Lotus – A potential nutraceutical source

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Scarcity of protein rich food and food supplements are responsible for protein-energy malnutrition particularly among children and lactating women in the developing countries. Legumes and some dicotyledonous seeds constitute important sources of protein as well as energy. Bioprospecting of such unconventional sources is able to provide food security, agricultural development, self-dependence and enhancement of economy of developing countries. Interestingly, seeds of lotus (*Nelumbo nucifera*) are edible, medicinally versatile and used as an important raw material of age-old traditional medical practices like Ayurveda and folk medicine. Tender rhizomes, stems and leaves of lotus are edible and its seeds are rich in protein as well as minerals. Petals are useful as garnish, while the stamens are used in flavoring the tea and the roasted seeds can be used as coffee substitute. Powdered popped seeds are eaten dry and useful in bread preparation. Seeds are raw material for Ayurvedic and folk medicines to treat many ailments such as tissue inflammation, cancer, diuretics, skin diseases and as poison antidote. Lotus plants provide several bioactive ingredients like alkaloids, flavonoids, antioxidants, antisteroids, antipyretic, anticancerous, antiviral and anti-obesity properties.

Key words: Lotus, nutrition, pharmaceutical, alkaloids, antioxidants, anticancerous, antiviral

Introduction

Protein-energy deficiency has been recognized as the most common form of malnutrition in regions where people mainly rely on starch-based diets and cereal porridges (FAO, 1994; Michaelsen and Henrik, 1998). Scarcity of protein rich food and food supplements has been responsible for the recurring problems associated with malnutrition in children and lactating women in developing countries. Exploration of non-conventional legumes and other dicots may meet the increasing demand of balanced nutrition. Legumes have been projected as a better and economically viable alternative source of proteins and calories against animal proteins in developing countries (Famurewa and Raji, 2005). Exploitation of nutritional value of seeds of wild legumes and other dicotyledonous seeds rich in nutrients assume importance to

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provide food security, agricultural development, self-dependence and enhancement of economy of developing countries. Little known dicotyledonous lotus seeds (*Nelumbo nucifera* Gaertn.) can play a significant role due to their edibility and medicinal properties. Nutritionally, lotus seeds are rich in proteins (10.6-14.8%) and essential minerals (Ibrahim and El-Eraqy, 1996; Indrayan *et al*., 2005). Lotus seeds are in high demand in Ayurvedic medicinal preparations and widely used in folk medicines to treat tissue inflammation, cancer, diuretics (Liu *et al.,* 2004), skin diseases and as poison antidote (Chopra *et al*., 1956). The main task of the present article is to document and emphasize the nutritional features and pharmaceutically value-added compounds of lotus seeds in view of future bioprospecting potential.

The lotus

In ancient times, lotus was common along the banks of the River Nile with closely related species known as 'sacred blue lotus' (*Nymphaea caerulea*). The Pharoic Egyptians worshipped the lotus flowers, fruit 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 lilly under the patronage of Sir Joseph Banks and nowadays it can be seen almost everywhere in modern botanical garden collections. Lotus plants are common in Australia, China, India, Iran and Japan (Anon., 1966). Lotus was introduced from China to Japan and cultivated for more than 1000 years (Komatsu *et al*., 1975). In China during 1999, it served as an industrial crop grown over 40,000 ha. In India, it is wide spread and known even from Himalayan lakes at altitude up to 1400 m (Polunin and Stainton, 1984). Lotus seeds sold in the Indian markets ('kamal gatta') as vegetable or raw material for Ayurvedic drug preparation (Anon., 1992). Seeds and roots of lotus are regarded as popular health food and the alkaloid (liensinine) extracted from them is effective to treat arrhythmia (Ling *et al*., 2005).

Nelumbo nucifera belongs to the family of Nelumbonaceae, which has several common names (e.g. Indian lotus, Chinese water lilly and sacred lotus) and synonyms (*Nelumbium nelumbo, N. speciosa*, *N. speciosum* and *Nymphaea nelumbo*). Lotus is a perennial, large and rhizomatous aquatic herb with slender, elongated, branched, creeping stem consisting of nodal roots; leaves are membranous, peltate (60-90 cm and above), orbicular and concave to cup-shaped; petioles are long, rough with small distinct prickles; flowers are white to rosy (Fig. 1a), sweet-scented, solitary, hermaphrodite, 10-25 cm diameter;

ripe carpels are 12 mm long, ovoid and glabrous; fruits are ovoid having nut like achenes; seeds are black, hard and ovoid (Fig. 1b,c).

