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Pharmacognostic & Preliminary Phytochemical Investigations of *Neptunia prostrata* L.

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ABSTRACT

The present study was taken to evaluate the pharmacognostic and phytochemical profile of *Neptunia prostrata* L., to access the quality standards. Various parameters like macroscopic and microscopic characters, physical constant values, behavior of powdered drug on treatment with different chemical reagents, fluorescence characteristics (under UV lamp at 366 nm) of the powder after treatment with different chemical reagents etc. were studied. Preliminary phytochemical screening and thin layer chromatography of different extractives has also been performed. These studies will help in future for identifying this plant for further research.

Keywords: *Neptunia prostrata* L., Microscopic characters, Powder analysis, Phytochemical screening, Thin Layer chromatography.

1. Introduction

Neptunia prostrata L. (Synonym: *Neptunia oleracea*. Family: Mimosaceae) is a miniature aquatic herb that floats by its white spongy structure. It has soft swollen stem with slender fibrous roots in flower bearing nodes [1]. As a medicinal plant it is well-known to the herbal doctors of Tripura and other states of North-Eastern region. The tribal people of Tripura cultivate this plant both as vegetables as well as medicinal plant. They prepare various tasty dishes with this vegetable. Aqueous extract of this plant is used in the treatment of jaundice, sores of tongue, diarrhoea with bleeding, epileptic convulsions, earache etc [2]. The paste of this plant is applied in leucorrhoea. Literature survey also reflects the laxative property of its pod [3]. The plant is considered refrigerant and astringent [4]. It is used to treat fever by applying the infusion of the whole plant on the body of the patient. Juice of the stem is dropped into ear to relieve earache. Roots are employed in late stage of syphilis. The powder root is applied over the nose to treat Syphilitic ulcer of the nose while decoction is taken to treat Syphilis [5]. Considering its versatile activities, Pharmacognostic and Phytochemical evaluation was carried out to identify the authentic genus and species. In the present investigation, we studied different pharmacognostical profiles along with thin layer chromatographic characterization of different extracts to fix some specific characteristics feature for quality standardization of this plant.

2. Materials & Methods**2.1 Plant Material**

The fresh *Neptunia prostrata* (whole plant) were collected from the local market (Lake Chowmuhani Bazar, Agartala, West Tripura) in the month of November, 2012 and identified by Prof. B. K. Datta, Taxonomist, Dept. of Botany (Plant Taxonomy & Biodiversity Research Laboratory), Tripura University. A voucher specimen of dried sample (NP001/TU/PHARMA/2012) has been deposited to the Dept. of Botany, Tripura University for the future reference and experiment.

2.2 Drugs & Chemicals

All the reagents and Chemicals used were laboratory and analytical grade and obtained from Rankem Lab. Okhla Industrial area, New Delhi. Chemicals were used without any further purification.

2.3 Pharmacognostic studies [6-12]

The macroscopic or organoleptic characters of the plant including size, shape, colour, odour, taste etc. were observed. The microscopic characteristics of leaf and stem (both intact and powdered) were studied. Powder analysis of both leaf and stem with different reagents were performed and fluorescence properties under UV lamp at 366 nm were observed. The physicochemical characteristics including moisture content, ash value etc. also determined.

2.4 Phyto-chemical studies [6-12]

Plant materials (whole plant) were dried in shaded floor and are powdered in a hand mill. Then the powdered materials were extracted with different solvents in a Soxhlet apparatus and concentrated in vacuum to get the dried crude extract. Extractive

values were calculated to get % yield for each extract. All the different extracts were subjected to a number of proximate qualitative phyto-chemical tests. Finally, thin layer chromatography of all the extracts with different standard solvent system was performed.

3. Results & Discussion

The macroscopic profile (Fig-1) i.e. organoleptic characteristics are given in the table-1, microscopic characteristics including leaf constants shown in the table-2, whereas TS of stem and leaf is shown in the Fig: 2 & 3 respectively. Powder microscopy of leaves and stems are shown in the Fig: 4 & 5 respectively. The powder characteristics with chemical reagents and fluorescence analysis under UV lamp are given in the table-3; different physical constants like ash value, moisture contents and extractive values are given in the table-4 & 5 respectively. Preliminary phytochemical screening and thin layer chromatography is reported in the table- 6 & 7 respectively.

Table 1: Morphological Characteristics of *Neptunia prostrata* L.

