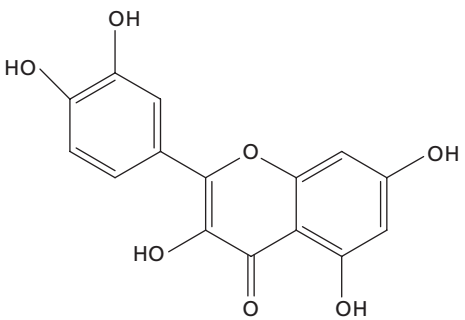
Figure 2 (*Continued*)



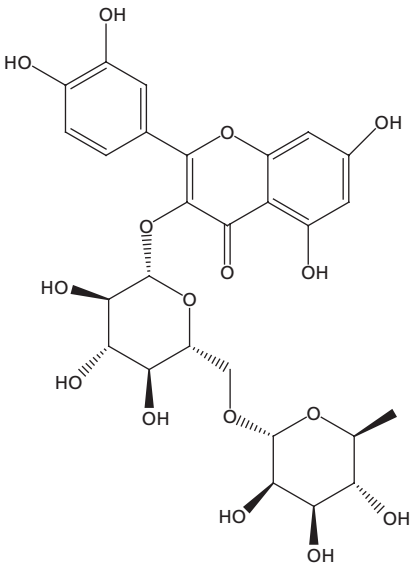
Nelumboside (**28**)



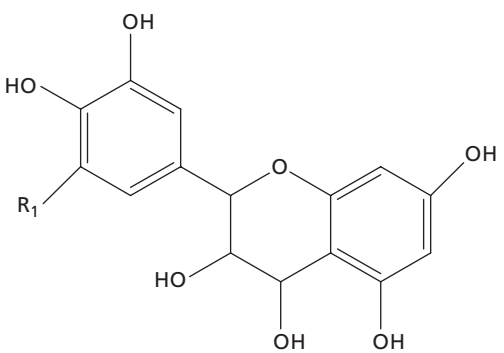
Quercetin-3-*O*-*β*-D-glucuronide (**32**)



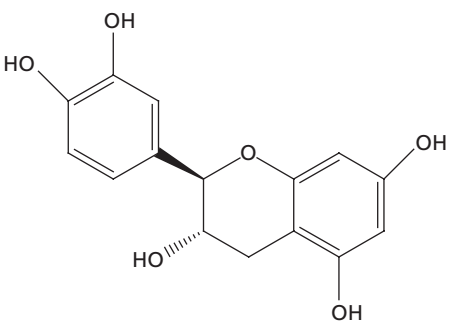
Quercetin (**29**)



Rutin (**33**)



Leucocyanidin (**30**): R_1 = H
Leucodelphinidin (**31**): R_1 = OH



(+)-Catechin (**34**)

Figure 2 (*Continued*)

