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Preliminary Phytochemical Evaluation and Antimicrobial Screening of Leaves Extract of *Neptunia prostrate* L

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The antimicrobial activity of crude methanolic extracts of *Neptunia prostrate* along with phytochemical screening was tested against two gram positive bacteria: *Bacillus pertusis and Staphylococcus aureus*; two gram negative bacteria: *Escherichia coli and Vibrio cholarae* and one strain of fungus, *Candida albicans* by using the paper disc agar diffusion method. The results showed activity against all bacterial pathogens, by recording zone of inhibition.

Key words: *Neptunia prostrate,* Antimicrobial, phytochemical potential.

Medicinal plants are finding their way into pharmaceuticals, neutraceuticals, cosmetics and food supplements. There is a continuous and urgent need to discover new antimicrobial compounds with diverse chemical structures and novel mechanisms of action because there has been an alarming increase in the incidence of new and re-emerging infectious diseases. Another big concern is the development of resistance to the antibiotics in current clinical use [1]. Some plants have been used by rural people in Tripura for the treatment of several diseases**,** including microbial infections for emetic and strengthening effects, and for increasing urine and decreasing blood pressure [2]. Most of the plants used for medicinal purpose have been identified, and their uses are well documented and described by different authors [3-5], but the efficacy of many of these plants is yet to be verified. Moreover, plant extracts and isolated individual compounds have been tested in the laboratory against bacteria, fungi and antiviral with antineoplastic activities [6, 7]. Attention has turned to natural antimicrobial agents in recent years [8]. Hence, there is an urgent need to study the screening of antimicrobial properties of herbs, which will be helpful in the treatment of several diseases caused by microorganisms.

In this study, we concentrate on the preliminary phytochemical evaluation and antimicrobial activity of leaves extract of *Neptunia prostrate* L (family – Mimosaceae), which is an aquatic herb. This plant is used as vegetable. Tribal cook it as '*sag*". They remove the spongy tissues of the stem and take the inner soft tender portion along with leaves. Leaves are used as condiments[9].

MATERIAL AND METHODS

Sample Preparation

The leaves of *Neptunia prostrata* L. were taken which were collected from Agartala in the month of May 2009 and were shed dried for seven

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days. Since certain compounds get denatured in sunlight, it is dried under shade to avoid decomposition. The dried leaves were then crushed to fine powder. The powdered content was extracted by methanol in a soxhlet apparatus by taking 100g powder in 100 ml of methanol.

Physico-chemical studies

The extracts were used for preliminary screening of physico- chemical parameters which include determination of density, specific gravity[10], colour, pH, etc.

The pH of the extract was determined by using a pH meter (ECPC TUTOR, Eutech Instruments, pH tutor, Singapore, Sl. No. 242510)where the meter was calibrated by using buffer tablets of pH 4, pH 7 and pH 9.

Colour of the extract was visualized by naked eye at sunlight. Density bottle was used to determine the density of the extract at room temperature by using an electronic balance (Anamed Electronic Balance, Anamed Instruments (P) Ltd., Model No. V300DR) the specific gravity was calculated accordingly. The values are depicted in Table 1.

Preliminary Phytochemical Studies

Phytochemical studies include R_f values determination[11, 12], qualitative chemical test[13], and component separation by column chromatography. The qualitative chemical tests were carried out for alkaloids, Saponin, Carbohydrate, Fixed oil, Starch, Amino acid, Tannin, Steroid[14,15]. The Rf values determined by adopting TLC, where silica gel G was taken as stationary phase and Benzene, Butanol : Water : Dioxan = 4 : 2: 1, Butanol : Acetic acid : Water = 4 : 2 : 1, Ethyl acetate were taken as mobile phase. The results obtained due to R_f values determination are tabulated in Table 2.

Table 1. Physicochemical characterization of the Extract

Plant Part of	Colour	pH	Density(g /cm^3)	Specific gravity
Neptunia prostrata	Deep green with brownish tint	6.19	0.80275	0.80511

Density of water at 25.5 ^{o}C = 0.99707 g / cm^3

Table 2. The R_f values

Sample	Solvent system (Mobile Phase)	Spot number	Distance traveled by components (cm)	Distance traveled by solvent (cm)	R_f value
Neptunia prostrata	B	1	0.4	6.2	0.065
		2	0.9	6.2	0.145
		3	1.3	6.2	0.209
		4	1.5	6.2	0.242
		5	1.9	6.2	0.306
		6	6.2	6.2	1.00
	BWD	1	3.5	6.7	0.522
		2	4.7	6.7	0.701
		3	5.2	6.7	0.776
		4	5.6	6.7	0.836
		5	6.4	6.7	0.955
		6	6.7	6.7	1.00
	BAW	1	4.7	5.9	0.796
		2	5.1	5.9	0.864
		3	5.9	5.9	1.00
	EA	1	0.4	6.2	0.065
		2	0.6	6.2	0.097
		3	2.6	6.2	0.419
		4	3.3	6.2	0.532
		5	6.2	6.2	1.00

BAW means Butanol : Water : Dioxan (4 : 2: 1), BWD means Butanol : Acetic acid : Water (4 : 2 : 1), B means Benzene, EA means Ethyl Acetate

Separation

Components present in the extract were separated by using a column chromatography taking ethyl acetate as mobile phase and silica gel for column chromatography as stationary phase. The column used was of 30 cm in length. Separated fractions were collected in individual beakers. The colour of the components and volume obtained is given in Table 3.

