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RESEARCH ARTICLE

TRADITIONAL AND BIOLOGICAL USES OF *NEPTUNIA OLERACEA* LOUR: AN OVERVIEW

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ABSTRACT

The present review highlights the multifaceted prospect of aquatic legume *Neptunia oleracea* Lour as food, medicinal plant and biofertilizer. The bibliographic data of the plant summarizes the parts used, its uses and country with references. The common chemical constituents of the plant responsible for the treatment of various ailments are given. It also describes the methodology and magnitude of its various biological activities. High nutritious value, high antioxidant an activity shown by the plant makes these plants an ideal food. The plant is also used as medicinal plant in various ailments in different countries. Pheophorbide *a* and its related compounds plant makes this plant a promising antitumor plant. The plant can also be used in sewage water treatment plants. The symbiotically associated bacteria efficiently fixed atmospheric nitrogen which significantly increases the N_2 status of the oligotrophic soil of wetland ecosystem.

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INTRODUCTION

Neptunia oleracea (Lour.) is an aquatic legume (Order – *Fabales* and Family – *Leguminoceae*) native to several continents of the humid tropics of both hemispheres particularly in Asia, Africa and South-America (Windler, 1966 and Gen and Eiji, 1974). The exact origin of the species is uncertain. It grows wild and cultivated as vegetable throughout Southeast-Asia, particularly in Indo-China and Thailand (Paisooksantivatana, 1993). In India, the plant is distributed in North-East India (Assam, Manipur and Tripura) (Sarma & Saikia, 2010; Sinha, 1996 and Koushik and Dutta. 2007). Its synonym names are *N. natans* (L.f.) Druce and *N. prostrata* (Lam.). It is commonly known as "water mimosa" and "water sensitive plant" in English, "garden puff" (United States), "neptunie potegère" (France), "juqueri manso" (Portugal), "kemon" (Indonesia), "kemon air" (Malaysia), "kânhchhnaèt" (Cambodia), "('phak) kas'ééd" (Laos), "phakkrachet" (Thailand), "rau nh[us]t" (Vietnam) and in India, "alambusa" Sanskrit, "lajjalu" Hindi, "ising-ekaithabi"(Manipur) and "nidrayam" Kanada.

Description

Neptunia oleracea is a perennial herb which is sometimes grown as an annual aquatic, floating or prostrate near water's edge (Fig. 1). The stems are terete, measure upto1.5 m long, rarely branched, become detached from the primary root system, form spongy-fibrous swollen internodes (to float) and produce fibrous adventitious roots at the nodes when growing in water. The leaves are arranged alternate, bipinnate and with 2-3(-4) pairs of pinnae. The petiole is 2-7 cm long and angled. The rachis is 3.5-8cm long and angled. The rachis of pinnae is 2.5-6.5cm long and winged. There are 8-20 pairs of leaflets per pinna which are oblong, measuring 5-1 mm×1.5-3.5mm, asymmetrical, hairless or with sparsely ciliate margins. The inflorescence is an axillary, erect or slightly nodding solitary spike which is obovoid in bud and 30-50 flowered. The peduncle is 5-30cm long. The flowers are small, sessile and yellow, with the lower ones sterile while the upper ones are bisexual. The bisexual flowers are with bell-shaped sepal of 2-3 mm and 5-lobed. The 5 regular petals are free and 3-4.5mm long. The 10 free stamens are 6-9 mm long. The pistil is up to 9 mm long and usually exserted beyond the stamens. The fruit is a legume, broadly oblongoid and flat, measuring 2-3 cm 2 1 cm and dehiscent along both sutures. The fruit stalk is longer than the persistent sepal. There are 4-8 seeds which are ovoid compressed, measuring 4-5mm3 2.5-3.5mm and brown (Windler, 1966). The leaves of *N. oleracea* are very sensitive to a touch (Darwin, 1880).

Traditional uses of various parts of the plants

Neptunia oleracea is an important medicinal plant. The plant as a whole is astringent, sweet, refrigerant, diuretic,

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antidiarrhoeal, antihelmintic and anodyne. It is useful in vitiated conditions of *pitta*, otalgia, cephalalgia, syphilis, burning sensation, dipsia, diarrhea, strangury and helminthiasis (Warrier *et al.*, 1995). It is cultivated in Asian countries for green manures for rice cultivation (Subha Rao *et al.*, 1995). The different ethnobotanical uses of this plant in different parts of the World are summarized in Table 1.

