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MINI REVIEW

Biological Importance and Phytochemistry of *Neptunia oleracea* Lour: A Mini Review

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Neptunia oleracea is an important medicinal plant used in treating various ailments in different countries. The plant contains chlorophyll-related compounds, amino acids, vitamins, *etc*. The presence of pheophorbide *a* and its related compounds make this plant a promising antitumor plant. The plant is used for sewage water treatment and is also utilized in nitrogen fixation in a wetland ecosystem. The present review emphasizes the traditional uses, biological activites and phytochemistry of *N. oleracea*.

Keywords: *Neptunia oleracea* Lour, Medicinal plant, Biofertilizer, Nitrogen fixation.

INTRODUCTION

Neptunia oleracea (Lour) (Fig. 1), an aquatic herb belonging to the family of Fabaceae. It is an aquatic macrophyte commonly consumed as a green leafy vegetable. It is synonym to *N. prostrata* (Lamk) Baillon and *N. natans* (L.f) Druce. It is native to Asia, Africa, and South America [1,2] and used as an edible medicinal plant grown in different countries [3]. In India, the plant is widely distributed in North-East India (Assam, Manipur and Tripura states) [4-6]. It can be found floating or prostrate near the edge of the water reservoir, water channel and ditches with stagnant to slow-moving water of pH 5.4-6.0 [7,8]. *Neptunia oleracea* is a perennial herb which is sometimes grown as an annual aquatic, floating or prostrate near water's edge. This plant has bipinnate leaves and stems made buoyant by their spongy white covering. The young shoot comprising of leaves, spongy stems and young seedpods can be eaten raw and cooked as green vegetables. The flowers are small, sessile and yellow, with the lower ones sterile while the upper ones are bisexual. The fruit is a legume, broadly oblongoid and flat and dehiscent along both sutures. The leaves of *N. oleracea* are very sensitive to touch and can be propagated *via* sexual and vegetative by seed and stem cutting, respectively [9].

The State of Manipur (India) belongs to the Indo-Burma Biodiversity Hotspot and endowed with rich diverse floral and faunal species in diverse. Its biodiversity includes 4000 angiosperms plant species, 430 medicinal plant species, 34 edible fungi species, 500 orchid species and 55 species of bamboos, 40 endemic rice cultivars, 160 fish species and 21 species of migratory aquatic birds [8]. Hence, a rich reserve of indigenous knowledge of herbal remedies for various ailments is expected to have accumulated especially in the rural areas of the country. Thus, in continuation of our interest in documentation of Manipuri medicinal plants for greater benefit to the scientific community [10-12], herein, this review based on the *Neptunia oleracea* locally known as ising-ekaithabi, emphasizes the traditional uses, phytochemistry and biological activities.

Traditional uses of various parts of the plants: This plant grows easily and spreads rapidly on the water surface, buoyed by the white spongy tissue in the stems. Apart from that, this plant is known for its use in human consumption and its usage also extends to herbal medicine [13,14]. The plant as a whole is astringent, sweet, refrigerant, diuretic, antidiarrheal, anthelmintic and anodyne. It is useful in vitiated conditions of pitta, otalgia, cephalalgia, syphilis, burning sensation, dipsia, diarrhea, strangury and helminthiasis [15]. It is cultivated in