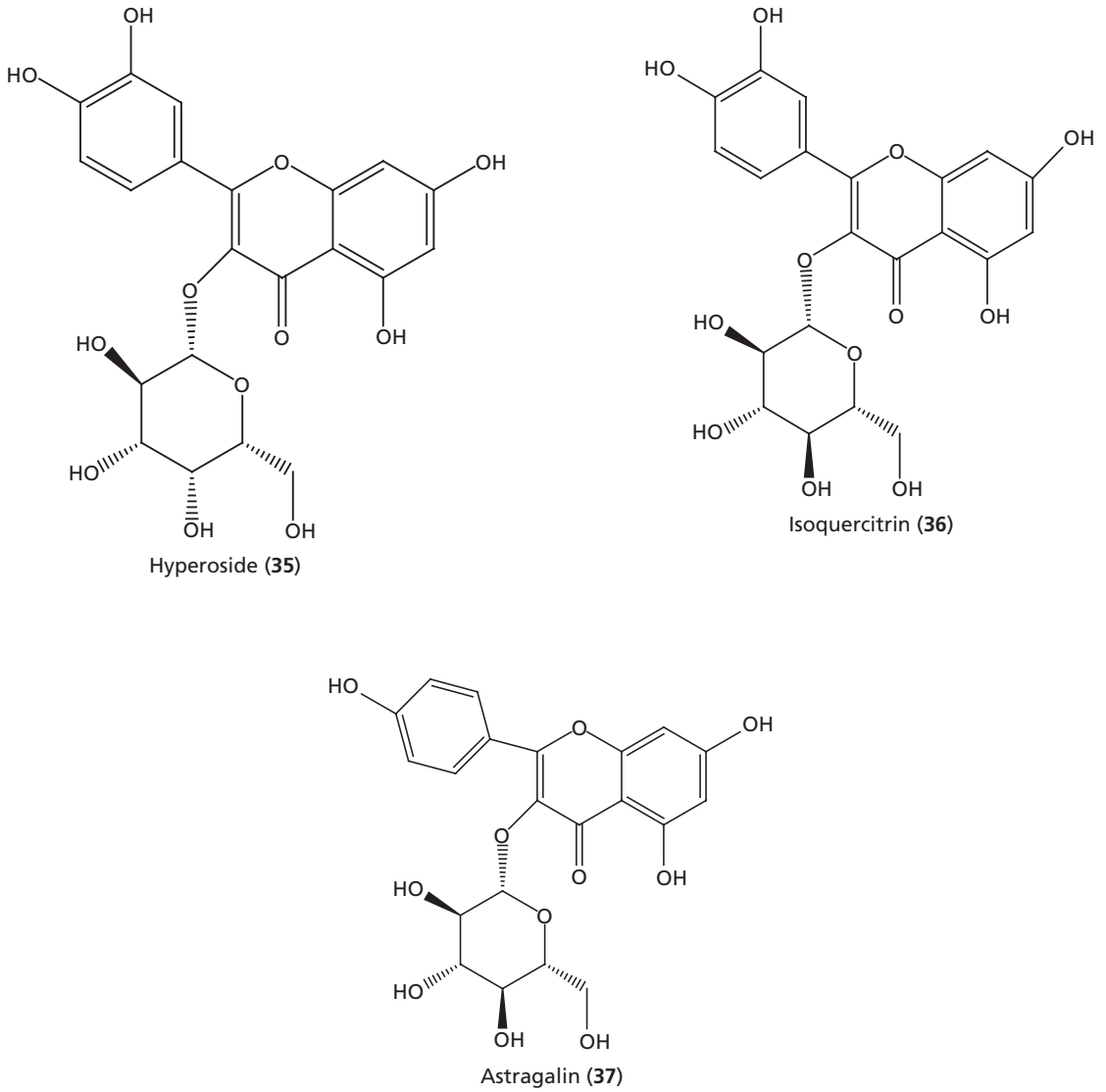


Figure 2 Major components of the leaf extract of *Nelumbo nucifera.*

(**19**), were also found in *N. nucifera* leaf extract.[69] Dehydroemerine (**20**), dehydronuciferine (**21**), dehydroanonaine (**22**), N-methylisococlaurine (**23**), anonaine, pronuciferine, *N*-nornuciferine, *O*-nornuciferine (**24**), nuciferine, remerine (**25**), roemerine, armepavine, liensinine, isoliensinine, negferine, asimilobine (**26**) and lirinidine (**27**) were isolated from leaves and petioles.[21,68,70–73] The leaves also contain a glycoside, nelumboside (**28**), and flavonoids such as quercetin (**29**) and leuco-anthocyanidin which were identified as leucocyanidin (**30**) and leucodelphinidin (**31**).[74,75] The presence of some other flavonoids in the leaves such as quercetin 3-O-α-arabinopyranosyl-(1→2)-β-galactopyranoside, quercetin-3-O-β-D-glucuronide (**32**), rutin (**33**), (+)-catechin (**34**), hyperoside (**35**), isoquercitrin (**36**) and astragalin (**37**) has also been reported.[21,23]

