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Assessment of polar and non-polar extract fractions of *Limnophila indica* for its *in-vitro* herbicidal efficacy

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Abstract
The present study was carried out for analysis of hexane and methanol extract of *Limnophila indica* and evaluated for its *in-vitro* herbicidal efficacy against *Raphanus sativus* in terms of three parameters that is inhibition of germination, inhibition of coleoptiles growth and inhibition of radical growth. The plant extracts of the plant *L. indica* exhibited the significant potential to suppress germination at all tested doses of the plant extracts having broad range percent inhibition for all the samples tested. The results were quiet satisfying as validated by their IC_{50} values in all the three parameters. Higher the IC_{50} value lower will be the herbicidal activity. The order in which the samples exhibited herbicidal potential is LIHE (413.26±90.10ppm) > LIME (620.18±86.86ppm) for % inhibition of germination, LIHE (366.67±59.39ppm) > LIME (448.86±19.78ppm) for % inhibition of coleoptiles growth and LIHE (416.93±69.76ppm) > LIME (349.25±120.56ppm) for % inhibition of radical growth. All the inhibition data were tested to be significantly different as analyzed via two factor analysis with replication ($p< 0.01$ and $p< 0.05$).

Keywords: *Limnophila indica*, hexane extract, methanol extract, herbicidal activity, pendimethalin

Introduction
Limnophila indica (L.) Druce (Plantaginaceae) is an aquatic, perennial herb found as submerged or emergent stem plant. Naturally it is inhabitant of fresh water reservoirs and marshy land. The submerged stems are smooth having feathery leaves while the emergent stems remain covered of flat shiny hairs. Flowers may be of pink, white, blue or purple to lavender coloured, stalkless, axillary or solitary, sessile or pedicellate and borne in the leaf axis. The fruit is generally capsulated containing around 150 seeds. The plant is known for its medicinal uses in traditional system of medicine such as antiseptic, anti-dysentry, anti-dyspesia, anti-filariasis, carminative, anti-shigella, antacid, antimicrobial, hepatoprotective and cytotoxic agent [1, 2].

Phytochemicals components phenolics, flavonoids, alkaloids, terpenoids and fatty acid esters were present in the various extracts of the *L. indica* [3, 4] and which were reported as an antibacterial, antifungal, antidiarrheal, antidysentery, antacid and hepatoprotective agent. Flavonoids, terpenoids, alcohols, aldehydes, acids and fatty acid derivatives also identified in the other species of *Limnophila* and found to possessed medicinal values such as antibacterial, antifungal, diuretic, antioxidant, anti-inflammatory, wound healing activity, cytotoxic and antitubercular [5].

The present investigation is about to assess and evaluate the difference in activity in context to the *in-vitro* herbicidal efficacy against *Raphanus sativus* in terms of three parameters that is inhibition of germination, inhibition of coleoptiles growth and inhibition of radical growth.

Materials and Methods
Isolation of essential oil from aerial plant part and various plant extracts of *L. indica*
Plant extracts were obtained with soxhlet type apparatus and the obtained extract were fractioned in methanol and hexane solvents. The samples were stored at 4^0C until analysis.

Evaluation of herbicidal activity
The effect of herbicidal action was assessed using various parameters such as inhibition of seed germination, inhibition of coleoptile growth and inhibition of radical growth.

Bioassay
Graded doses of the plant extracts (250, 500, 750 and 1000 ppm) were used to assess the bioassay of herbicidal action against *Raphanus sativus*.

The seeds against which the herbicidal action is to be assessed was firstly surface sterilized using 0.25% hypochloride solution for 15 min.

The experiment was conducted in petri plates using moisturizing paper at the bottom to maintain sufficient moisture for the germination and growth of the seeds. Ten seeds were placed in each petri plates for the assessment and the solution containing the essential oil and the plant extract were poured in the petri plates. After each consecutive time intervals of 24 hours the number of seeds germinated were counted at 24, 48, 72 and 96 hours after the application of the treatment. The experiment was stopped after 96 hours when all the seeds were germinated in the control and length of the coleoptile and the radicle was measured. The activity was assessed in comparison to control and the standard pendimethalin. The formulae used for determination of inhibition of seed germination, inhibition of coleoptile growth and inhibition of radical growth were as follows:

Inhibition of seed germination

% Inhibition =100 × (1- Gt/Gc)

Where, Gt – no. of seeds germination in treatment,

Gc – No. of seeds germination in control.

Inhibition of coleoptile growth

% Inhibition =100 × (1- Ct/Cc)

Where, Ct – Coleoptile growth in treatment,

Cc – Coleoptile growth in control.

Inhibition of radicle growth

% Inhibition =100 × (1- Rt/Rc)

Where, Rt – Radicle growth in treatment,

Rc – Radicle growth in control.

