Elucidation of the antioxidant and antimicrobial activity of extracts of leaves of *Neptunia prostrata Linn*

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ABSTRACT

The herb *Neptunia prostrata Linn.* which belongs to Mimosaceae family has been used in folkloric medicine in the North-eastern states of India, Assam and Tripura by indigenous herbal healers since time immemorial but there is a scarcity of any background study documenting its use as an antimicrobial herb. This unmet need led to the present study being conceptualised with the objective to evaluate any antioxidant property and antimicrobial activity of the leaf extracts of this herb in the selected strains of bacteria. For the same, plants were collected and authenticated. Following identification of these herbs, methanolic, ethanolic, pet ether and chloroform extracts were prepared using soxhletion. Acute toxicity study as per OECD guidelines 420 was assessed in wistar albino rats and in swiss albino mice (n=5) of both sexes at doses of 2000 mg/kg body weight and did not reveal any morbidity or mortality in the animals within the stipulated period. Phytochemical screening was performed on all four extracts of *Neptunia prostrata*. Phytochemical constituents depicted the presence of glycoside, and flavonoids in only ethanolic, methanolic and chloroform extracts. Alkaloids were present in the chloroform extract. The antimicrobial activity was performed by disc agar diffusion method with respect to amoxicillin at standard doses against ATCC strains of Gram positive *Staphylococcus aureus*, *Bacillus subtilis* and Gram negative *Salmonella typhi*. The herbs showed antioxidant activity comparable to standard antioxidants in-vitro such as Ascorbic acid (Vitamn C) with comparable IC_{50} values. The results of the antibacterial assay on the basis of the zone of inhibition (mm) and MIC values of the extracts of (NPHE) under study suggest these two indigenous herb have conspicuous and potent putative role in the therapeutics of a vast plethora bacterial infections that need to be corroborated for the expansion of future prospective in-vivo studies with larger sample size.

Key Words: *Neptunia prostrata, antioxidant, antimicrobial activity, MIC.*

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INTRODUCTION

The practice of indigenous medicine has time and again focused on herbs for their innate antimicrobial activity against a plethora of bacteria and moulds since ages [1, 2]. *Neptunia Prostrata, is one such herb growing in the states of Assam, Tripura of India and is regarded as* a variant of the ''Touch-me not plant" *Mimosa pudica* known locally by the synonym "Water- Mimosa". The leaves of this *herb* have found use in folkloric medicine and ancient vedic texts [3] since time immemorial.

Neptunia prostrata Linn. *(Neptunia oleracea*) belonging to Mimosaceae family is macroscopically a miniature aquatic herb that floats by its white spongy structure and has been

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reported to be a source of antioxidants [4-8]. Antioxidants in aerial parts of many herbs have reported a close link to antimicrobial or antibacterial properties, and this led us to the objective of the study to elucidate any such properties associated with leaves of this herb.

For the evaluation of the antimicrobial activity of the leaf extract of *Neptunia prostrate*, literature review has revealed that the indigenous ethnic groups of Tripura cultivate this plant as vegetable and medicinal plant and also prepare different dishes using this vegetable. In fact, this plant is useful for different types of remedies like acidity, gastritis, constipation, and dysentery. It has been reported that it possesses analgesic, hepatoprotective, and antimicrobial activity [9-11].

MATERIALS AND METHODS

Acute toxicity study as per OECD guidelines 420

The standard method of oral acute toxicity study was contacted on rodents as per OECD Guideline No. 420. Wistar albino rats of both sexes (n=5) administered with 2000 mg/ kg dose of the Neptunia prostrata extract (NP-1) were used in the study and observed for 14 days for mortality of any behavioural abnormalities.

Identification of plant materials

The herbarium containing the dried plant materials were processed and identified as *Neptunia prostrata* by the office of the Botanical Survey of India, Shibpur and a voucher was accorded the number BST/Herb/2016/003. A copy of the same was maintained for further use.

Phytochemical Screening of extracts

Phytochemical screening is routinely performed in investigations to ascertain the first line of tests dealing with the chemical identification of the medicinally active substances found in medicinal herbs. Concerning the bioactive substances which can be derived from plants, one can name alkaloids, flavonoids, tannin, carotenoids, antioxidants and phenolic compounds [12-15].

Dried leaf extracts (methanolic, ethanolic and hydro-alcoholic) of *Neptunia prostrata* were subjected to phytochemical screening to check for the broad chemical categorisation.