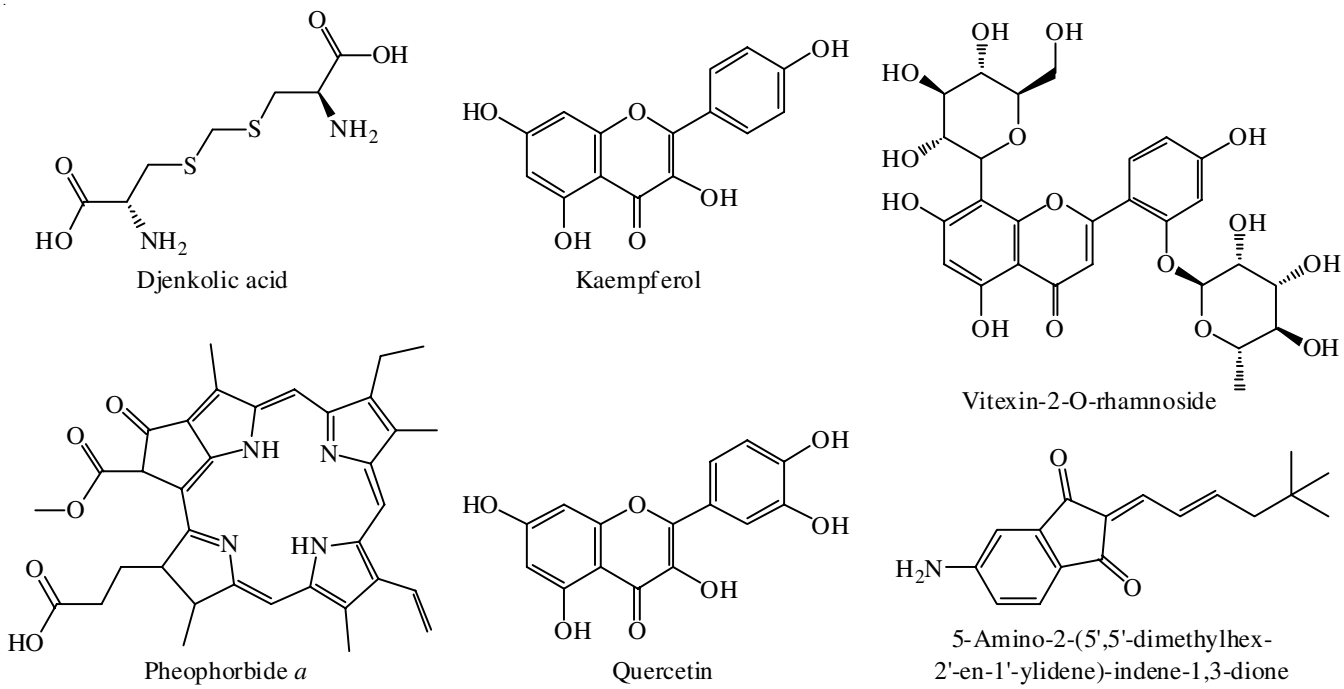

Fig. 1. Representative compounds found in *N. oleracea*

Asian countries for green manures for rice cultivation [16]. The different ethnobotanical uses of this plant in different parts of the World are summarized in Table-1.

Chemical constituents: Lee *et al*. [33] identified 18 metabolites (10 primary metabolites, 5 flavonoids, and 3 phenolic acids) using NMR spectroscopy from the leaf and stem of *N. oleracea* extracts. Further, Lee *et al*. [33] also reported the presence of 37 metabolites from leaves. The other phytochemicals screening of the plant yields various compounds such as chlorophyll-related compounds, vitamins, carotene, *etc*. (Table-2). The shoot and leaves also contain elements like Na, K, Ca, Mg, Fe, Mn, Zn, Pb, Ni, Cu and P [34,35]. The nutritional qualities of *N. oleracea* shoot were also revealed the presence of high amounts of moisture, carbohydrates, fibre, protein, fat, ash and Ca, P, Fe (in the ranges of 38.7 mg, 7 mg, 5.3 mg, respectively). The shoot contained vitamin A, vitamin B_1, vitamin B_2, vitamin B_3 (niacin) and vitamin C (5155 IU, 0.12 mg, 0.14 mg, 8.2 mg and 1.8 mg/100 g, respectively) [32].

Saupi *et al*. [36] reported the mineral and proximate compositions of *N. oleracea* to be sequenced as follows: K > P > Ca