Fig. 1. Lotus (*Nelumbo nucifera*) flower (a), dry seeds (b) and cut open seeds (c) with embryo within seed (arrow) (scale: cm).

Germination of any economically valued seeds assumes importance for the purpose of propagation or germplasm preservation. Some reports are available on germination of lotus seeds, where the 237 years old lotus seeds (*Nelumbium* sp.) collected during 1705 from Hans Sloane of the British Museum were germinated in 1942 (Ramsbottom, 1942) indicating their long-term viability. Ushimaru *et al*. (2001) observed that seeds of anoxia-tolerant lotus (*N. nucifera*) grown in the ponds of Japan showed germination under water revealing that seedlings are tolerant to oxygen deficient conditions (hypoxia). Lotus plants propagated vegetatively through rhizomes. Arunyanart and Chaitrayagun (2005) worked on the callus induction and somatic embryogenesis of *N. nucifera*. Callus was initiated by culturing bud, cotyledon and young leaf explants on Murashige and Skoog (MS) medium containing 0, 4, 8 and 10 μM 2,4-D and 0, 1, 2 and 3 μM kinetin or substituting 0, 0.5 and 1 mM benzyladenine (BA) for kinetin. Bud explants cultured on medium containing 4 μM 2,4-D and 1 μM BA gave the best callus growth. For somatic embryogenesis, the calli initiated on MS medium containing a combination of 2,4-D (4, 6, 8, and 10 μM) and BA (1 μM) and subsequent transfer to media with 2,4-D (2-4 μM) and BA (0 or 0.5 μM) had produced the somatic embryos. Cultures of 12-week-old callus on medium with 2,4-D (6 μM) and BA (1 μM) showed the best growth as well as regeneration somatic embryos.

Nutritional value

Tender rhizomes, stems and leaves of lotus are edible (Anon, 1992) and can be cooked along with other vegetables, soaked in syrup or pickled in vinegar (Phillips and Rix, 1995). Rhizomes consist of 1.7% protein, 0.1% fat, 9.7% carbohydrate and 1.1% ash (Reid, 1977) and exhibit mild flavour and extensively used in Chinese recipe, while stem on cooking as food and the taste like beet (Hedrick, 1972; Tanaka, 1976). Ogle *et al*. (2001) reported the use of lotus stem (consists of 6, 2.4, 0.2 mg/100 g calcium, iron and zinc respectively) as a vegetable used in salads at Vietnam. As home remedy, lotus leaves are useful to treat summer heat syndrome in Japan and China and used to treat obesity in China (Ono *et al.,* 2006). Petals of lotus are floated in soups or used as a garnish, while the stamens are used in flavoring the tea (Facciola, 1990). Ibrahim and El-Eraqy (1996) reported that Egyptian lotus seeds consist of 14.8% crude protein. The green embryos in the seeds are bitter and usually removed prior to selling in the markets as food product. The seeds can be popped like popcorn, ground into powder and eaten dry or used in bread making. The roasted seeds are good coffee substitute and possess saponins, phenolics and carbohydrates in appreciable quantities (Anon., 1992; Ling *et al*., 2005). Seeds of *N. nucifera* consist of 10.5% moisture, 10.6-15.9% protein, 1.93-2.8% crude fat, 70-72.17% carbohydrate, 2.7% crude fibre, 3.9-4.5% ash and energy 348.45 cal/100 g (Reid, 1977; Indrayan *et al*., 2005). Minerals of lotus seeds consists of chromium (0.0042%), sodium (1%), potassium (28.5%), calcium (22.1%), magnesium (9.2%), copper (0.0463%), zinc (0.084%), manganese (0.356%) and iron (0.199%).