Statistical analysis

All the experimental procedure was conducted in three replications and the data were expressed in terms of mean±standard deviation. Data illustrated in the tables and the graphs were subjected to ANOVA at 1% level of significance ($p< 0.01$) and 5% level of significance ($p< 0.05$) for herbicidal activity with two factor analysis with replication via. SPSS 12.0 software. Data analyzed were found to be significantly different at the respective level of significance. Regression line method was used to calculate IC_{50}.

Results and Discussion

Inhibition of seed germination

The inhibition of seed germination was assessed as the measure of herbicidal activity. The number of seeds germinated was counted and accordingly the percent inhibition of seeds germinated was calculated on per day basis till the 100% germination is achieved at various concentrations range of 250, 500, 750, 1000 ppm for both the plant extracts.

On day 1 the percent inhibition for LIME the percent inhibition was measured as 16.66%, 41.11%, 75.55% and 87.77% at various increasing concentration ranges while in case of LIHE was recorded as 65.55%, 87.77%, 100% and 100% respectively from lower to higher concentrations (Table 1).

On day 2 the percent inhibition for LIME the percent inhibition was measured as 30.09%, 37.96%, 69.44% and 69.44% at various increasing concentration ranges while in case of LIHE was recorded as 49.53%, 73.14%, 88.42 and 96.29% respectively from lower to higher concentrations (Table 1).

On day 3 the percent inhibition for LIME was measured as 30.00%, 40.00%, 60.00% and 70.00% at various increasing concentration ranges while in case of LIHE was recorded as 40.00%, 50.00%, 73.33 and 80.00% respectively from lower to higher concentrations (Table 1).

On day 4 the percent inhibition for LIME was measured as 13.33%, 30.00%, 46.00% and 70.00% at various increasing concentration ranges while in case of LIHE it was recorded as 16.66%, 40.00%, 66.66 and 70.00% respectively from lower to higher concentrations (Table 1).

IC_{50} was calculated at the time when 100% germination was achieved in the control and is used to compare the relative herbicidal activities of all the samples as lower is the herbicidal activity higher will be its IC_{50} values. The order in which the activity was observed was LIHE (413.26±90.10ppm) > LIME (620.18±86.86ppm) (Table 4).

Inhibition of coleoptile growth

The inhibition of coleoptile growth was assessed as the measure of herbicidal activity. The percent coleoptile growth inhibition of seeds germinated was calculated at the time when 100% germination is achieved at various concentrations range of 250, 500, 750, 1000 ppm for both the plant extracts.

The percent inhibition of coleoptile growth for LIME the percent inhibition was measured as 29.78%, 58.05%, 78.95% and 94.85% at various increasing concentration ranges while in case of LIHE was recorded as 28.88%, 72.72%,94.31% and 95.65% respectively from lower to higher concentrations (Table 2).

IC_{50} was calculated at the time when 100% germination was achieved in the control and is used to compare the relative herbicidal activities in terms of inhibition of coleoptile growth of all the samples as lower is the herbicidal activity higher will be its IC_{50} values. The order in which the activity was observed was LIHE (366.67±59.39ppm) > LIME (448.86±19.78ppm) (Table 4).

Inhibition of radicle growth

The inhibition of radicle growth was assessed as the measure of herbicidal activity. The percent radicle growth inhibition of seeds germinated was calculated at the time when 100% germination is achieved at various concentrations range of 250, 500, 750, 1000 ppm for both the plant extracts.

The percent inhibition of radical growth for LIME the percent inhibition was measured as 11.25%, 53.62%, 83.32% and 93.30% at various increasing concentration ranges while in case of LIHE was recorded as 16.21%, 60.26%,90.11% and 95.22% respectively from lower to higher concentrations (Table 3).

IC_{50} was calculated at the time when 100% germination was achieved in the control and is used to compare the relative herbicidal activities in terms of inhibition of coleoptile growth of all the samples as lower is the herbicidal activity higher will be its IC_{50} values. The order in which the activity was observed was LIHE (416.93±69.76ppm) > LIME (349.25±120.56ppm) (Table 4).

Significant herbicidal activity obtained may be attributed to the presence of aristolone and beta-monolinolein in the methanol and hexane extracts respectively and which is already published in our previous communication [8, 9]. The current investigation totally supports the previous reports that the phytotoxic ability in the botanicals presumably may be

due to the presence of phytochemical components in the extracts and the essential oil [12]. Lu *et al.* [6] also stated that the herbicidal or phytotoxicity appeared may be due to the high phytochemical content in the botanicals that is phenols, flavonoids, terpenoids, alkaloids etc.

