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Allelopathic and antimicrobial evaluation of two Indian weeds- *Heliotropium indicum* L. and *Synedrella nodiflora* (L.) Gaertn with phytochemical studies

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ABSTRACT

In the last two decades, antibiotic resistance has become a severe problem worldwide. Herbal antimicrobials have enormous potential as they can serve purpose with lesser side effects than synthetic antimicrobials. Weeds release different allelochemicals which can act as antimicrobials or herbicides. Leaves of two important Indian weeds viz. *Heliotropium indicum* L. (Boraginaceae) and *Synedrella nodiflora* (L.) Gaertn. (Asteraceae) were taken in this allelopathic and antimicrobial study. Seed germination is considered to be the most significant stage especially under stress conditions and thus acts as an excellent model bioassay technique. Aqueous and ethanolic leaves extract of both plants progressively affected germination parameters of tested plants. But it affected seeds of *Lactuca sativa* more than those of *Vigna mungo*. *Lactuca sativa* Germination % declined to 40%-52% in case of ethanolic extract of these weeds. Ethanolic extracts are more effective in inhibiting germination in both indicator plants. The weed extracts were very effective against *Escherichia coli*, *Staphylococcus aureus* and fungus *Aspergillus niger*. *Salmonella enterica* is the most resistant strain tested against these extracts. *Heliotropium indicum* L. was found to have tannin, alkaloid, and saponin and glycoside in both types of extracts. *Synedrella nodiflora* L. contain phenols, alkaloids and flavonoids in both aqueous and ethanolic extracts. Terpenoids and tannin were found in ethanolic extract of *Synedrella nodiflora*. The present study concludes that both plants contain herbicidal and antimicrobial constituents. Further studies regarding the isolation and identification of these antimicrobial and herbicidal constituents are necessary.

Keywords: *Heliotropium indicum*, *Synedrella nodiflora*, weed, allelopathy, antimicrobial, herbicide, Phytochemical

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INTRODUCTION

In the last two decades, antibiotic resistance is emerging as a serious problem worldwide [1,2]. This has lead to the exploration for alternative, safe and efficient antimicrobial compounds from natural resources like plants. At the same time, there is a growing demand among consumers for natural food preservatives or herbicides in agricultural industries [3]. Herbal extracts are fast becoming popular as natural antimicrobial or preservatives [4-6].

Herbal antimicrobials have huge prospect as these are less harmful than synthetic antimicrobials [7]. Promising antibacterial activities of extracts of different plants against various microorganisms have been reported by many scientists and this has raised the confidence of scientists about the potential of phyto-antimicrobial agents [7-11]. Some medicinal herbs have also been assessed for antimicrobials [12-16].

Weeds are the plants which interfere with agricultural operations and compete with crop plants for light, water, nutrients and space and reduce the crop growth and yield [17]. Weeds release different phytotoxins as leachates, exudates and decomposition products. These chemicals (mostly secondary metabolites and byproducts of primary metabolic processes) are referred to as allelochemicals and the effect is· known as allelopathy [18]. Allelopathy may have beneficial or harmful influence on nearby plants or microorganisms [19]. Such allelochemicals have significant impact on growth and development of neighbouring plants [20]. Allelochemicals may be present in any plant organs like leaves, flowers, fruits, roots, rhizomes, stems or seeds [19, 21]. The inhibitory or stimulatory action of allelochemicals can affect various cellular or physiological processes like germination, respiration, photosynthesis, enzymatic activity, water relations, mineral availability, cell division and elongation, and structure and permeability of cell membranes and walls [22, 23]. These allelochemicals could be helpful to control weeds, insects, microbes, nematodes and plant pathogens. Many such weeds were tested for their antimicrobial and Allelopathic effects [24-26]. While some plants have been screened for this purpose, many are yet to be screened.