TABLE-1
BIBLIOGRAPHIC DATA ON TRADITIONAL USES OF *N. oleracea*

Entry	Country	Part	Uses	Ref.
1	India	Whole plant	Very good tonic and given for 4-12 days regularly in empty stomach	[17]
2	India	Whole plant	Nose bleeding, sore tongue, diarrhea with blood. White discharged and epilepsy	[18]
3	India	Whole plant	Plant is refrigerant, astringent, the juice of the plant stem is used for earache, and root is for syphilis.	[19]
4	India	Young shoots and leaves	For human consumption as food	[20]
5	India	Leaves and flower	To cure earache and syphilis	[21]
6	India	Young shoot	Crushed twigs are mixed with a paste of unboiled rice and made into large-sized pills, fried and taken orally or with meals to prevent gastritis, acidity and constipation	[22]
7	India	Stem and root	Stem juice is instilled into the ear to cure earache. The root and stem decoction are used to cure gonorrhea.	[23]
8	Manipur, India	Leaves	The leaf is eaten raw in dysentery and intestinal infection	[24]
9	India	Root	Root extract is taken with curd to cure dysentery	[25]
10	Australia	Whole plant	Animal feed	[26]
11	Nigeria	Whole plant	Yellow fever and Guinea worm infection	[27]
12	Nigeria	Stem	The stem is cut and chewed. It is used as a stimulant.	[28]
13	Malaysia	Roots	Necrosis of the nose and hard palate. Treating advanced stage of syphilis.	[3]
14	Vietnam	Shoot, stem, and leaves	Used in uterine infections and discharge	[29]
15	Thailand	Whole plant	A vegetable that is most often used in yam phak ka ched, a spicy and sour salad with kinds of seafood or kaeng som soup	[30]
16	Thailand	Young leaf and stem	Use as a detoxifier to treat fever, food poisoning, and severe allergic reactions.	[31]
17	Laos	Leaf and stem	Eaten raw and as soup	[32]

TABLE-2
PHYTOCHEMICAL CONSTITUENTS OF *Neptunia oleracea*

Entry	Sources	Constituents	Ref.
1	Leave and stem	α-Glucose, β-glucose, sucrose, fructose, malic acid, choline, fatty acids, amino acids, vitexin-2-*O*-rhamnoside, catechin, and derivatives of quercetin, kaempferol and myricetin, gallic acid, 3,4-*O*-dimethylgallic acid, and caffeic acid.	[33]
2	Leaves	Phytosterol, fatty acids, alanine, leucine, valine, α-glucose, β-glucose, fructose, oleanolic acid, catechin, gallic acid, 3,4-*O*-dimetgylgallic acid, caffeic acid, quercetin 3-*O*-α-L-rhamnoside-7-*O*-β-D-glucoside, quercetin 3-*O*-(2, 6-di-orhamnosylglucoside), quercetin-3,7-di-*O*-glucoside, quercetin-3-*O*-rutinoside (rutin), quercetin-3-*O*-glucoronide, quercetin-3-*O*-glucoside, quercetin-3-*O*-xyloside, isorhamnetin-3-*O*-rutinoside, quercetin-3-*O*-arabinoside, quercetin-3-*O*-rhamnoside, quercetin, apigenin-6,8-di-*C*-β-*D*-glucopyranoside (vicenin 2), vitexin-2"-*O*-rhamnoside, vitexin, isovitexin, myricetin-3-*O*-rutinoside, myricetin-3-*O*-glucoside, myricetin-3-*O*-rhamnoside, myricetin-3-*O*-arabinoside, kaempferol 7-*O*-(2"-rhamnosyl)-glucoside, kaempferol-3-*O*-glucoside, kaempferol-3-*O*-arabinoside, kaempferol-3-*O*-rhamnoside, kaempferol, luteolin-6,8-di-*C*-β-*D*-glucopyranoside (leucenin 2), catechin, (–)-epigallocatechin-3-gallate, luteolin-8-*C*-glucoside (orientin), gliricidin-*O*-hexoside, cinnamic acid derivative, monogalloylglucose, syringic acid derivative, protocatechuic acid-*O*-pentoside, digalloylglucose, methylgallate dimer, hexose polymer and hydroxytyrosol hexoside.	[37]
3	Leaves	Pheophorbide *a* and related compounds	[38]
4	Leaves	5-Amino-2-(5',5'-dimethylhex-2'-en-1'-ylidene)-indene-1,3-dione	[39]
5	Seeds	Dichrostachinic acid (*L*-form) and Djenkolic acid (*R*-*R*) form, *N*-Ac	[40]
6	Dried plant	Vitamin C, vitamin E, carotenes, xanthophylls, tannins and phenolics	[41]
7	Leaves	Carotene	[42]

> Na > Mg > Mn > Zn > Cu and moisture content > crude protein > crude fibre > ash > crude fat, respectively. The moisture amount was considerably high in the month of October (86.26% ± 0.62%) and April (83.75% ± 0.55%). Mineral contents for phosphorous (395.67 ± 26.5 and 405.92 ± 43.67 mg/100 g), calcium (348 ± 14.93 and 381.42 ± 9 mg/100 g), Ca/P (0.88 ± 0.09 and 0.95 ± 0.11) and copper (2.58 ± 0.29 and 2.97 ± 0.12 mg/100 g) were substantially lower in April than in October ($p \leq 0.05$). Thus, the edible part of the plants is a rich source of crude fibre, crude protein, ash, mineral (*e.g.* potassium) and calories.