Experimental investigation by Tiwari *et al.* [11] and Park *et al.* [7] also suggests that the activity like herbicidal effect of the plant extracts and the essential oil might be possibly occurred due to the various active components present in the essential oil and the extracts or even may be due to the interaction of the major and the minor components present in the botanicals.

Table 1: % Inhibition of germination of plant extracts of aerial plant part of *L. indica*.

S.N.	Samples		% Inhibition of germination (Day 1)			
	Plant extracts		250 ppm	500 ppm	750 ppm	1000 ppm
1.	LIME	R_1	33.33	50.00	83.33	83.33
		R_2	0.00	40.00	60.00	80.00
		R_3	16.66	33.33	83.33333	100.00
		Avg.	16.66 ±16.66	41.11 ±8.38	75.55 ±13.47	87.77 ±10.71
2.	LIHE	R_1	66.66	83.33	100.00	100.00
		R_2	80.00	80.00	100.00	100.00
		R_3	50.00	100.00	100.00	100.00
		Avg.	65.55 ±15.03	87.77 ±10.71	100.00 ±0.00	100.00 ±0.00
3.	Pendimethalin	R_1	100.00	100.00	100.00	100.00
		R_2	100.00	100.00	100.00	100.00
		R_3	100.00	100.00	100.00	100.00
		Avg.	100.00 ±0.00	100.00 ±0.00	100.00 ±0.00	100.00 ±0.00
S.N.	Samples		% Inhibition of germination (Day 2)			
	Plant extracts		250 ppm	500 ppm	750 ppm	1000 ppm
1.	LIME	R_1	55.55	44.44	88.88	66.66
		R_2	22.22	44.44	44.44	66.66
		R_3	12.50	25.00	75.00	75.00
		Avg.	30.09 ±22.58	37.96 ±11.22	69.44 ±22.73	69.44 ±4.81
2.	LIHE	R_1	55.55	77.77	88.88	88.88
		R_2	55.55	66.66	88.88	100
		R_3	37.5	75	87.5	100
		Avg.	49.53 ±10.42	73.14 ±5.78	88.42 ±0.80	96.29 ±6.41
3.	Pendimethalin	R_1	100.00	100.00	100.00	100.00
		R_2	100.00	100.00	100.00	100.00
		R_3	100.00	100.00	100.00	100.00
		Avg.	100.00 ±0.00	100.00 ±0.00	100.00 ±0.00	100.00 ±0.00
S.N.	Samples		% Inhibition of germination (Day 3)			
	Plant extracts		250 ppm	500 ppm	750 ppm	1000 ppm
1.	LIME	R_1	40.00	40.00	70.00	70.00
		R_2	30.00	50.00	40.00	70.00
		R_3	20.00	30.00	70.00	70.00
		Avg.	30.00 ±10.00	40.00 ±10.00	60.00 ±17.32	70.00 ±0.00
2.	LIHE	R_1	40.00	50.00	70.00	90.00
		R_2	50.00	50.00	70.00	70.00
		R_3	30.00	50.00	80.00	80.00
		Avg.	40.00 ±10.00	50.00 ±0.00	73.33 ±5.77	80.00 ±10.00
3.	Pendimethalin	R_1	100.00	100.00	100.00	100.00
		R_2	100.00	100.00	100.00	100.00
		R_3	100.00	100.00	100.00	100.00
		Avg.	100.00 ±0.00	100.00 ±0.00	100.00 ±0.00	100.00 ±0.00
S.N.	Samples		% Inhibition of germination (Day 4)			
	Plant extracts		250 ppm	500 ppm	750 ppm	1000 ppm
1.	LIME	R_1	10.00	30.00	50.00	70.00
		R_2	10.00	30.00	40.00	70.00
		R_3	20.00	30.00	50.00	70.00
		Avg.	13.33 ±5.77	30.00 ±0.00	46.66 ±5.77	70.00 ±0.00
2.	LIHE	R_1	20.00	50.00	70.00	80.00
		R_2	20.00	30.00	70.00	70.00
		R_3	10.00	40.00	60.00	60.00
		Avg.	16.66 ±5.77	40.00 ±10.00	66.66 ±5.77	70.00 ±10.00
3.	Pendimethalin	R_1	100.00	100.00	100.00	100.00
		R_2	100.00	100.00	100.00	100.00
		R_3	100.00	100.00	100.00	100.00
		Avg.	100.00±0.00	100.00±0.00	100.00±0.00	100.00 ±0.00

Note: LIME- *Limnophila indica* methanol extract**,** LIHE-*Limnophila indica* hexane extract

Table 2: % Inhibition of coleoptile growth of plant extract of aerial plant part of *L. indica*