Chemical constituents

The phytochemical compounds of *N. oleracea* reported in literatures are summarized in Table 2.

a. Floating mat with white spongy aerenchymatous tissue

b. Erect inflorescence

c. Mature pod

d. Dehisce pods showing seeds

Fig.1 (a-d). ***Neptunia oleracea*** **showing different stages of its life cycle**

Table 1. Bibliographic data on traditional uses of *N. oleracea*

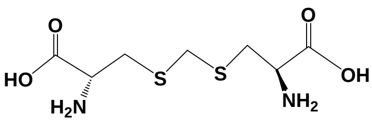
Entry	Part use	Uses	Country	Reference
1.	Root	Root extract is taken with curd to cure dysentery	India	Dinesh Jadhav, 2006.
2.	Young shoots and leaves	For human consumption as food	India	Jugindro Singh, A., 1982
3.	Whole plant	Nose bleeding, sore tongue, diarrhea with blood. White discharged and epilepsy	India	D'Souza Marie, 1985.
4.	Leaves and flower	To cure earache and syphilis	India	Santosh kumar and Satya Narain, 2010.
5.	Young shoot	Crushed twigs are mixed with paste of un- boiled rice and made into large sized pills, fried and taken orally or with meals to prevent gastritis, acidity and constipation	India	Himangshu Bikash Das *et al.*, 2009
6.	Whole plant	Plant is refrigerant, astringent, the juice of the plant stem is used for earache, and root is for syphilis. The young stem is eaten as a pot herb and pods are consumed as vegetable	India	R. Vardhana, 2008
7.	Stem and root	Stem juice is instilled into the ear to cure earache. The root and stem decoction is used to cure gonorrhea.	India	Dangwal *et al.*, 2010.
8.	Leaves	Leaf is eaten raw in dysentery and intestinal infection	Manipur, India	Singh, H.B *et al.*, 2003.
9.	Whole plant	Animal feed	Australia	Wildin, JH *et al*., 1996.
10.	Whole plant	Yellow fever and Guinea worm infection	Nigeria	Ita, EO, 1994.
11.	Stem	The stem is cut and chewed. It is used as stimulant.	Nigeria	Igoli et al., 2005.
12.	Roots	Necrosis of the nose and hard palate. Advance stage of syphilis	Malaysia	Paisooksantivatana, Y., 1993.
13.	Shoot, stem and leaves	Used in uterine infections and discharge	Vietnam	Ogle, 2003.
14.	Whole plant	Vegetable that is most often used in *yam phak ka ched*, a spicy and sour salad with sea foods or *kaeng som* soup	Thailand	Jones and Csurhes, 2008.
15.	Young Leaf and Stem	It is used medicinally as a detoxifier to treat fever, food poisoning, and severe allergic reactions.	Thailand	Salguero, C.P., 2003.

Table 2. Phytoactive constituents of *Neptunia oleracea*

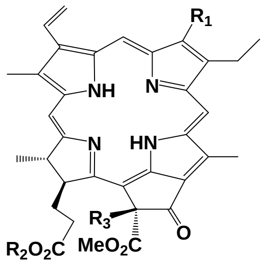
Entry	Sources	Constituents	Reference
1.	Leaves	Pheophorbide *a* and related compounds	Nakamura, *et al.*, 1996
2.	Seeds	Dichrostachinic acid(*L*-form) and Djenkolic acid(*R-R*) form, *N*-Ac	Krauss and Reinbothe, 1973
3.	Shoot and leaves	Na, K, Ca, Mg, Fe, Mn, Zn, Pb and Ni	Prusty, 2007
4.	Leaves	Na, K, Mg, Ca, Fe, Cu, Zn and P	Abulude, F.O., 2005
5.	Dried plant	Vitamin C, vitamin E, carotenes, xanthophylls, tannins and phenolics	Chanwitheesuk, A., 2005
6.	Leaves	Carotene	Tee and Lim., 1991.

The energy value of *N. oleracea* is 184 kj/100 g. The edible portion of shoots contain (per 100 g) (Jones and Csurhes, 2008).

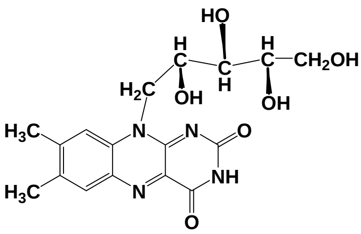
Entry	Constituents	Percentage
1.	Moisture	89.4 g
2.	Protein	6.4 g
3.	Fat	0.4 g
4.	Carbohydrates	0.8 g
5.	Fibre	1.8 g
6.	Ash	1.2 g
7.	Ca	38.7 mg
8.	P	7 mg
9.	Fe	5.3 mg
10.	Vitamin A	5155 IU
11.	Vitamin B_1	0.12 mg
12.	Vitamin B_2	0.14 mg
13.	Niacin(Vitamin B_3)	8.2 mg
14.	Vitamin C	1.8 mg