Biological activity

Antimicrobial activity: The antimicrobial activity of leaf extracts of *N. oleracea* [43] was performed by disc agar diffusion method concerning amoxicillin at standard doses against ATCC strains of Gram-positive *Staphylococcus aureus*, *Bacillus subtilis* and Gram-negative *Salmonella typhi*. The IC_{50} values of 15, 18 and 19 mcg/mL were comparable to amoxicillin and gave a clearer picture of the potency and strong antibacterial property of the extracts.

Antibacterial activity: The leaves of *N. oleracea* were reported for the antimicrobial activity [44]. The *in vitro* antimicrobial activity of *N. oleracea* extracts was tested against *Helicobacter pylori* by disc diffusion and agar dilution methods. The minimum inhibitory concentration (MIC) value in petroleum ether was 10.5 ± 0.8, chloroform (10.7 ± 2.0) and methanol (28.3 ± 4.1).

Antioxidant activity: For the stems and leaves, the antioxidant activity of *N. oleracea* has an IC_{50} of 29.72 and 35.45 µg/mL, respectively [45]. The scavenging activity of DPPH free radicals was analyzed to determine the antioxidant activity of the dried stem and leaves of *N. oleracea*, which has IC_{50} of < 30 mg/mL. Its stems and leaves showed a high scavenging activity for DPPH free radicals [33]. The leaf methanolic extract exhibited an antioxidant activity of 13.1 mg butylhydroxyanisole (BHA) equivalent/g dry weight. Extracts from *N. oleracea* exhibited five peaks (P-1, P-2, P-3, P-4, and P-5), indicating their antioxidant activity in HPLC.

α-Glucosidase inhibition activity: *N. oleracea* showed a potential activity of α-glucosidase inhibition with an IC_{50} of 19.74 and 19.09 µg/mL for stems and leaves, respectively [46]. The IC_{50} of *N. oleracea* extracts exhibited higher α-glucosidase inhibition than that of quercetin. *N. oleracea* leaves exhibited stronger α-glucosidase inhibition activity than its stems. The difference in the α-glucosidase inhibition activity between stems and leaves was significant ($p < 0.05$) [33].

Analgesic and anti-inflammatory activity: Paul *et al.* [39] studied the anti-inflammatory and analgesic activities of *N. oleracea*. The methanolic extracts (200 mg/Kg) highly suppressed writhing response induced by acetic acid. The results of the samples were more significant ($p < 0.001$) than those of the control. The extracts exhibited a higher percentage of protection than other samples and aspirin (3.8 ± 0.387). An extract exhibited the average writhing of 4.2 ± 0.7348. Two samples showed higher anti-inflammatory activities than the control after 3 h. Similar to diclofenac sodium, both the extracts continuously provided a substantial percentage of protection after 4 h.

Antitumor activity: Bhumireddy *et al*. [47] investigated the antiproliferative activities of the extracts of methanolic leaves of *N. oleracea* on a haematological cancer cell line. The extract activity was determined using the apoptosis assays and cell cycle analysis. Additionally, the effect of extracts on PErk1/2 and *c*-Myc modulation was analyzed. In cancer cells, the extracts caused cell death and spared normal cells. Cleaved PARP and BCL-2 levels increased and decrease, respectively, after the treatment, which caused a decrease in pERK1/2 protein and *c*-Myc levels. Nakamura *et al*. [38] isolated pheophorbide a (PPBa) and related compounds (EtPPBa, PPBb, MePPBa, EtPPBb and 10-OHPPBa) from *N. oleracea* leaves. PPBa and PPBb exhibited an excellent inhibition activity towards the activation of Epstein-Barr virus at a concentration of 5 µM with IC_{50} of 3.3 and 4.5 µM, respectively. The inhibition potentials are comparable to those of curcumin (IC_{50} = 3.1 µM), a natural

antitumour promoter acquired from turmeric. Thus, *N. oleracea* can be utilized as a promising antitumour promoter.