Scanning electron microscopy and chemical analysis of the chloroform extract of leaves showed that the wax was composed of a mixture of aliphatic compounds, principally nonacosanol and nonacosanediols. Analysis of gas chromatography spectra of lotus leaves waxes showed a much lower proportion of the secondary alcohol nonacosan-10-ol (16.2% by weight) compared with nonacosanediols (64.7%). Gas chromatographic analysis of the extracted leaf waxes revealed nonacosan-10-ol (16.2 ± 1.1%), triacontan-7-ol (2.4 ± 0.4%), nonacosane-4, 10-diol (18.6 ± 0.5%), nonacosane-5, 10-diol (34.1 ± 1.9%), nonacosane-10, 13-diol (12.0 ± 0.7%), hentriacontane-12, 15-diol (1.8 ± 0.0%), tritriacontane-9, 10-diol (0.7 ± 0.0%) and octadecanoic acid (0.7 ± 0.0).[76]

Pharmacology and toxicology

Cardiovascular activity

Two alkaloids that act as serotonin antagonists, namely asimilobine and lirinidine, were isolated from the leaves of *N. nucifera*. Both alkaloids inhibited serotonin-induced

contractions in isolated rabbit aorta.[73] Another alkaloid, nelumbine, which acts as a cardiac poison, was also reported to be present in leaves and petioles of the plant.[66]

Antioxidant activity

Wu *et al.*[77] used hydrogen peroxide-mediated cytotoxicity in Caco-2 cells to investigate the potential antioxidant activity of the methanol extract from the lotus leaf. A dose-dependent protective effect against reactive oxygen species (ROS)-induced cytotoxicity was observed when Caco-2 cells were treated with 10 mM hydrogen peroxide in combination with the methanol extract of the lotus leaf (0.1–0.3 mg/ml). The extract also exhibited concentration-dependent antioxidant activities against haemoglobin-induced linoleic acid peroxidation and Fenton reaction-mediated plasmid DNA oxidation.[77]

Antiviral activity

The 95% ethanol extract has been reported to show anti-HIV activity (EC50 < 20 μg/ml). Some anti-HIV principles, including (+)-1(R)-coclaurine, (–)-1(S)-norcoclaurine and quercetin 3-O-β-D-glucuronide, were found in *N. nucifera* leaves.[21] Both (+)-1(R)-coclaurine and (–)-1(S)-norcoclaurine showed potent anti-HIV activity, with EC50 values of 0.8 and < 0.8 μg/ml, respectively, and therapeutic index values above 125 and 25, respectively, whereas quercetin 3-O-β-D-glucuronide was less potent (EC50 2 μg/ml). Other potent anti-HIV bisbenzylisoquinoline alkaloids such as nuciferine, liensinine, negferine and isoliensinine have also been isolated from the leaves of *N. nucifera,* with EC50 values below 0.8 μg/ml and therapeutic index values of 36, > 9.9, > 8.6 and > 6.5, respectively.[21]

Anti-obesity activity

Ono *et al.*[22] have reported the effects of leaf extract on digestive enzymes, lipid metabolism and theromogenesis, together with the anti-obesity effect using mice with obesity induced by a high-fat diet. The extract showed a concentration-dependent inhibition of the activities of α-amylase and lipase, and up-regulated lipid metabolism and expression of uncoupling protein-3 mRNA in C2C12 (mouse myoblast cell line) myotubes. It also prevented increases in body weight, parametrical adipose tissue weight and liver triacylglycerol levels.[22]

Lipolytic activity

A 50% ethanol extract of *N. nucifera* leaves was reported to stimulate lipolysis in the white adipose tissue of mice. Chromatographic analysis of the extract showed that the phytomolecules responsible for lipolytic activity included quercetin-3-O-α-arabinopyranosyl-(1→2)-β-galactopyranoside, catechin, hyperoside, isoquercitrin and astragalin.[23]