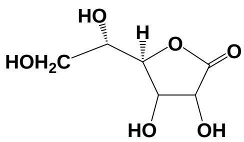
Djenkolic acid



Pheophorbide *a*



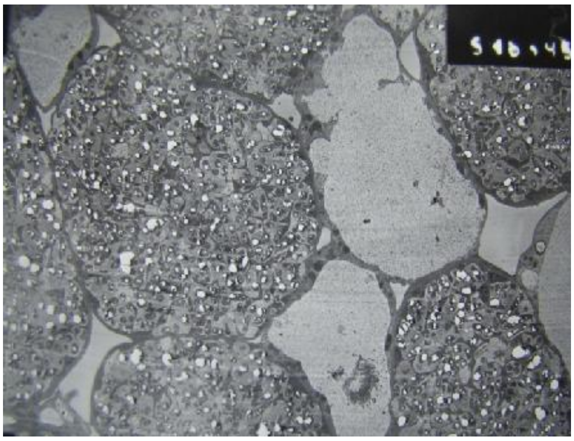
Vitamin B_1



Vitamin C

Fig. 2. Some Phytoactive compounds of *Neptunia oleracea*

3a. Root nodules



3b. Transmission electron micrograph showing a section of root nodule with endosymbiotic bacteria. (1400x).

Biological activity

Anti-tumour activity

Nakamura *et al.,* 1996 reported the isolation of Pheophorbide *a* (PPB*a*) and its related compounds (PPB*b*, EtPPB*a*, EtPPB*b*, MePPB*a*, 10-OHPPB*a***)** as a possible anti-tumor promoters in the leaves of *N. oleracea*. Where, PPB*a* and PPB*b* showed marked inhibitory activity toward EBV activation at a concentration of 5μM and IC_{50} were 3.3 and 4.5μM respectively. These inhibitory potentials are comparable to that of curcumin (IC_{50} = 3.1μM, a representive anti-tumor promoter from turmeric. (Huang, *et al.,* 1998). Wherease, the inhibitory activities of others compounds were 5 to 10 times lower than those of PPB*a* and PPB*b*. As chlorophyll related compounds such as PPB*a* and 10-OHPPB*a* are known for dietary photosensitizers (Endo, *et al.,* 1982). The photocytotoxicity of PPB*a* and 10-OHPPB*a* in Raji cells was evaluated, the logarithmic values of the 50% lethal concentrations (pLC_{50} s) of both exhibited potent photocytotoxicity (pLC50> 6) with irradiation. In the lipid peroxidation test, both PPB*a* and 10-OHPPB*a* significantly accelerated lipid peroxidation (OD_{532} = 0.574 and 0.426, respectively). Subsequently, the enhancing effect by photo-irradiating PPB*a* and 10-OHPPB*a* on the inhibitory potent towards EBV activation was examined. PPB*a* had an inhibitory effect on EBV activation even under dark conditions. Although, PPB*a* and 10-OHPPB*a* exhibited a similar photosensitizing effect, the inhibitory effects with irradiation were distinct from each other. Chlorophyll-related compounds have been reported to possess antioxidative (Cahyana, *et al*., 1993) and anti-inflammatory activity (Hirota, *et al*., 1993).

Antioxidant activity

Neptunia oleracea exhibited antioxidant activity (Abulude, 2005). The antioxidant activity of methanolic extract of leaves was studied using the β-carotene bleaching method (Thalang, *et al.,* 2001), the methanolic extract of leaves showed activity of 13.1mg butylhydroxyaniso (BHA) equivalent/g dry weight. Also, extract from *N. oleracea* give five peaks (P-1, P-2, P-3, P-4 and P -5), which showed an antioxidant activity in HPLC analysis.

Anti-bacterial

The leaves of *Neptunia oleracea* possess antimicrobial activity (Uyub, et al., 2010). The *in-vitro* antimicrobial activity of *N. oleracea* extracts were 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 highest in methanol (28.3±4.1).