S.N.	Samples		% Inhibition of coleoptile growth			
	Essential oil		**50ppm**	**100ppm**	**150ppm**	**200ppm**
	Plant extracts		**250 ppm**	**500 ppm**	**750 ppm**	**1000 ppm**
1.	LIME	R_1	25.84	60.64	82.17	96.19
		R_2	29.41	57.86	72.90	94.29
		R_3	34.09	55.65	81.79	94.05
		Avg.	29.78 ±4.13	58.05 ±2.50	78.95 ±5.24	94.85 ±1.17
2.	LIHE	R_1	31.93	84.78	94.91	98.28
		R_2	29.34	60.69	95.29	95.69
		R_3	25.37	72.69	92.72	92.96
		Avg.	28.88 ±3.30	72.72 ±12.04	94.31 ±1.38	95.65 ±2.66
3.	Pendimethalin	R_1	100.00	100.00	100.00	100.00
		R_2	100.00	100.00	100.00	100.00
		R_3	100.00	100.00	100.00	100.00
		Avg.	100.00 ±0.00	100.00 ±0.00	100.00 ±0.00	100.00 ±0.00

Note: LIME- *Limnophila indica* methanol extract**,** LIHE-*Limnophila indica* hexane extract

Table 3: % Inhibition of radicle growth of plant extract of aerial plant part of *L. indica*

S.N.	Samples		% Inhibition of radicle length growth			
	Plant extracts		**250 ppm**	**500 ppm**	**750 ppm**	**1000 ppm**
1.	LIME	R_1	10.56	50.77	85.88	93.77
		R_2	1.127	49.43	80.62	94.53
		R_3	22.08	60.66	83.46	91.60
		Avg.	11.25 ±10.49	53.62 ±6.13	83.32 ±2.63	93.30 ±1.51
2.	LIHE	R_1	16.86	66.72	91.27	96.57
		R_2	26.17	54.02	90.77	95.90
		R_3	5.59	60.04	88.30	93.19
		Avg.	16.21 ±10.30	60.26 ±6.34	90.11 ±1.58	95.22 ±1.79
3.	Pendimethalin	R_1	100.00	100.00	100.00	100.00
		R_2	100.00	100.00	100.00	100.00
		R_3	100.00	100.00	100.00	100.00
		Avg.	100.00 ±0.00	100.00 ±0.00	100.00 ±0.00	100.00 ±0.00

Note: LIME- *Limnophila indica* methanol extract**,** LIHE-*Limnophila indica* hexane extract

Table 4: IC_{50} of plant extract of aerial plant part of *L. indica.*

S.N.	Samples	IC_{50}values in triplicate			Mean IC_{50} values
		1st	2nd	3rd	
1.	LIME-1	520.83	681.81	657.89	620.18±86.86ppm
2.	LIHE-1	441.17	312.5	486.11	413.26±90.10ppm
3.	LIME-2	450.83	467.59	428.17	448.86±19.78 ppm
4.	LIHE-2	299.03	410.32	390.67	366.67±59.39 ppm
5.	LIME-3	345.75	230.48	471.54	349.25±120.56ppm
6.	LIHE-3	457.70	456.73	336.37	416.93±69.76ppm

Note: LIME- *Limnophila indica* methanol extract**,** LIHE-*Limnophila indica* hexane extract, 1- inhibition of germination, 2- inhibition of coleoptiles growth, 3- inhibition of radical growth.

Conclusion

The plant extracts of the plant *L. indica* when assessed for herbicidal activity in terms of inhibition of germination exhibited the significant potential to suppress germination at all tested doses of the plant extracts having broad range percent inhibition for all the samples tested. The results were also validated by IC_{50} values, having substantially higher IC_{50} values of the plant extracts. Higher the IC_{50} value lower will be the herbicidal activity. The order in which the samples exhibited herbicidal potential is LIHE (413.26±90.10ppm) > LIME (620.18±86.86ppm). The plant extracts of the plant *L. indica* when assessed for herbicidal activity in terms of inhibition of coleoptile growth exhibited the significant potential to suppress germination at all tested doses of the plant extracts having broad range percent inhibition for all the samples tested. The results were also validated by IC_{50} values, having substantially higher IC_{50} values of the plant extracts. Higher the IC_{50} value lower will be the herbicidal activity. The order in which the samples exhibited herbicidal potential is LIHE (366.67±59.39ppm) > LIME (448.86±19.78ppm). The plant extracts of the plant *L. indica* when assessed for herbicidal activity in terms of inhibition of radicle growth exhibited the significant potential to suppress germination at all tested doses of the plant extracts having broad range percent inhibition for all the samples tested. The results were also validated by IC_{50} values, having substantially higher IC_{50} values of the plant extracts. Higher the IC_{50} value lower will be the herbicidal activity. The order in which the samples exhibited herbicidal potential is LIHE (416.93±69.76ppm) > LIME (349.25±120.56ppm).

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