Heliotropium indicum Linn. (Indian heliotrope) is one such weed species which belongs to the family Boraginaceae. The plant is an annual, erect, branched hirsute plant of 0.5-1.5 ft height [27]. Its leaves are alternate to opposite, oblong, hairy and acute. Its flowers are small, white and in scorpioid cyme [28]. It is found in many tropical and subtropical countries. It contains Pyrrolizidine alkaloids (heliotrine, indicine N-oxide) tannins. Indicine, AC- indicine, indicinine, indicinine-N-oxide, lupeol, rapnone, Estoadiol etc. The whole plant is used in different ailments like high

fever, throat infection, gonorrhea, inflammation, reheumatism, ring worm, ulcers & wounds [29, 30]. *Synedrella nodiflora* (L.) Gaertn (Asteraceae) is a small, annual pubescent herb of about 0.5-1 ft height. It is found in the plains of India and in the Andamans also [31]. Leaves are opposite and toothed. Flower-heads small, axillary or terminal, rayed; ray florets 1 – 2 seriate, fertile, yellow; disk florets fertile, tubular, limb 4-toothed [28]. Leaves extract is known to be used in rheumatism, earache, toothache, headache, and as laxative. Active phytochemicals are alkaloids, sesquiternenes and flavonoids [31]. The present work was an attempt to explore the allelopathic and antimicrobial effects of these two common Indian weeds.

MATERIALS AND METHODS

Plants collection and preparation

Aerial portions including the inflorescence of *Heliotropium indicum* L. (*Boraginaceae*) and *Synedrella nodiflora* (L.) Gaertn. (Asteraceae) were collected in February 2011, from Kalyani (approximately 88°44´E and 22°97´N), Nadia district, West Bengal, India. The plants were identified and authenticated at Department of Botany, University of Kalyani. The plants were cleaned to make these completely free from any possible contamination. The leaves were dried at 50 ºC for 3-5 days, then separately ground into fine powder using a mechanical grinder and sieved. The powder was kept in dark colored glass bottles and subsequently used.

Preparation of solvent extraction

40 g of each dry powder was mixed in 100 ml sterile double distilled water or ethanol (100%) for 48 hours at 24ºC with stirring [32]. The extracts were centrifuged and filtered through Whatman No.1 filter paper and bacterial 0.45µm filter (Millipore). Then extracts were evaporated using vacuum rotary evaporator to near dryness and stored in glass vials in dark at 4ºC. Extractive values were calculated in terms of percentage considering the weight of plant material as 100%. These crude solvent extracts were diluted with either sterile double distilled water or 10% dimethyl sulphoxide (DMSO) which are to be used as negative control respectively to obtain required concentration before experiments [11].

Indicator plants

Two types of plant seeds were chosen as model indicator for germination bioassays. Seeds of *Vigna mungo* L. (Black gram, Papilionaceae) and *Lactuca sativa* L. (Lettuce, Asteraceae) were procured from reputed agricultural seed farm, Kolkata. Uniform and visibly perfect seeds were surface sterilized with 0.1% mercuric chloride for 2 min. and then washed repeatedly with sterile distilled water [33].

Allelopathy bioassay

Seeds of indicator plants were incubated for 24 hours in extracts of 0, 5, 10, 20% (w/v) concentrations and then ten to twelve selected seeds were put in each Petridish lined with sterile Whatman No. 1 filter papers. Respective extract of desired dilution was added to each Petridish of respective treatment daily just to keep the seeds moist enough to get favorable condition for germination and growth [34]. The growth parameters like germination percentage, root and shoot length (mm), fresh weight and dry weight (after drying at 60°C) (mg) were determined 5 days after germination[33, 35]. A seed was considered as germinated if a radical of 2mm length emerged. Distilled water was used as a control. Each treatment was replicated thrice under temperature of 28°C and 12/12 hrs. light/dark cycles.