Habit	Annual, floating in water.
Colour	Leaf- green; Stems and roots- brown.
Odour	Characteristic.
Taste	Astringent.
Surface	Smooth, sign of shrinkage after drying.
Leaves	Abruptly bi-pinnate; stipule obliquely ovate-cordate, acute; common petioles 3.2-4.5 cm long; pinnate 2-3 pairs, opposite, shortly stalked.
Leaflets	8-15 pairs, sessile, 8-13 by 2.5-3 mm, narrow oblong, obtuse glabrous.
Flowers	Minute, sessile, in oblong beads 1.3-1.9 cm long; peduncles 7.5-15 cm long, stout erect tapering, glabrous, with frequently a membranous ovate-cordate bract about the middle.
Stem	Elongate soft, swollen, not much branched.
Root	Emitting, slender fibrous roots in abundance from leaf and flower bearing nodes.
Pods	Stalked deflexed, 1.3-2.5 by 0.8 cm, slightly curved, oblique at the base, depressed between the seeds, beaked, dry, and dehiscent soon by the upper structure.
Seeds	6-9, ovoid-oblong, slightly compressed, 5 by 4 mm, brown in colour.



Fig 1: Fresh *Neptunia prostrata* L. (Whole plant)

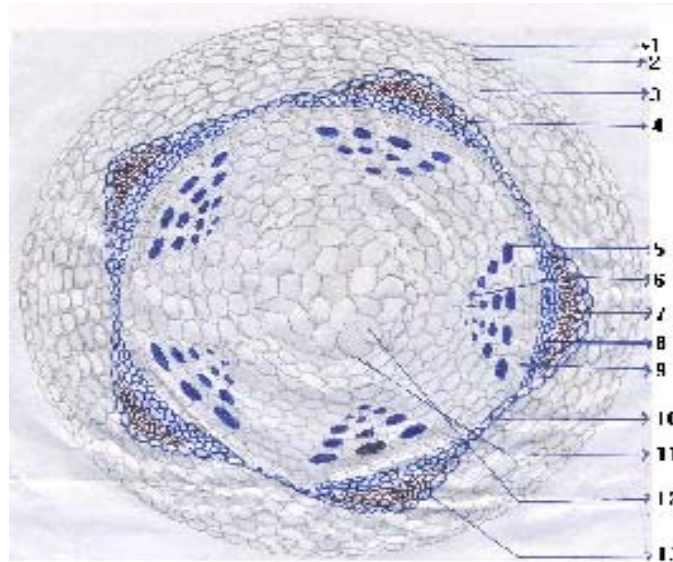


Fig 2: Transverse Section of Stem of *Neptunia prostrata* L.

1-epidermis, 2-hypodermis, 3-general cortex, 4-starch sheath, 5-metaxylem, 6- protoxylem, 7-bundle cap, 8-phloem, 9-cambium, 10- medullary ray, 11-pith, 12-benzene shaped cell-sap with tannin solution, 13-starch granules present in starch sheath.