Antibacterial Studies (Agar diffusion method)

The antibacterial screening[16] was carried out for the extracts two gram positive bacteria: *Bacillus pertusis and Staphylococcus aureus*; two gram negative bacteria : *E. coli* and *vibrio cholarae* by Agar diffusion method in particular paper disc method. The nutrient agar media was first prepared, whose composition is given below. The prepared media was sterilized by autoclaving at 121°C (15lb/ sq. inch) for fifteen minutes. The slants were prepared and the test organisms were subcultured in the fresh media, which were then incubated for 24 hours at 37 ± 1°C. Then they were stored in refrigerator and considered as stock culture. Bacterial inoculums were prepared by transferring a loopful of stock culture to sterilized nutrient broth taken in the test tubes, which were incubated at 37 ± 1°C for 18 hours before the experiments were carried out.

Table 3. The colour of the components and volume obtained after separation by column chromatography

Name of the plant	Components separated (eluted)	Colour	Volume collected (ml)
Neptunia prostrate	NP_1 (1^{st})	Green	19.0
	NP_2 (2^{nd})	Brown	1
	NP_3 (3^{rd})	Deep Green	15.5
	NP_4 (4^{th})	Brownish green	12.0
	Absorbed	-	2.5

Table 4. Antimicrobial studies

S. No.	Name of the Plant/ Standard	Leaves Extract (methanolic)/ Components	Zone of inhibition in mm against bacteria strains and fungus strain				
			Bacillus ertusis	*Staphylococcus aureus*	*Escherichia coli*	*vibrio cholarae*	*Candida albicans*
1.	*Neptunia prostrate*	Extract	10	11	7	10	12
		NP_1	9	10	9	9	11
		NP_2	12	14	9	12	15
		NP_3	11	9	10	8	10
		NP_4	10	9	7	7	10
2.	Tetracycline (Standard)	-	23	24	24	23	-
3.	Amphotericin B (Standard)	-	-	-	-	-	16

NP_1, NP_2, NP_3, NP_4 are the separated components

Petridishes, test tubes, flasks plugged with cotton were all first sterilized in Hot Air Oven for one hour. Fresh sterilized nutrient agar media for bacteria was again prepared, which in molten condition was transferred aseptically into the sterilized petridishes and left at the room temperature to be solidified. Two ml of inoculated nutrient broth was then transferred in each of the petridishes aseptically. The broth was then spread uniformly throughout the entire media.

In each of the plates, paper disc of 6 mm diameter soaked with the standard drug solution (tetracycline: 200 µg /ml in DMF), dried extract and their separated dried components dissolved in DMF (100 mg/ ml) were placed aseptically and accordingly. The plates were kept undisturbed for at least two

hours at room temperature to allow diffusion of the solution properly into the nutrient media. After incubation of the plate at 37 ± 1°C for 24 hours, the diameter of zone of inhibition surrounding each of the discs were measured. Average data from experiments are depicted in Table 4.

Antifungal Studies

The antifungal studies[16] were carried out for one strain of fungus *Candida albicans* by Agar diffusion method, in particular paper disc method. The zones of inhibition were observed and recorded. The test organism was sub-cultured in fresh Sabaraud Dextrose Agar (SDA) media, composition of which is given below. The SDA media prepared was first sterilized at 121°C (15 lb/ sq. inch) for 15 minutes by autoclaving .

The slant was prepared by sterilized SDA media, which was then sub-cultured with the test organism. The slant was then maintained at 22-25° C for 48 hours. From this loopful of fungus strain was then transferred aseptically in nutrient broth media and again maintained at 22-25°C for 48 hours. The sterilized SDA media was then placed in the sterilized Petri dishes in molten condition and were allowed to solidify at room temperature. The inoculated broth of 2 ml was then spread uniformly and aseptically over the prepared plates. In each plate paper disc of 6 mm diameter soaked in the standard drug solution (Amphotericin B), dried extract and their separated dried components dissolved in solvent (DMF) were placed aseptically and accordingly. The results obtained from antifungal studies are given in Table 4.

RESULTS AND DISCUSSION

The Study of preliminary Physico-chemical parameters like pH indicated that it had slightly acidic characteristic. The qualitative chemical tests performed in studying preliminary phyto-chemical parameters indicated the presence of Alkaloid and Tannin. Four components were separated performing column chromatography using ethyl acetate as the mobile phase. Presence of components was observed in the separation in TLC indicated by their individual R_f – values in the mobile phases such as Butanol : Water : Dioxan (4 : 2: 1), Butanol : Acetic acid : Water (4 : 2 : 1), Benzene, Ethyl Acetate. The extract and the four separated components exhibited variable degrees of antimicrobial activity, but not at par to the standard. Comparatively the second component eluted (NP_2) showed better activity against *Bacillus pertusis, Staphylococcus aureus, vibrio cholarae* and *Candida albicans* while other components and the extract showed moderately mild activity against the five microorganism.

The present investigation reveals the antibacterial and antifungal properties of the extract and its component against *Bacillus pertusis, Staphylococcus aureus, vibrio cholarae, E. coli* and *Candida albicans.*

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