Hypocholesterolaemic activity

The aqueous extract of lotus leaves was studied for its effects on serum lipids in a rat model. The rats were fed a high-fat diet containing 1.5% cholesterol and 1% cholic acid. Subsequent oral treatment with a crude aqueous extract of lotus leaves resulted in sharp decreases in serum total cholesterol, free cholesterol and phospholipids compared with the high-fat-loaded control group.[24]

Flowers

The flowers are solitary, large, 10–25 cm in diameter, white, pink or pinkish white, fragrant and have peduncles arising from the nodes of the rhizome, and 1–2 cm long sheathing at the base. The sepals, petals and stamens are spirally arranged, passing gradually one into another.[78]

Traditional uses

Flowers are traditionally used to treat diarrhoea, cholera, fever, hepatopathy, hyperdipsia and many bleeding disorders.[4] The flower stalks of *N. nucifera* have been used as one of the ingredients of 'Madhucasava', a unique fermenting medium used for microbiological screening.[79] The flowers are used in the treatment of premature ejaculation, abdominal cramps and bloody discharges, and as a cardiac tonic. The flower stalk is used for the treatment of bleeding gastric ulcers, excessive menstruation and post-partum haemorrhage.[2] The lotus honey is used as a tonic and for the treatment of eye infections.[80]

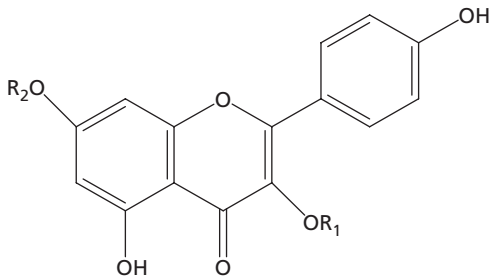
Phytochemistry

Several flavonoids have been identified in the stamens of *N. nucifera* (Figure 3). These include kaempferol (**38**) and seven of its glycosides: kaempferol 3-O-β-D-galactopyranoside (**39**), kaempferol 3-O-β-D-glucopyranoside (**40**), kaempferol 7-O-β-D-glucopyranoside (**41**), kaempferol 3-O-α-L-rhamnopyranosyl-(1-6)-β-D-glucopyranoside (**42**), kaempferol 3-O-α-L-rhamnopyranosyl-(1-2)-β-D-glucopyranoside (**43**), kaempferol 3-O-α-L-rhamnopyranosyl-(1-2)-β-D-glucuronopyranoside (**44**), kaempferol 3-O-β-D-glucuronopyranoside (**45**), kaempferol 3-O-β-D-glucuronopyranosyl methylester (**46**), myricetin 3′,5′-dimethylether 3-O-β-D-glucopyranoside (**47**), quercetin 3-O-β-D-glucopyranoside (**48**), nelumboroside A (**49**) and nelumboroside B (**50**). It also contains two isorhamnetin glycosides: isorhamnetin 3-O-β-D-glucopyranoside (**51**) and isorhamnetin 3-O-α-L-rhamnopyranosyl-(1→6)-β-D-glucopyranoside (**52**).[16,18,81] Some non-flavonoid compounds, including adenine, myo-inositol, arbutin (**53**) and β-sitosterol glucopyranoside (**54**), have also been identified in stamen extract.[81]