Pharmaceutical value

Traditional knowledge

Traditional knowledge reveals many medicinal uses of lotus plant. The whole plant serves as astringent, emollient, diuretic and sudorific and possesses antifungal, antipyretic and cardiotonic. Different parts of the lotus plant are useful in treatment of diarrhea, tissue inflammation and haemostasis (Yu and Hu, 1997). The rhizome extract has anti-diabetic (Mukherjee *et al*., 1997a) and anti-inflammatory properties due to presence of asteroidal triterpenoid (Mukherjee *et al*., 1997b). Rhizomes are used for pharyngopathy, pectoralgia, spermatorrhoea, leucoderma, small pox, diarrehoea, dysentery and cough. The stem is used in indigenous Ayurvedic medicines as diuretic, anthelmintic and to treat strangury, vomiting, leprosy, skin disease and nervous exhaustion. Young leaves with sugar are useful to treat rectal prolapse and the leaves boiled with *Mimosa pudica* in goat's milk can be used to treat diarrhea. Leaf paste can be applied to the body during fever and inflammatory skin conditions. Leaves are used as effective drug for hematemesis, epistaxis, hemoptysis, hematuria and metrorrhagia (Ou, 1989). Hyperlipidaemia in rodents can be treated with lotus leaves (La Cour *et al*., 1995; Onishi *et al*., 1984). Leaves also possess diuretic and astringent properties and help to treat fever, sweating and strangury and as styptic (Chinese Materia Medica, 1977). The leaves and flowers are useful to treat many bleeding disorders and consumption of flowers is recommended to promote conception. Flowers are useful to treat diarrehoea, cholera, fever, hepatopathy and hyperdipsia. In folk medicines, seeds are used in the treatment of tissue inflammation, cancer, skin diseases, leprosy, poison antidote and generally prescribed to children as diuretic and refrigerant (Chopra *et al*., 1956). The fruits and seeds of lotus are astringent and used to treat hyperdipsia, dermatopathy, halitosis, menorrhagia, leprocy and fever (Nadkarni, 1982). Seed powder mixed with honey is useful in treating cough, while roots with ghee (melted fresh butter), milk and gold promote strength, virility and intellect. Lotus seeds have been reported to possess rich antimicrobial properties (Mukherjee *et al*., 1995; Mukherjee, 2002). Embryo of lotus seeds are used in traditional Chinese drug called 'Lian Zi Xin', which primarily helps to overcome nervous disorders, insomnia, high fevours (with restlessness) and cardiovascular diseases (e.g. hypertension, arrhythmia) (Chen *et al.,* 2007).

Alkaloids and flavonoids

Lotus alkaloids dilate the blood vessels and reduce the blood pressure. Leaves are bitter, sweet and consist of several flavonoids and alkaloids (Shoji *et al*., 1987). The embryos possess small amount of alkaloids, which are antispasmodic for the intestines and alleviates diarrhea. The embryos within lotus seeds possess an alkaloid isoquinoline, which is sedative, antispasmodic and beneficial to heart. It dispels pathogenic heat from the heart and spontaneous bleeding due to heat. The major phytochemicals present in lotus seeds are alkaloids (e.g dauricine, lotusine, nuciferine, pronuciferine, liensinine, isoliensinine, roemerine, nelumbine, neferine) (Tomita *et al*., 1961; Furukawa *et al*., 1965; Wang *et al*., 1991; Qian, 2002). Dauricine and neferine block the Na^+, K^+ and Ca^{2+} transmembrane currents in cardiac cells (Qian, 2002). As anti-arrhythmic, neferine significantly inhibit the rabbit platelet aggregation (Li *et al*., 1990; Yu and Hu, 1997).

Lim *et al.* (2006) worked on the rat lens aldose reductase (RLAR) (a principal enzyme of polyol pathway associated with diabetes) inhibitory constituents of stamens of *N. nucifera* in rat lens. Methanol extract of the stamens exert an inhibitory effect on RLAR. Thirteen flavonoids and seven of its glycosides were isolated from lotus plants along with four non-flavonoid compounds. Among the isolated flavonoids, those possessing 3-O-alpha-l-rhamnopyranosyl - (1 → 6) – beta – d - glucopyranoside groups in their C rings, including kaempferol3-O-alpha-l-rhamnopyranosyl-(1→6)-beta-d-glucopyranoside and isorhamnetin 3-O-alpha-l-rhamnopyranosyl-(1→6)-beta-d-glucopyranoside exhibited the highest degree of RLAR inhibitory activity *in vitro* evidencing IC_{50} of 5.6 and 9.0 μM respectively.