Bio fertilizers

Wetlands are often subject to annual net losses of N from the system via leaching of the soil, which are not balanced by inputs of N via the mineralisation of organic matter. These oligotrophic ecosystems are largely dependent on N inputs from biological nitrogen fixation, and legume-rhizobial symbioses are some of the main contributors. In tropical wetlands, many of the plant species are nodulated legumes. These flooding tolerant leguminous plants have shown that not only will these fix substantial amounts of N2 whilst flooded, but there may even be positive selection pressure for them (Loureiro *et al.,* 1994; James *et al.,* 1992a). This selection pressure may be due to the inherently low N-status of the heavily leached soils in tropical wetlands, and also because, under flooded conditions, there is a decrease in the mineralisation of organic matter, which consequently results in a shortage of available N (Bennett & Albrecht, 1984; Walter & Bien, 1989; Barrios & Herrera, 1993b). *N. oleracea* fix their own nitrogen via a symbiotic relationship with soil bacteria stored in specialised root nodules (Fig 3 a & b). A variety of bacteria have been isolated from such nodules, particularly from *Neptunia oleracea*, including a species closely related to Rhizobium, *Allorhizobium undicola* (de Lajudie et al., 1998a), and the alphaproteobacterium *Devosia neptuniae* (Rivas et al., 2002, 2003). In addition to these ‘exotic’ bacterial species, more ‘conventional’ rhizobia have also been isolated from *Neptunia* nodules, for example *Rhizobium tropici* strains UPRM8033 and DUS239 from *Neptunia plena* (Zurdo-Pineiro *et al*., 2004), *Labrys*, Liujia-146T (Vasilyeva & Semenov, 1984; Yi-Ju Chou *et al*., 2007; Subha Rao *et. al.,* 1995 reported from India that nodules on this legume as *Rhizobium* sp and Romesh S., *et al.,* 2010. When cultivated as a vegetable, highest yields of these plants are achieved when an effective rhizobium is used for inoculation (Yanasugondha & Buranakarl 1981). Schaede (1940) describe nodules as being formed on adventitious roots arising from floating stems. He argued against *Neptunia* bearing true stem nodules. Subha Rao *et al* (1995) studied *Neptunia-Rhizobium* symbiosis in greater details. In two studies in Brazil, there were differences in the δ15N values for *N. oleracea* which are an indication of the amount of nitrogen derived from the atmosphere. In both studies, nodulation was present and abundant on *N. oleracea* but in one case the δ15N value was surprisingly high and not dissimilar to non-legumes (Kern et al. 2000; Kreibich et al. 2006). This variation may be attributed to differences in nitrogen fixing ability of the nodulating bacteria or the amount of mineral nitrogen available in the water in which *N. oleracea* was growing.

Not only are wetland legumes ecologically important, but they are also of agricultural importance. For example, there has been much recent interest in using nodulated wetland legumes, particularly stem-nodulated ones, as green manures in the cultivation of lowland rice due to their high rates of N2 fixation under flooded conditions (Dreyfus *et al.*, 1985; Morris *et al.,* 1989; Ladha *et al,* 1992a). As N_2-fixing legumes contribute significantly to the N-balance of tropical wetlands (Salati *et al.,* 1982; Barrios & Herrera, 1993b), they are of obvious importance in studies on their preservation and sustainability (Sprent & Sprent, 1990; Sprent, 1995).

Water treatment

Suppadit *et al*., 1995 reported the treatment of effluent from shrimp farm using *Neptunia oleracea*. Again, the quality of water contaminated with distilled slop was evaluated after treatment with *Neptunia oleracea* (Suppadit *et al.,* 2008). The experiments were done in artificial housing and were carried out in 5 x 3 factorial arrangements with four replications for the water quality indicators, the biomass of water mimosa and heavy metal contamination. Concentrations of 0% (control), 5%, 10%, 15% and 20% distilled slop in contaminated water at 10, 20 and 30 d treatment periods were evaluated. The initial biomass of the water mimosa for all treatments was 0.200 kg per 50 L of effluent. The removal efficiency of suspended solids at all concentrations of distilled slop and treatment time was 89.3 - 96.3%. The removal efficiencies of biological oxygen demand and chemical oxygen demand at all concentrations of distilled slop were optimal at 10 d treatment (42.9 - 70.4% and 8.27 - 25.0%, respectively); higher efficiency was obtained when the concentration of the distilled slop decreased from 20% to 0%. The removal efficiency of total dissolved solids and total kjeldahl nitrogen and the pH neutral control efficiency were not found in this study. pH, total dissolved solids and total kjeldahl nitrogen increased with treatment time and with an increase in the concentration of distillery slop. The biomass of water mimosa at all concentrations of distilled slop increased at 10 d but tended to decrease with longer treatment period and an increase in the concentration of the distilled slop. The biomass of water mimosa at all concentrations of distilled slop was higher than that of the control at all treatment times. Nitrogen volatilization and transformation affected the kjeldahl nitrogen (KN) removal rate of distilled slop at all concentrations, whereas adsorption of nutrients in the water mimosa had no effect on the KN removal rate because the death rate of water mimosa was high. The concentration of the heavy metals Pb, Cd and Hg in water contaminated with distilled slop was acceptable.

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