Antioxidant assay

Antioxidants can be considered as health protecting factors. Chronic diseases rate such as heart diseases and cancer can be reduced by antioxidants according to scientific evidence. The source of antioxidants are whole grains, fruits and vegetables. Moreover, plant sourced antioxidants including vitamin E, vitamin C, phenolic acids, and carotenes have been considered as having the potential to reduce disease risk. The antioxidant compounds are mostly derived from plant sources belonging to different classes of compounds which have chemical and physical properties [16, 17].

The methanolic extract for DPPH, nitric oxide, hydroxyl, superoxide, and hydrogen peroxide (H_2O_2) radical was found to have high free radical scavenging activity. The content of total phenols, flavonoids and tannins was also found to be high in methanolic extract which may be correlated to its antioxidant activity [18- 20].

Determination of DPPH radical scavenging activity:

DPPH radical scavenging assay is a method which is widely used for evaluating the free radical scavenging ability of natural compounds. In fact, it is based on the measurement of the scavenging ability of antioxidant substances towards the stable radical. Using DPPH radical, the free radical scavenging activity of the extracts was investigated in vitro [21, 22].

The free radical DPPH (1,1-Diphenyl 1-2-pieryl-hydrazil) 0.1 mM solution of DPPH in ethanol was prepared and 1ml of this solution was added to 3ml of various concentrations of extracts of *Neptunia prostrata (25, 50, 75, 100, 125, 150 μgm/ml)* of ethanol extracts. After 30mints, it was put in the incubators. Then absorbance was measured at 517nm. The percentage of inhibition was calculated by comparing the absorbance of controls and test samples [23]. Log dose inhibition curve was used for calculating the IC 50 value of the sample, which is the concentration of sample required to inhibit 50% of the DPPH free radical. Higher free radical activity was indicated by lower absorbance of the reaction mixture indicated [24].

Where a control is the absorbed of the control reaction A and A control and A test is the absorbance in the presence of the samples of the extracts. The antioxidant activity of the extract was expressed as IC 50. The IC 50 values are defined as the concentrations in (microgram/ml) of extracts that inhibits the formation of DPPH radicals by 50% [25].

RESULTS

Diphenyl pycryl hydrazyl (DPPH) assay of the extracts (Summarised in Figure 1)

The IC_{50} value of the extract was 11.2 µg/ml; whereas, the same for Ascorbic acid was 18.02 µg/ml.

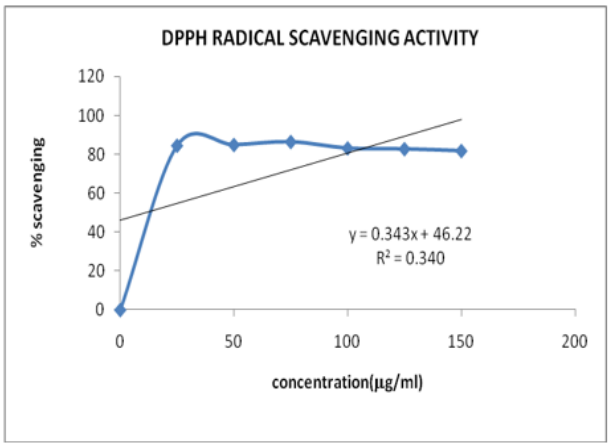


Figure 1: IC $_{50}$ value of NP-1 extracts (11.02 µg/ml) (IC $_{50}$ Ascorbic acid=18.02 µg/ml)

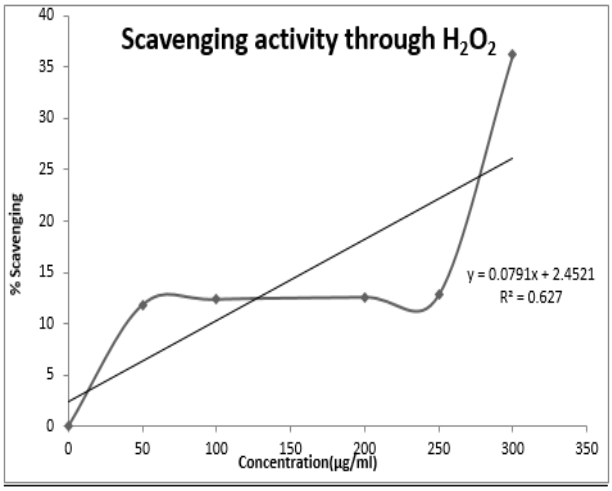


Figure 2: IC$_{50}$ value of extract of *Neptunia prostrata* (NP-2) = 101.03 µg/ml, IC$_{50}$ value of Ascorbic acid (Vitamin C) = 93 µg/ml

Table 1. The extracts used for the phytochemical screening

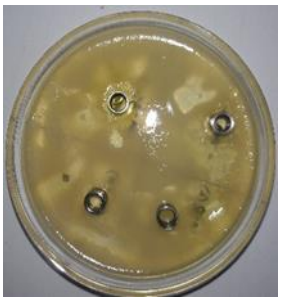
Phytoconstituents	NP1 extract	NP2 extract
Tanins	-	-
Alkaloids	++	++
Flavonoids	+++	+
Glycosides	+++	++

The table indicated the presence of alkaloids, glycosides and flavonoids in NP 1 and NP 2 leaf extracts, respectively.