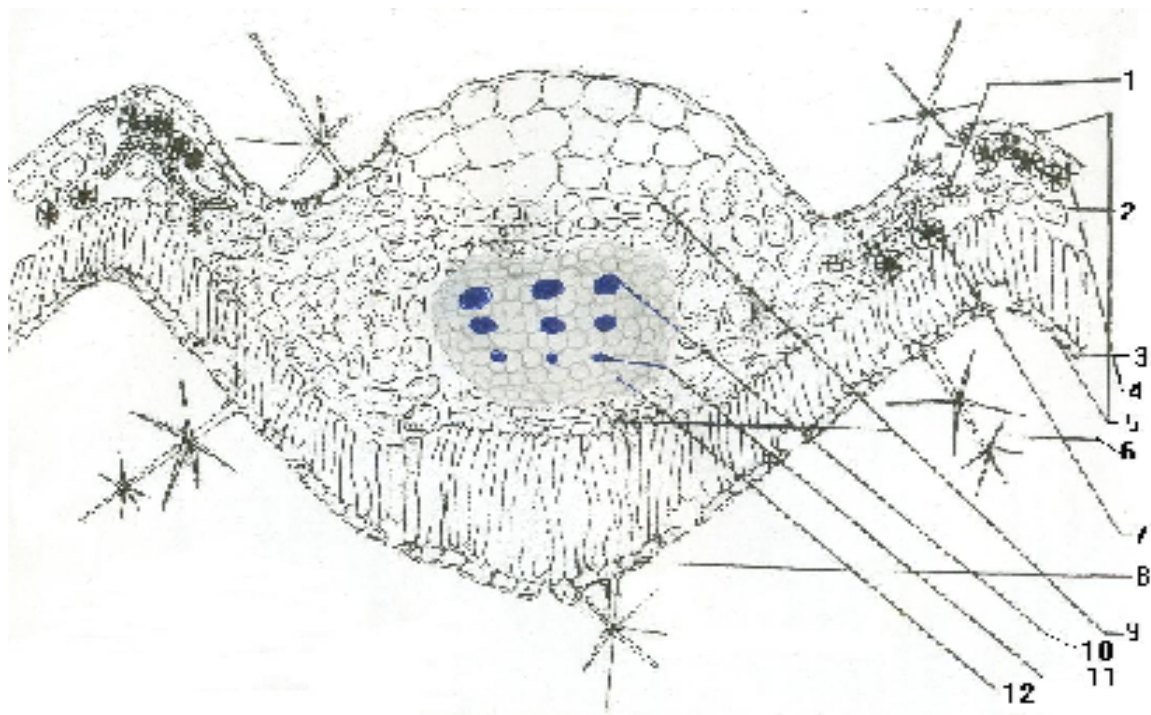


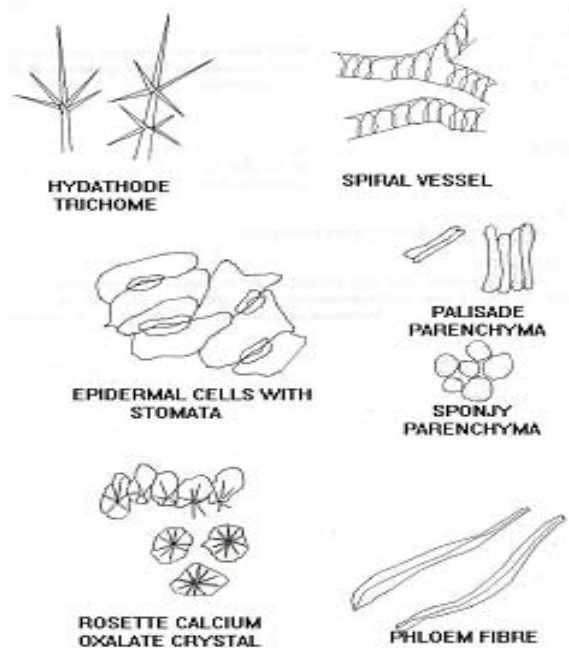
Fig 3: Transverse Section of Leaves of *Neptunia prostrata* L.

1-vessel, 2-spongy parenchyma, 3-upper epidermis, 4-lower epidermis, 5-cuticle containing hydathode trichome, 6-hydathode, 7-rosette calcium oxalate crystals, 8- upper epidermis, 9-collenchyma, 10-meta xylem, 11-proto xylem, 12-phloem.

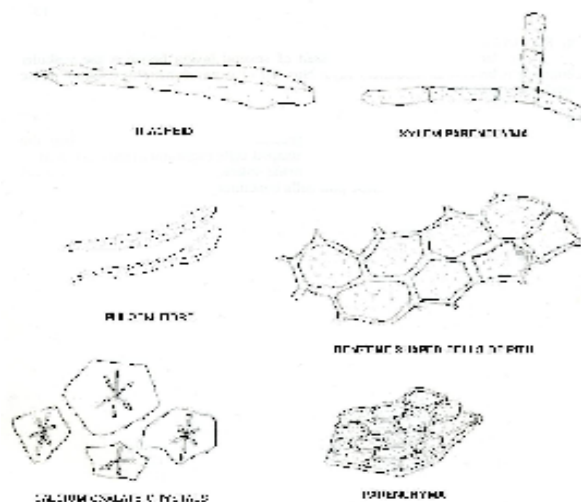
Table 2: Various Leaf constants of *Neptunia prostrata* L.