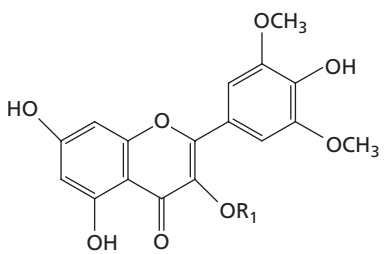
Pharmacology and toxicology

Hypoglycaemic activity

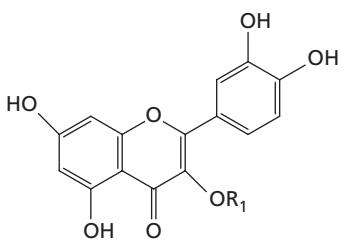
Sun-dried flower powder of *N. nucifera*, as well as the aqueous and alcoholic extract of the flower, produced significant hypoglycaemia in fasting normal albino rabbits. There was no significant difference in the activities of 1000 mg/kg of the test drug (sun-dried powder of the flower) and equivalent amounts of the extracts; the effect was approximately 50% of that produced by 250 mg/kg tolbutamide.[82] In normal rabbits, the extract at a dose of 1000 mg/kg significantly lowered hyperglycaemia induced by subcutaneous injection of 0.5 mg/kg adrenaline hydrochloride.[82] In-vitro studies with rat hemidiaphragm revealed that the sun-dried flower powder significantly enhanced the effect of insulin. The improvement of glucose tolerance may also be due to increased peripheral glucose utilisation caused by increased sensitivity of skeletal muscle to endogenous insulin.[83]



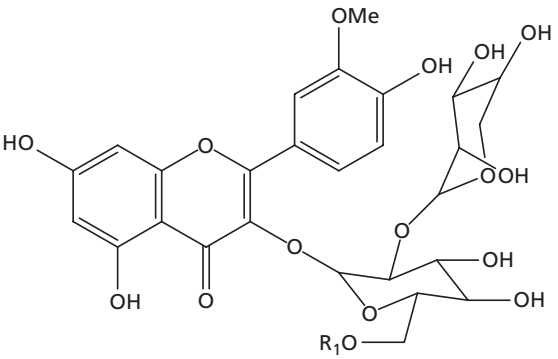
Kaempferol (**38**): $R_1 = R_2 = H$
Kaempferol 3-O-β-D-galactopyranoside (**39**): R_1 = Gal; $R_2 = H$
Kaempferol 3-O-β-D-glucopyranoside (**40**): R_1 = Glc; $R_2 = H$
Kaempferol 7-O-β-D-glucopyranoside (**41**): $R_1 = H$; R_2 = Glc
Kaempferol 3-O-α-L-rhamnopyranosyl-(1→6)-β-D-glucopyranoside (**42**): R_1 = Rha-(1→6)-Glc; $R_2 = H$
Kaempferol 3-O-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranoside (**43**): R_1 = Rha-(1→2)-Glc; $R_2 = H$
Kaempferol 3-O-α-L-rhamnopyranosyl-(1→2)-β-D-glucuronopyranoside (**44**): R_1 = Rha-(1→2)-Gln; $R_2 = H$
Kaempferol 3-O-β-D-glucuronopyranoside (**45**): R_1 = Gln; $R_2 = H$
Kaempferol 3-O-β-D-glucuronopyranosyl methylester (**46**): R_1 = Gln-Me; $R_2 = H$



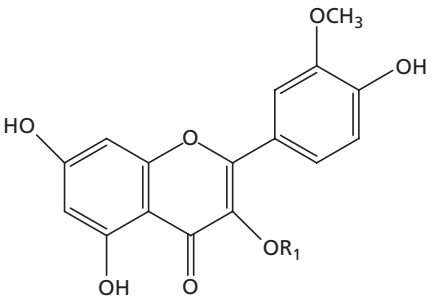
Myricetin 3′,5′-dimethylether 3-O-β-D-glucopyranoside (**47**)



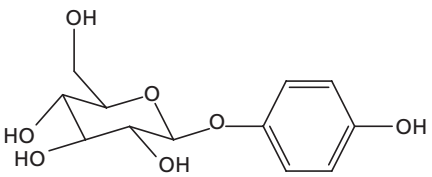
Quercetin 3-O-β-D-glucopyranoside (**48**): R_1 = Gln



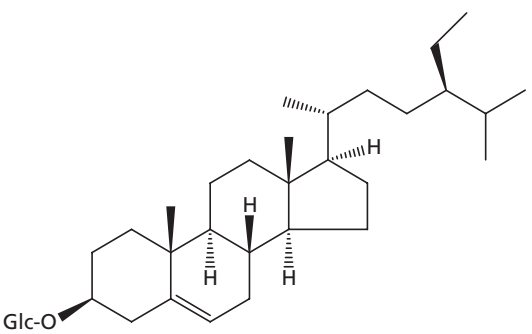
Nelumboroside A (**49**): $R_1 = H$
Nelumboroside B (**50**): R_1 = Rha