Test micro-organisms

Four enteropathogenic, three food-spoiler and one probiotic bacterial strains were selected for the antimicrobial activities of these weeds study. The strains used were *Salmonella enterica* serovar typhimurium MTCC 3224, *Serratia marcescens* MTCC 4822, *Staphylococcus aureus* MTCC 7405, *Escherichia coli* MTCC 3221, *Klebsiella pneumoniae* subsp. pneumoniae MTCC 6644, *Proteus vulgaris* MTCC 7299, *Bacillus cereus* MTCC 6909, *Lactobacillus brevis* MTCC 4460 and those strains were obtained from MTCC, IMTECH, Chandigarh, India. All bacterial cultures were maintained on Muller Hinton agar (HiMedia) and subcultured regularly. The fungal strain *Aspergillus niger* was taken from laboratory collection (isolated from bread) and grown on Sabouraud dextrose agar (HiMedia).

Standard inoculum was prepared by sub-culturing 4-5 freshly grown isolated colonies of each strain in Muller Hinton broth (MHB) and incubated at 35-37 ºC for 24 hours. Inocula were standardized with sterile MHB to give final cell load of 10^6-10^7 CFU/ml.

Phytochemical evaluations

The extracts were tested to phytochemical evaluation using standard techniques of plant secondary metabolites according to Harborne and Turner [36], Evans [37] and Thenmozhi et al.[38]. Extracts were tested for phenolics, tannin, flavonoids, alkaloids, triterpenoids, saponin, anthraquinone and glycosides.

Disc diffusion bioassay

The disc diffusion test was performed as described by Jorgensen et al [39]. A 0.5 ml standardized inoculum suspension of each bacterial strain was spread on MHA plates with a sterile bent glass rod spreader. Sterile 6-mm Whatman no.1 filter paper discs were aseptically placed on plates. Sample decoctions or extracts of standard concentrations (10 mg dry weight) were aseptically

poured on the discs along with sterile double distilled water or 10% DMSO as negative and ampicillin as positive controls. Plates were allowed to stand for 30 minutes at room temperature prior to incubation at 35-37 ºC for 24 hours. The inhibition zone diameters were measured three times and means were represented to nearest mm.

Statistical analyses

The experiments were done at least twice and their mean values were represented. All statistical analyses including ANOVA and Tukey's Post Hoc test were done in SPSS Version 16.0. Differences were considered significant when $p \leq 0.05$.

RESULTS AND DISCUSSION

One of the most significant physiological phases of a plant is seed germination and so it acts as an excellent model bioassay technique for allelopathic tests. Certain crucial metabolic changes and enzymatic activity just after the imbibition of water are vital for germination. Allelo chemicals may inhibit or influence seed germination of crop plants by influencing these enzymatic activities [40].

Table 1: Allelopathic effect of aqueous (Hi-AE) and ethanolic (Hi-EE) extracts of *Heliotropium indicum* on *Vigna moonga* and *Lactuca sativa*

Extract		***Vigna moonga***				
Concn.(%)		**Germination %**	**RL[1]**	**SL**	**FW**	**DW**
Hi-AE	0	100 ab[2]	6.8 a	5.8 a	49 a	10 a
	5	93 ab	7.1 a	5.4 a	46 ab	9 a
	10	86 abc	6.5 a	5.2 a	42 ab	8 a
	20	78 bc	6.1 a	4.8 a	36 b	7 a
Hi-EE	0	78 a	5.9 a	5.3 a	38 a	6 a
	5	82 a	5.5 ab	5.2 ab	35 ab	5 a
	10	77 a	5.0 bc	4.8 bc	31 bc	5 a
	20	72 a	4.6 bc	4.2 c	27 bc	5 a
		Lactuca sativa				
Extract	Concn.(%)	Germination %	RL	SL	FW	DW
Hi-AE	0	96 ab	8.6 a	6.4 a	64 a	18
	5	95 ab	8.0 a	6.1 a	60 ab	12 abc
	10	84 abc	6.8	6.0 a	52 bc	11 abc
	20	72 bc	5.5	5.2	47 bc	8 abc
Hi-EE	0	84 a	8.2	6.7 a	55 a	10 a
	5	74 ab	7.2	6.4 ab	52 ab	9 ab
	10	64 bc	6.4	5.9 abc	46 b	7 bc
	20	52 bc	5.0	5.2 bc	38	6 c

[1] RL- Root length, SL-shoot length (cm); FW- fresh weight, DW- dry weight (mg).

values in the same columns for each parameter followed by same letters are not significantly different ($p \leq 0.05$) according to Tukey's test.