Sl. No.	Parameters	Range		Mean	
		Up. Ep.	Lo. Ep.	Up. Ep.	Lo. Ep.
1.	Stomatal Index	23.4 – 25.50	25.8 – 27.00	24.45	26.40
2.	Vein islet number	10.2 – 36.70	20.4 – 30.60	23.45	25.50
3.	Palisade ratio	7.5 – 9.00	Nil	8.25	Nil

(Up. Ep.: Upper Epidermis; Lo. Ep.: Lower Epidermis)

**Fig 4:** Powder Microscopy of Leaves of *Neptunia prostrata* L.

1. Trichome: hydathode. **2. Spiral vessel** as xylem components. **3. Phloem fibre:** longitudinal shape of about 240-336 μm . **4. Epidermal cells with stomata:** rubiaceous stomata where subsidiary cells are parallel to the stomata. **5. Mesophyll tissue:** (a) palisade and (b) spongy parenchyma. **6. Calcium oxalate crystals:** rosette crystals.

**Fig 5:** Powder Microscopy of Stems of *Neptunia prostrata* L.

1. Xylem Components: a. **Tracheids** with wide cellular axis and needle like axis of about 184.8 μm . b. **Xylem parenchyma** with parenchymatous cells having pit in between. **2. Phloem Components:** phloem fibre of about 137.5 μm . **3. Pith** containing benzene shaped cells. **4. Calcium Oxalate Crystals:** rosette crystals. **5. Parenchyma** in cortex.

Table 3: Behavior of powder after treatment with different chemical reagents

CHEMICALS USED	LEAF POWDER		STEM POWDER	
	Visible Light	UV Light (366 nm)	Visible Light	UV Light (366 nm)
Powder as such	Dark Green	Black	Creamy White	Black
Powder + Conc. HCl	Reddish	-----	-----	Light green fluorescence
Powder + NaOH	Yellowish	Light green	Yellowish	Light green
Powder + Conc. HNO ₃	Orange	-----	Orange	-----
Powder+ H ₂ SO ₄	Brown	Light green fluorescence	Cheery red	Florescent green
Powder + I ₂ Solution	-----	-----	-----	-----
Powder + Glacial acetic acid	Yellow	Saffron colour fluorescence	Yellow	Saffron colour fluorescence
Powder + NH ₃	Yellow	Florescent green	Yellow	Florescent green
Powder + Mayer's reagent	-----	-----	-----	-----
Powder + Picric acid	-----	-----	-----	-----
Powder+FeCl ₃	Yellow green	-----	Yellow green	-----
Powder + 5% KOH	Yellowish	Light green	Yellowish	-----
Powder + AgNO ₃	-----	-----	-----	-----
Powder + Lead Acetate	-----	-----	-----	-----
Powder + Liebermann Burchard reagent	Light green	Light orange fluorescence	Brown	Creamy white fluorescence
Powder + Aniline H ₂ SO ₄	Creamy light green	Saffron fluorescence	Yellow	Creamy white fluorescence
Powder + + Ethanolic KOH	Brown	Saffron fluorescence	Yellow	Light orange
Powder + Methanolic KOH	Yellow green	Saffron fluorescence	Yellow	Mud Yellow fluorescence
Powder + Methanolic NaOH	Yellow green	Saffron fluorescence	Yellow	Mud Yellow fluorescence
Powder + Ethanolic NaOH	Light green	Saffron fluorescence	Light yellow	Light green fluorescence

Table 4: Ash value & Moisture contents of *Neptunia prostrata* L.

ASH VALUE		LEAF	STEM
Total Ash		9.404 ± 0.12 %	9.621 ± 0.04 %
Acid (HCl) insoluble ash		0.860 ± 0.04 %	0.274 ± 0.01%
Alcohol insoluble ash		8.075 ± 0.07 %	6.505 ± 0.12%
Water insoluble ash		4.596 ± 0.04 %	3.152 ± 0.14 %
MOISTURE CONTENT			
LEAF	4.848 ± 0.03 %	STEM	4.560 ± 0.04 %

Table 5: Extractive values of *Neptunia prostrata* L. with different solvents

Extracts	Percentage Yield (w/w)	Colour	Fluorescence under UV
MeOH	17.5 %	Dark green	Dark red
EtOH	12.22 %	Dark green	Orange
CHCl ₃	6.45%	Dark green	Orange
Pet. Ether.	1.09 %	Dark green	Light pink
EtOAc	3.11 %	Dark green	Saffron red
Acetone	2.47 %	Dark green	Saffron red

Table 6: Preliminary Phytochemical Investigation *N. prostrata* L. different extracts