Isorhamnetin 3-O-β-D-glucopyranoside (**51**): R_1 = Glc
Isorhamnetin 3-O-β-L-rhamnopyranosyl- (1→6)-β-D-glucopyranoside (**52**): R_1 = Rha-(1→6)-Glc



Arbutin (**53**)



β-sitosterol glucopyranoside (**54**)

Figure 3 Flavonoids identified in the stamens of *Nelumbo nucifera*. Glc = glucose; Rha = rhamnose; Gln = glutamine; Gal = galactose.

Antioxidant activity

Jung *et al.*[16] examined the potential of *N. nucifera* stamens to scavenge DPPH free radicals and peroxynitrites ($ONOO^-$), and the inhibition of total ROS generation by kidney homogenates using 2′,7′-dichlorodihydrofluorescein diacetate. The methanol extract showed strong antioxidant activity in the $ONOO^-$ system and marginal activity in the DPPH and total ROS systems. In a similar fashion, seven known flavonoids were isolated from lotus stamens, most of which also showed potent antioxidant activity.[16] The glycosides nelumboroside A, nelumboroside B, isorhamnetin glycoside and isorhamnetin rutinoside isolated from *N. nucifera* stamens showed potent antioxidant activity in DPPH and $ONOO^-$ assays.[18] 'Yunyupju', a liquor made from the blossoms and leaves of lotus, has been reported to have antioxidant activity, with an IC50 value of 1.07 ± 0.04 mg.[84]

Antipyretic activity

The ethanol extract of stalks of *N. nucifera* was evaluated for its antipyretic potential on normal body temperature and yeast-induced pyrexia in rats. The stalk extract showed significant activity in both models at oral doses of 200 and 400 mg/kg. In the model of yeast-provoked pyrexia, the extracts showed dose-dependent lowering of body temperature up to 4 h; the results were comparable to those with paracetamol.[25]

Aldose reductase inhibitory activity

Two glycosides, namely kaempferol 3-O-α-L-rhamnopyranosyl-(1→6)-β-D-glucopyranoside and isorhamnetin 3-O-α-L-rhamnopyranosyl-(1→6)-β-D-glucopyranoside, isolated from the methanol extract of stamens of *N. nucifera* exhibited a high degree of inhibitory activity against rat lens aldose reductase *in vitro,* with IC50 values of 5.6 and 9.0 μM, respectively.[81]

Hepatoprotective activity

Oral administration of a 50% hydroalcoholic extract of *N. nucifera* flowers (200 and 400 mg/kg) showed significant dose-dependent protective effects against carbon-tetrachloride- and paracetamol-induced hepatotoxicity in rats. A 400 mg/kg oral dose of 50% aqueous ethanolic extract of flower exhibited the most significant protective effect. The hepatoprotective mechanisms of flower extract might be due to prevention of lipid peroxidation, inhibition of cytochrome P450 activity, stabilising of the hepatocellular membrane, and enhancement of protein synthesis.[26]

Rhizome

The rhizomes are 60–140 cm long, 0.5–2.5 cm in diameter, yellowish white to yellowish brown, smooth, with longitudinal striations and brown patches, and with nodes and internodes. Transverse section of the rhizome shows an outer layer of epidermis, surrounded by cuticle followed by a dense sub-epidermal layer, a spongy layer and an inner dense layer, continuous with the parenchyma cells.[85] When freshly cut, the rhizome exudes mucilaginous juice and shows a few large cavities surrounded by several larger ones. Fracture is tough and fibrous, and the odour is indistinct.[42]