Analysis of variance of the data showed that the germination process of indicator plants showed diverse response to weed extracts. Aqueous and ethanolic extract of *Heliotropium indicum* L. affected germination parameters of tested plants (Table 1). With the increase of concentration, the inhibitory effect was progressively increased. Ethanolic extract is more effective in inhibiting germination and growth of seedlings. But it affected seeds of *Lactuca sativa* more than those of *Vigna mungo*. *Lactuca sativa* Germination % decreased to 52% in case of ethanolic extract. The mechanism of inhibition on the seedling germination caused by allelochemicals can be the result of reduced cell division and/or cell elongation [41]. The inhibitory effect resulted in decrease in fresh as well as dry weight of seedlings. Allelochemicals might have interfared with imbibition of water and other physiological processes [42].

Table 2: Allelopathic effect of aqueous (Sn-AE) and ethanolic (Sn-EE) extracts of *Synedrella nodiflora* on *Vigna moonga* and *Lactuca sativa*

		Vigna moonga				
Extract	**Concn.(%)**	**Germination %**	**RL[1]**	**SL**	**FW**	**DW**
Sn-AE	0	100 ab[2]	6.8 a	5.8 a	49 a	10 a
	5	88 ab	6.2 ab	5.0 ab	38 a	8 a
	10	74 bc	5.2 bc	4.7 ab	35 ab	7 a
	20	64 c	4.8 c	4.1 b	30 ab	7 a
Sn-EE	0	78 a	5.9 a	5.3 a	38 a	7 a
	5	70 ab	5.4 ab	4.9 a	35 ab	6 a
	10	58 bc	4.8 bc	4.6 ab	32 ab	5 a
	20	44 c	4.2 c	3.9 b	28 b	5 a
		Lactuca sativa				
Extract	Concn.(%)	Germination %	RL	SL	FW	DW
Sn-AE	0	96 a	8.6 a	6.4 a	64 a	18 a
	5	92 a	8.2 a	5.9 ab	60 ab	14 ab
	10	82 ab	6.9	5.3 b	52 b	11 b
	20	68 b	5.2	4.8 b	47 b	8 b
Sn-EE	0	84 a	8.2 a	6.7 a	55 a	10 a
	5	72 a	7.6 a	6.2 ab	52 ab	8 ab
	10	50 b	6.1 b	5.7 bc	46 b	8 ab
	20	40 b	5.3 b	5.1 c	38	6 b

[1] RL- Root length, SL-shoot length (cm); FW- fresh weight, DW- dry weight (mg).

[2] values in the same columns for each parameter followed by same letters are not significantly different ($p \leq 0.05$) according to Tukey's test.

Germination of *Vigna mungo* as well as of *Lactuca sativa* became affected by extracts of *Synedrella nodiflora* at different concentrations (Table 2). In both the indicator plants ethanolic extracts showed more inhibition of seed germination than aqueous extracts. Root and shoot

lengths of the plants showed variable response to different concentrations of extract. There was gradual decline in different germination parameters of both the plants. But root showed greater sensitivity to the extracts. Greater sensitivity of root growth than shoot growth to the plant extracts have also been demonstrated in other plant species [43]. Earlier studies showed that lower concentrations of certain plant extract may be stimulatory to the test plant growth [44]. No such stimulatory effect was found in case of these two plants.