Table 2: The findings of the antimicrobial activity of *Neptunia prostrata*

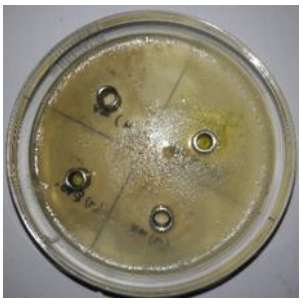
Organism	NP1 Low dose 100 mg/ml	NP1 High dose 400 mg/l	Amoxicillin Low dose 100 mg/l	Amoxicillin High dose 400 mg/l
	(Diameter in mm representing zones of inhibition)			
S. aureus	26 mm	28 mm	27 mm	31 mm
S. typhi	16 mm	18 mm	14 mm	17 mm
B. subtilis	12 mm	18 mm	21 mm	25 mm

Amoxicillin was selected as the standard antibiotic for the study.



S. aureus
ATCC No: 267688

S. typhi
ATCC No: 243234



B. subtilis
ATCC No: 276788

Fig 3: The antimicrobial assay of the extract of Neptunia prostrata

The antimicrobial study using the extracts revealed that the plant possesses chemical constituents in the extract and has definite antimicrobial activity against the three strains of bacteria and their laboratory cultures as evident from the Nutrient agar method. IC $_{50}$ values of 15, 18 and 19 mcg/ml were comparable to amoxicillin and gave a clearer picture about the potency and strong antibacterial property of the extracts.

DISCUSSIONS

There is indeed a great dearth of credible literature related to report about the use of *Neptunia prostrata* from North

eastern states of India. Only a handful number of studies focussed on the antioxidant nature of the leaves and other aerial parts but the real novelty of the study lies in linking the antibacterial effect on select strains of bacteria (Both Gram positive and negative) to it which may justify the robust antioxidant profile of the herb.

The study was aimed at assessing the plausible antibacterial and antimicrobial role of extracts of leaves of Neptunia prostrata Linn (NP). Gross phytochemical screening tests revealed the presence of flavonoids and tannins predominantly in the leaves of the plant. However, as a limitation of the study, it may be not isolating and characterising any lead moiety that is responsible for the bioactivity of NP and study of any isolated medicinal lead moiety could have been facilitated and was a ground for connecting the potential antioxidant nature and antibacterial property of the herb extract on the basis of its structure and chemistry.

The study stands out to report the safety of the herb through oral acute toxicity study as per OECD guidelines-420 and secondly with respect to the strong antioxidant and practically comparable IC_{50} values with standard antioxidants (such as ascorbic acid) and antibacterial property comparable to the beta lactam antibiotic amoxicillin) through the disc diffusion assay methodology in the selected strains of bacterium.

CONCLUSION

In this study, we came to know about the antimicrobial effects of the plant against bacterial strains as well as the preliminary phytochemical screening of the extracts of the plant. The study results revealed that the plant has some antimicrobial activities as evident from the study. The IC_{50} value of the extract was 11.2 µg/ml; whereas, the same for Ascorbic acid was 18.02 µg/ml for the DPPH assay. With regard to the Hydrogen peroxide antioxidant assay IC_{50} value of extract of *Neptunia prostrata* (NP-2) was 101.03 µg/ml; while, IC_{50} value of Ascorbic acid (Vitamin C) was found to be about 93 µg/ml.

Acute toxicity study as per OECD guidelines 420 was assessed in wistar albino rats and in Swiss albino mice (n=5) of both sexes at doses of 2000 mg/kg body weight and did not reveal any morbidity or mortality in the animals within the stipulated period pointing out towards its possible safety profile. Antimicrobial studies on other strains of bacteria may be also performed to understand the spectrum of antimicrobial activity of the plant extracts under study. The pharmacological in-vivo activities of the plant and its toxicity studies as per OECD guidelines need to be ascertained using a large sample size in animal models of disease to corroborate the present findings and reaffirm its putative role in therapeutics

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Ethics clearance

BST/IAEC/Feb-2018/02

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Conflict of interest

None.

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