Sl. No.	Phytochemical Tests	MeOH	EtOH	Pet. Ether	CHCl ₃	EtOAc	Acetone
1.	Alkaloids	-	-	-	-	-	-
2.	Glycosides	+	+	-	+	+	-
3.	Saponins	-	+	-	+	+	-
4.	Flavonoids	+	+	-	+	+	+
5.	Tannins	+	+	-	-	+	+
6.	Steroids	+	+	+	+	+	+
7.	Terpenoids	+	+	+	+	+	+
8.	Carbohydrate	+	+	-	-	+	-
9.	Protein	+	+	-	-	-	+
10.	Fat	+	+	+	+	+	+

Table 7: Thin Layer Chromatography of *Neptunia prostrata* L. different extracts

Sl. No.	Extract	Solvent system	No. of spots	R _f Value
1.	MeOH	EtOAc : MeOH (1:1)	4	0.34, 0.68, 0.79, 0.99
		Pet. Ether : Acetone (9:1)	8	0.13, 0.21, 0.3, 0.35, 0.45, 0.54, 0.78, 0.97
		CHCl ₃ : Acetone (8:2)	4	0.12, 0.42, 0.87, 1
		C ₆ H ₆ : EtOH (9:1)	7	0.15, 0.33, 0.5, 0.59, 0.72, 0.86, 0.98
		EtOAc : n-BuOH: H ₂ O (4:4:3)	5	0.19, 0.40, 0.78, 0.86, 0.96
		Pet. Ether : EtOAc (2:1)	5	0.08, 0.25, 0.6, 0.88, 0.97
		CHCl ₃ : EtOH (96 : 4)	5	0.09, 0.16, 0.34, 0.83, 0.96
2.	EtOH	EtOAc : MeOH (1:1)	7	0.1, 0.31, 0.36, 0.64, 0.75, 0.84, 0.96
		Pet. Ether : Acetone (9:1)	5	0.11, 0.27, 0.37, 0.50, 0.66
		CHCl ₃ : Acetone (8:2)	4	0.43, 0.7, 0.92, 0.96
		C ₆ H ₆ : EtOH (9:1)	7	0.18, 0.28, 0.5, 0.6, 0.8, 0.86, 0.95
		EtOAc : n-BuOH: H ₂ O (4:4:3)	5	0.35, 0.40, 0.78, 0.9, 0.96
		Pet. Ether : EtOAc (2:1)	5	0.5, 0.63, 0.73, 0.82, 0.94
		CHCl ₃ : EtOH (96 : 4)	4	0.48, 0.78, 0.92, 0.98
3.	CHCl ₃	EtOAc : MeOH (1:1)	5	0.05, 0.27, 0.39, 0.45, 0.95
		Pet. Ether : Acetone (9:1)	9	0.05, 0.11, 0.13, 0.27, 0.31, 0.37, 0.45, 0.53, 0.98
		CHCl ₃ : Acetone (8:2)	3	0.6, 0.66, 0.73
		C ₆ H ₆ : EtOH (9:1)	3	0.64, 0.7, 0.75
		EtOAc : n-BuOH: H ₂ O (4:4:3)	2	0.77, 0.94
		Pet. Ether : EtOAc (2:1)	1	0.99
		CHCl ₃ : EtOH (96 : 4)	2	0.18, 0.99
4.	Pet. Ether	EtOAc : MeOH (1:1)	2	0.07, 0.39
		Pet. Ether : Acetone (9:1)	5	0.09, 0.25, 0.43, 0.66, 0.98
		CHCl ₃ : Acetone (8:2)	4	0.74, 0.78, 0.8, 0.96
		C ₆ H ₆ : EtOH (9:1)	3	0.03, 0.78, 0.91
		EtOAc : n-BuOH: H ₂ O (4:4:3)	2	0.23, 0.86
		Pet. Ether : EtOAc (2:1)	2	0.7, 1
		CHCl ₃ : EtOH (96 : 4)	1	0.81

(MeOH- Methanol; EtOH- Ethanol; EtOAc- Ethyl acetate; CHCl₃- Chloroform; n-BuOH- n-Butanol; C₆H₆- Benzene)

4. Conclusion

Different diagnostic morphological and microscopical characteristics of leaves, stems and their powder form will help to distinguish the original drug and other adulteration. Different physicochemical and phyto-chemical investigations were performed to access the different chemical constituents, which is in the future plan for isolation and characterization.

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