The weed extracts were very effective against *Escherichia coli, Staphylococcus aureus* and fungus *Aspergillus niger* (Table 3). Highest DIZ was shown against *Staphylococcus aureus* treated with ehanolic extracts of these weeds. *Salmonella enterica* is the most resistant strain tested against these extracts. Earlier study showed that methanolic extract of *Heliotropium indicum* has broad spectrum activity but ineffective against *Salmonella typhi* [45]. Ethanolic extracts of *Heliotropium indicum* and *Synedrella nodiflora* showed better antimicrobial activity than aqueous extracts. It is thus concluded that *both weeds* possess good antibacterial and antifungal activity. In a similar study with *Synedrella nodiflora, Bacillus subtilis* was found to be most resistant to methanol and aqueous extracts showing no inhibition [46]. Probiotic strain *Lactobacillus brevis* was to some extent resistant so it may be expected that these extracts would not interfare with growth of *Lactobacillus brevis* in human gut [47].

Table3: Antibacterial activities, indicated by diameter of inhibition zone (DIZ, mm, for 10 mg dry wt./ disc, Mean±SD) of extracts of herbs against the micro-organisms [- means <6mm DIZ]

Microorganisms	***Heliotropium indicum***		***Synedrella nodiflora***	
	Aqueous	**Ethanolic**	**Aqueous**	**Ethanolic**
E.coli	9±1.527	10±1	10±1	9±1
S. aureus	10±1	11±0.577	7±0.577	11±1
S. enterica	-	-	-	-
S. marcescens	-	7±1.527	-	10±1
K. pneumoniae	7±1	9±1	7±1	9±1
P.vulgaris	-	9±1.527	8±0.577	7±1.527
B. cereus	-	10±1	8±1	10±1
L.brevis	7±1.527	7±0.577	8±1.527	7±0.577
A.niger	9±0.577	11±1	9±1	10±0.577

Plant materials were screened for different secondary metabolites (Table 4). *Heliotropium indicum* L. was found to have tannin, alkaloid, and saponin and glycoside in both types of extracts. Triterpenoids, anthraquinone and phenolics were found in neither extract but flavonoid was found in aqueous extract. Oluwatoyin et al. [45] showed the presence of Alkaloids, saponins, tannins, glycosides and flavonoids in *Heliotropium indicum* while steroids, coumarin and cardiac

glycosides were absent. Extracts of *Synedrella nodiflora* L. contain phenols, alkaloids and flavonoids in both aqueous and ethanolic extracts. Triterpenoids and tannin were found in ethanolic extract. Saponin and glycosides were found present in a study by Bhogaonkar *et al.*[46] though not found in present study.

Table 4: Phytochemical qualitative evaluation of extracts of *Heliotropium indicum* and *Synedrella nodiflora*

	Heliotropium indicum		***Synedrella nodiflora***	
	Aqueous	**Ethanolic**	**Aqueous**	**Ethanolic**
Alkaloids	+	+	+	+
Tannin	+	+	-	+
Phenolics	-	-	+	+
Flavonoids	+	-	+	+
Saponin	+	+	-	-
Glycosides	+	+	-	-
Triterpenoids	-	-	-	+
Anthraquinone	-	-	-	-

In the present study, extract of both the weeds exhibited variable herbicidal and antimicrobial activity. Herbicidal or antimicrobial activity of the extracts varies depending on the chemicals extracted in different types of solvents [48, 49]. The results showed that presence of such allelopathic weeds in the agricultural fields may pose a serious threat to the crop production as these plants will release allelochemicals to the soil environment and thus could result in failure of seed germination of crops. On the other hand, these weeds as potential source of herbicides may be used for weed management as mentioned by Cheema and Khaliq [50].

CONCLUSION

The present study concludes that both plants contain herbicidal and antimicrobial constituents. Isolation and identification of allelochemical and antimicrobial compounds from these plants could provide means to minimize their negative effects over the crops and potentially could provide alternative models for the development of herbicides or antimicrobials.

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