Antiulcer activity: The antiulcer properties of *N. oleracea* ethanol extract of leaves were evaluated in pyloric ligated rats [48]. In this method, the rats (24 h fasted) were subjected to pyloric ligation for 4 h. The leaves of *N. oleracea* has shown antiulcer properties in pyloric ligated rats by increasing mucin content, free and total acidity, pH, and decreased ulcer index, the volume of gastric juice, total proteins and pepsin content. The experimental results were significant when compared with control groups using ranitidine.

Biofertilizers: *N. oleracea* fixes their nitrogen *via* a symbiotic relationship with soil bacteria stored in specialized root nodules. A variety of bacteria have been isolated from such nodules, particularly from *N. oleracea*, including a species closely related to rhizobium, *Allorhizobium undicola* [34] and the α-proteobacterium *Devosia neptuniae* [48,49]. Two studies conducted in Brazil have shown different δ15N for *N. oleracea*, indicating the content of nitrogen obtained from the atmosphere. In these studies, nodulation on *N. oleracea* was abundant; however, in a case, the δ15N value was similar to non-legumes and considerably high [50,51]. This variation may result from different amounts of mineral nitrogen available in the water where the plant was growing or the nitrogen fixing capacities of nodulating bacteria.

Water treatment: *N. oleracea* is widely used in reduction or decontamination of toxic contaminants, including arsenic, in waters in countries such as Malaysia [52]. With a short life cycle and easy growth, *N. oleracea* is promising for phytoremediation and suitable for planting in areas with contaminated water [53]. From the comparison with above ground tissues, *N. oleracea* roots can accumulate heavy metals [54]. Suppadit *et al*. [13,55] treated the effluent obtained from shrimp farms by employing *N. oleracea*. Atabaki *et al*. [52] assessed the removal efficiency of *N. oleracea* for the phytoremediation of arsenic polluted water. The results of micromorphological analyses confirmed the tolerance of *N. oleracea* of up to 30 ppm for the arsenic treatment. The chlorophyll and gas exchange content results showed serious arsenic damage at doses of >30 ppm. Additionally, the highest arsenic removal and accumulation efficacies were observed in 30-60 ppm. The analyses of lipid and proline peroxidation contents confirmed the tolerance of water mimosa for up to 30 ppm of arsenic. X-ray spectroscopy and scanning electron microscopy confirmed arsenic accumulation, indicated by water mimosa tissue deformation. Water mimosa is a dependable bioremediator for arsenic removal from water systems.

Toxicology: Bhoomannavar *et al*. [56] reported an acute toxicity of ethanol extracts of *N. oleracea* leaves in mice. Various groups comprising two mice each were created. These mice were orally administered the 0.5, 1, 1.5, and 2.0 g/kg p.o. ethanol extracts. The control group only received propylene glycol (vehicle). For the initial 4 h and for the subsequent 6 h, the general behaviour and mortality of the mice were observed continuously and intermittently, respectively. Then, after 24 h and 48 h the oral administration, behaviour and mortality were observed. No behavioural changes were observed and the leaf extracts indicated no mortality after orally administration with the maximum dose of up to 2 g/kg body weight. Acute toxicity was studied according to the OECD guidelines in Swiss albino mice (n = 5) and Wistar albino rats of both sexes. The doses used were 2000 mg/kg body weight. Within the stipulated period, the results did not show mortality or morbidity in these animals [43]. Thus, up to 2000 mg/kg body weight is safe for consumption.

Conclusion

The review highlights the prospect of aquatic legume *Neptunia oleracea* Lour as an essential food, medicine and biofertilizer. More studies of other constituents and their activities need to be explored, which would be useful in promoting research aiming at the development of new agents for medicinal and agro-industries. Also, identification of other bioactive components would be fruitful for further research. This review would be useful for advocating its increased utilization as vegetable crop and medicinal plant.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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