Traditional uses

The rhizome extract is used as a tonic.[42] The rhizome nodes are used in the treatment of nasal bleeding, haemoptysis, haematuria and functional bleeding of the uterus. It is included in Chinese herbal prescriptions for the treatment of tissue inflammation, cancer and chronic liver cirrhosis.[86] The rhizome is also used for arresting bleeding, dissipating blood stasis, improving appetite, haematemesis and haemoptysis.[87] Powdered rhizome is prescribed as a demulcent for haemarrhoids and is beneficial in dysentery and chronic dyspepsia.[36,88] External application in the form of a paste is useful in scabies and ring worm. The rhizome yields a nutritious arrowroot that is used for diarrhoea, dysentery and dyspepsia in children.[35,89]

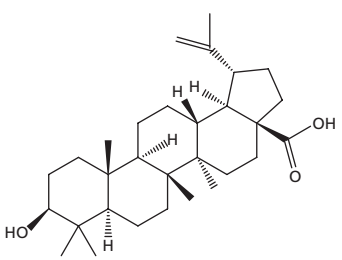
Phytochemistry

The rhizomes of lotus are consumed as a vegetable in Asian countries. They are used as health foods because of their mineral content. Abundant starch grains are present throughout the tissue. Fresh rhizome contains 31.2% starch, which shows no characteristic taste or odour.[90] The binding and disintegration properties of isolated *Nelumbo* starch have been compared with maize and potato starch; *Nelumbo* starch was found to be superior as an adjuvant in the preparation of tablets.[91] It has been reported that 50% (v/v) alcohol is required for maximum extraction of the constituents.[92] The methanol extract of the rhizome has been found to possess a steroidal triterpenoid – betulinic acid (**55**; Figure 4).[8,93] Fresh rhizome contains 83.80% water, 0.11% fat, 1.56% reducing sugar, 0.41% sucrose, 2.70% crude protein, 9.25% starch, 0.80% fibre, 1.10% ash and 0.06% calcium. The vitamins thiamine (0.22 mg/100 g), riboflavin (0.6 mg/100 g), niacin (2.10 mg/100 g) and ascorbic acid (1.5 mg/100 g) and an asparagine-like amino acid (2%) are also present in the rhizomes. The oxalate content of rhizome was found to be 84.3 mg/100 g.[88,94] The following elements were also found: calcium – 1.15%, copper – 0.0015%, iron – 0.053%, magnesium – 0.398%, zinc – 0.0032%, barium – 0.00064%, potassium – 0.756% and sodium – 0.10%.[95]

Pharmacology and toxicology

Antidiarrhoeal activity

The antidiarrhoeal potential of *N. nucifera* rhizome extract has been reported. The extract produced significant inhibitory effects against castor-oil-induced diarrhoea and PGE_2-induced enteropooling; the propulsive movements of a charcoal meal



Betulinic acid (**55**)

Figure 4 The triterpenoid – betulinic acid found in the methanol extract of the rhizome of *Nelumbo nucifera.*

were also reduced significantly.[28] The observed antidiarrhoeal effect was reconfirmed by Talukder and Nessa in the rat.[96]

Antimicrobial activity

Different extracts of rhizome showed significant antibacterial effects against *Staphylococcus aureus*, *Escherichia coli*, *Bacillus subtilis*, *B. pumilis* and *Pseudomonus aeruginosa*. The chloroform extract was found to be the most effective when compared with the standard drug chloramphenicol.[29] Antifungal and anti-yeast activities of the rhizome extract were evaluated against five different strains of fungi and yeast, including *Candida albicaus*, *Aspergillus niger, A. fumigatus* and *Trichophytum mentagopyhtes*; the extract showed potential activity in all the strains tested and the effect was comparable to that of griseofulvin, used as standard drug for comparison.[30]

Hypoglycaemic activity

The oral hypoglycaemic effect of *N. nucifera* was demonstrated using an ethanol extract of the rhizome, which markedly reduced the blood sugar level of normal, glucose-fed hyperglycaemic and streptozotocin-induced diabetic rats, when compared with control animals. The extract improved glucose tolerance and potentiated the action of exogenously injected insulin in normal rats. The extract exhibited activity corresponding to 73% and 67% of that of tolbutamide in normal and diabetic rats, respectively.[27,33] An anti-diabetic constituent (tryptophan) has been isolated from the nodes of lotus rhizome.[97] In glucose-fed hypoglycaemic mice, the methanolic extract of nodes at a dose of 400 mg/kg and 100 mg/kg of isolated tryptophan showed potential anti-diabetic activities.[97]