Antioxidants

Ushimaru *et al.* (2001) studied the antioxidative enzyme changes in seedlings of *N. nucifera*, which responds to oxygen deficiency by germination under water. They reported that the activity of superoxide dismutase, dehydroascorbate reductase and glutathione reductase was lower in seedlings germinated under submerged condition in the darkness (SD) than those found in seedlings germinated in air and the darkness (AD). Ascorbate peroxidase activity was higher in SD than AD seedlings and the activity of catalase and monodehydroascorbate reductase in SD seedlings was nearly same as in AD seedlings. Leaf stalk extract possess antipyretic effect (Sinha *et al*., 2000), while antioxidants effect is found in leaves and stamen (Jung *et al*., 2003; Wu *et al*., 2003).

Lotus seed extract possess hepatoprotective, free radical scavenging properties and antifertility properties (Sohn *et al*., 2003). Yen *et al*. (2006) reported the free radical scavenging and protective effects of lotus seed extracts (LSE) against reactive nitrogen, sodium nitroprusside (SNP), peroxynitrite-induced cytotoxicity and DNA damage in macrophage RAW 264.7 cell lines. Inhibitory effects of the seeds extracted with water (LSWE), ethyl acetate (LSEAE) and hexane (LSHE) were evaluated. Results showed that all the extracts inhibit nitric oxide accumulation in LPS-activated RAW 264.7 cells. The extracts (0.01-0.2 mg/ml) showed dose-dependent inhibitory effect on the accumulation of nitric oxide upon decomposition of SNP. The potency of inhibitory activity was high in LSEAE followed by LSWE and LSHE. Results on the effect of three seed extracts on macrophage DNA damage revealed that RAW 264.7 cells treated with extracts (0.01-0.2 mg/ml) and incubated at 37°C for 2 hr showed no significant difference in the tail movement between the control and sample indicating that seed extracts do not induce DNA damage in RAW 264.7 cells. Results of the comet assay indicated that all extracts could inhibit DNA damage in macrophage RAW 264.7 cells induced by SNP. The LSWE, LSEAE and LSHE (at 0.2 mg/ml) showed DNA damage in macrophage RAW 264.7 cells at 63, 59 and 38% inhibition by peroxynitrite respectively. Similarly, the extracts tested were potent peroxynitrite scavengers in preventing nitration of tyrosine. At higher concentration (0.2 mg/ml), percentage inhibition of 3-nitrotyrosine formation against control was 29.0, 21.0 and 8.0% for LSWE, LSEAE and LSHE, respectively. These results imply that the extracts derived from various solvents are potent scavengers of peroxynitrite due to their ability to prevent the nitration of tyrosine. Rai *et al*. (2006) studied the antioxidant activity of hydro-alcoholic extract of lotus seeds using *in vitro* and *in vivo* models. Total phenolics in extract was 7.61% and exhibited strong free radical scavenging activity as evidenced by the low IC_{50} values (16.12 μg/ml) in 1,1-diphenyl-2-picryl hydrazyl, which was comparable to rutin (IC_{50}, 18.95 μg/ml). In nitric oxide method, the extract showed more activity (IC_{50}, 84.86 μg/ml) than standard rutin (IC_{50}, 152.17 μg/ml). No signs of acute toxicity were evident up to oral dose of 1,000 mg/kg body weight in Swiss Albino mice.