Psychopharmacological activity

The methanol extract of the rhizome of *N. nucifera* produced significant psychopharmacological actions in rats and mice: reduction in spontaneous activity, and a decrease in exploratory behaviour in the head dip and Y-maze tests. Thus, the extract possesses most of the pharmacological characteristics of a minor tranquilizer.[98]

Diuretic activity

The diuretic activity of *N. nucifera* rhizome was reported by Mukherjee and coworkers.[31] The methanol extract of the rhizome induced significant diuresis in rats at doses of 300, 400 and 500 mg/kg. There was a dose-dependent increase in the volume of urine, with Na^+ and Cl^- excretion, accompanied by a significant excretion of K^+. The increase in volume of urine was less than with the standard diuretic Furosemide (20 mg/kg).[31]

Anti-inflammatory activity

We have reported the anti-inflammatory activity of *N. nucifera* rhizome and isolated betulinic acid against carrageenin- and serotonin-induced rat paw oedema.[8] The rhizome extract at doses of 200 and 400 mg/kg, and betulinic acid at doses of 50 and 100 mg/kg (administered orally) showed significant anti-inflammatory activity; the effect was comparable to that of the standard drugs phenylbutazone and dexamethasone.[8]

Antioxidant activity

Yang and coworkers have performed in-vitro studies of the antioxidant activity of methanol and acetone extracts of the *N. nucifera* rhizome using the DPPH assay and β-carotene bleaching assay.[86] The methanol and acetone extract showed highest DPPH scavenging activity, at 66.7 and 133.3 mg/l, respectively; the methanol extract exhibited a higher antioxidant activity coefficient than ascorbic acid. The rhizome knot also exhibited radical scavenging activity, measured spectrophotometrically and by electron spin resonance.[14]

Antipyretic activity

The methanolic extract of *N. nucifera* rhizome showed antipyretic activity in rats with yeast-induced pyrexia. Oral doses of the extract of 200, 300 and 400 mg/kg produced significant dose-dependent lowering of normal body temperature and yeast-provoked elevation of body temperature in rats. The result was comparable to that of the standard antipyretic drug paracetamol (150 mg/kg intraperitoneally).[88]

Conclusions

Different parts of *N. nucifera,* including the leaves, rhizomes, seeds and flowers, have been reported to have therapeutic potential in traditional medicine for the treatment of various diseases. Pharmacological activities of different extracts of rhizomes, seeds, leaves and flowers, and the compounds isolated from these extracts, have been demonstrated through numerous in-vitro and in-vivo test models. These include antioxidant, anti-inflammatory, antimicrobial, anti-arrhythmic, antipyretic, anti-ischaemic, anti-diabetic, hypoglycaemic, anti-diarrhoeal, immunomodulatory and other activities. The responsible bioactive compounds belong to several chemical groups; mostly they are alkaloids (like dauricine, lotusine, nuciferine, liensinine, roemerine, neferine, armepavine), flavonoids (like kaempferol, quercetin, leucocyanidin, leucodelphinidin, catechin, isoquercitrin, astragalin), glycosides (nelumboroside A, nelumboroside B, isorhamnetin glycoside and isorhamnetin rutinoside), triterpenoid (like betulinic acid), vitamins and minerals. Thus, the pharmacological effects and various active ingredients of different parts of *N. nucifera* are well understood. This review highlights several pharmacological and phytochemical studies that have demonstrated the therapeutic potential of *N. nucifera.* This needs to be explored further through clinical studies on human volunteers to provide evidence-based therapeutics.

Declarations

Conflict of interest

The Author(s) declare(s) that they have no conflicts of interest to disclose.

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