Procyanidins (or condensed tannins) are widely distributed in the plants, which represent ubiquitous group of plant phenols. Ling *et al*. (2005) performed pioneering work on the procyanidins of non-edible parts of lotus. The procyanidins of lotus pods have been extracted up to a purity of 98% in Me_2CO-H_2O and purified by Sephadex LH-20 column chromatography. The ESI-MS analysis showed that the main molecular weight distribution of procyanidins ranged from 291 to 1155, with M + H peak values of 291.1, 579.2, 731.2, 867.2, 1019.4 and 1155.3 indicating that the extract contains monomers, dimers and tetramers of procyanidins with greatest amount of dimmers, catechin and epicatechin base units. The extracted procyanidin of lotus pod was light red brown amorphous powder. The spectrometric quantifications of the extract showed 90.7% total polyphenols and 98.3% procyanidin. Based on the calibration curve of procyanidin B2, procyanidin of lotus pod extract was 63.2% indicating the differences in procyanidin. The scavenging effect of different concentrations of lotus pod procyanidin on superoxide free radicals (O_2^{-}) were expressed as IC_{50} as 17.6 mg/l, which is equivalent to 0.3 mg/l vitamin C. The procyanidin extract showed scavenging effect on ·OH that raised with increasing concentration and the IC_{50} was 10.5 mg/l which is equal to 4.1 mg/l vitamin C. Function of lotus pod procyanidin extract such as antioxidant activity in lard oil and soybean oil systems were further investigated. The results revealed that the peroxide values of the control and lard oil system containing 0.05, 0.1 and 0.2% procyanidin extract of lotus seed pod after 11 days under the same conditions were 54, 22, 18.04 and 20 mM/kg respectively. At 0.1% procyanidin, the extract showed a strong antioxidant activity than the others at 60°C and different concentrations of the procyanidin. Procyanidin extract possess the same effect as butylated hydroxytoluene (BHT) on inhibiting auto-oxidation of lard on the eighth day. The data indicated that the procyanidin partly inhibit the autoxidation of lard and thus 0.1% procyanidin possess a strong antioxidant property.

Antisteroids

Gupta *et al*. (1996) have reported antisteroidogenic effect of seed extract of *N. nucifera* in the testis and ovary of the rat. Fractions of petroleum ether extract were administered orally to sexually immature female rats and mature male rats on alternate days up to 15 days. The treatment caused a remarkable delay in sexual maturation in pre-pubertal female rats as evidenced from age of vaginal opening and first estrus (cornified smear) and reduction in sperm count and motility in mature male rats. The treatments resulted in accumulation of cholesterol and ascorbic acid, while reduction in delta-5-3-beta-hydroxysteroid dehydrogenase and glucose-6-phosphate dehydrogenase activity in the ovary and testis. These observations revealed that the petroleum ether extract results in suppression of genesis of steroids in ovary as well as testis.

Antipyretic

Chopra *et al*. (1958) have reported the antipyretic potential of *N. nucifera*. Similarly, Sinha *et al*. (2000) also reported the antipyretic potential of ethanol extract of lotus stalks on normal body temperature as well as yeast-induced pyrexia in rats. The extract (200 mg/kg) significantly lowered the body temperature up to 3 hr after administration, while 400 mg/kg up to 6 hr. In yeast model, provoked elevation of body temperature showed dose-dependent lowering up to 4 hr at both the doses and results were comparable to paracetamol, the standard antipyretic agent (150 mg/kg).

Anticancerous

Liu *et al*. (2004) reported that the ethanolic extracts of lotus inhibit the cell proliferation and cytokines in primary human peripheral blood mononuclear cells activated by phytohemagglutinin (a specific mitogen for T lymphocytes). Liu *et al*. (2006) studied the effects of (S)-Armepavine ($C_{19}H_{23}O_3N$; MW313) of lotus in suppression of T cells proliferation. The potential benefit of (S)-armepavine on systemic lupus erythematosus (SLE) was undertaken on MRL/MpJ-lpr/lpr mice, which had similar disease feature to human SLE. The MRL/MpJ-lpr/lpr mice treated orally with (S)-armepavine for 6 weeks and their SLE characteristics were evaluated. The results revealed that (S)-armepavine prevented lymphadenopathy and extended the life span of MRL/MpJ-lpr/lpr mice. The (S)-armepavine treatment resulted in a marked dose-dependent decrease in both cytokines (T lymphocyte-mediated cytokines) production. The result was: 83.9±5.1 pg/ml in mice treated with control vs. 54.5±4.7 pg/ml in mice treated with 10 mg/kg/day (S)-armepavine for IL-2, Pb0.01; 44.3±4.5 pg/ml in mice treated with control vs. 12.0±1.3 pg/ml in mice treated with 10 mg/kg/day (S)-armepavine for IFN-γ, Pb0.01. Further, the data revealed that (S)-armepavine impaired IL-2 and IFN-γ transcripts in human peripheral blood mononuclear cells. Liu *et al*. (2006) concluded that (S)-armepavine could be targeted as an immunomodulator for the management of autoimmune diseases like SLE.

Antiviral

Kashiwada *et al*. (2005) isolated anti-HIV benzylisoquinoline alkaloids and flavonoids from lotus leaves [-(+)-1(R)-Coclaurine, (-)-1(S)-norcoclaurine and quercetin 3-O-b-D-glucuronide]. The first two compounds possess potent anti-HIV activity [EC_{50}, 0.8 and <0.8l μg/ml; therapeutic index (TI), >125 and

>25 respectively], while the third was less potent (EC_{50} 21 μg/ml). Other benzylisoquinoline, aporphine and bisbenzylisoquinoline alkaloids (liensinine, negferine and isoliensinine) isolated from leaves and embryo of lotus exhibited potent anti-HIV activities (EC_{50}, <0.81 μg/ml; TI, >9.9, >8.6, and >6.5 respectively). An aporphine alkaloid, nuciferine also showed EC_{50}, 0.81 μg/ml and TI, 36.

Inhibitory effects of ethanolic extracts of seeds of lotus on herpes simplex type 1 (HSV-1) have been reported by Kuo *et al*. (2005). Ethanolic extracts (100 μg/ml) significantly suppressed HSV-1 replication (IC_{50} for replication, 50.0 μg/ml). The HSV-1 inhibitory effects of subfractions separated from seeds indicated that NN-B-5 (out of nine main fractions: NN-B-1 to NN-B-9) extracted from the bioactive NN-B fraction obtained from butanol had the highest suppresser activity. The ethanolic extracts prepared from fresh seeds showed anti-HSV-1 activity (IC_{50} 62.0±8.9 μg/ml). To understand whether NN-B-5 reduced the acyclovir-resistant HSV-1 propagation or not, the TK HSV-1 was strain employed as target and performed the plaque reduction assay. The NN-B-5, at 50 μg/ml had inhibited TK HSV-1 replication in HeLa cells up to 85.9%. These results suggested clearly that NN-B-5 attenuates the acyclovir-resistant HSV-1 propagation.

Anti-obesity

Ono *et al*. (2006) reported the pharmacological mechanism of the anti-obesity effect of lotus *N. nucifera* leaf extract (NNE) in mice and rats. The effect of leaf extract on digestive enzyme activity, lipid metabolism and theromogenesis in mice treated for five weeks caused concentration dependent inhibition of α-amylase and lipase activities and upregulated lipid metabolism and expression of UCP3 mRNA in C2C12 myotubes. The NNE also inhibited the activities of α-amylase and lipase activity *in vitro*. Inhibition of lipase by NNE (IC_{50}, 0.46 mg/ml) was stronger than α-amylase (IC_5, 0.82 mg/ml). The extracts prevented the increase of body weight, parametrial adipose tissue weight and liver triacylglycerol levels in mice with obesity induced by high fat diet and the UCP3 mRNA expression in skeletal muscle tended to be high.

Future outlook

Bioprospecting of lotus seeds hold promising future as an alternate protein supplement and potential pharmaceutical source. As there are no detailed reports on the toxic effects on long-term consumption of lotus seeds and their products, further experiments are warranted. Although nutraceutical value of lotus seeds is established, further precise exploration of value-added compounds might be beneficial in health promotion. Similar to other edible seeds, lotus seeds are also prone to microbial contamination and spoilage. Studies on contaminants and spoilage microflora (bacteria and fungi), their toxins and control measures will be useful in popularizing the use of lotus seeds. As lotus seeds have potential nutraceutical advantage, blending its flour with other nutritionally rich legumes (e.g. soybean) or millets (e.g. finger millet) will be of immense value to develop low cost proteinaceous and health food supplements to combat malnutrition as well as specific ailment.

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