Journal of Entomology and Zoology Studies

Available online at www.entomoljournal.com

E-ISSN: 2320-7078
P-ISSN: 2349-6800
JEZS 2019; 7(2): 594-599

Received: 01-01-2019
Accepted: 04-02-2019

Randeep Kumar
Department of Chemistry, College of Basic Sciences and Humanities, G.B. Pant University of Agriculture & Technology, Pantnagar, U.S. Nagar, Uttarakhand, India

Bahaar Anjum
Department of Chemistry, College of Basic Sciences and Humanities, G.B. Pant University of Agriculture & Technology, Pantnagar, U.S. Nagar, Uttarakhand, India

Ravendra Kumar
Department of Chemistry, College of Basic Sciences and Humanities, G.B. Pant University of Agriculture & Technology, Pantnagar, U.S. Nagar, Uttarakhand, India

Om Prakash
Department of Chemistry, College of Basic Sciences and Humanities, G.B. Pant University of Agriculture & Technology, Pantnagar, U.S. Nagar, Uttarakhand, India

Ganesh Panday
Ganesh Panday, School of Agriculture, Graphic Era Hill University, Clement Town, Dehradun, Uttarakhand, India

AK Pant
Department of Chemistry, College of Basic Sciences and Humanities, G.B. Pant University of Agriculture & Technology, Pantnagar, U.S. Nagar, Uttarakhand, India

RM Srivastava
Department of Entomology, College of Agriculture, G.B. Pant University of Agriculture & Technology, Pantnagar, U.S. Nagar, Uttarakhand, India

Correspondence
Randeep Kumar
Department of Chemistry, College of Basic Sciences and Humanities, G.B. Pant University of Agriculture & Technology, Pantnagar, U.S. Nagar, Uttarakhand, India

Chemical composition, total phenolics assay and biological activities of chloroform extract from *Limnophila indica* (L.) Druce

Randeep Kumar, Bahaar Anjum, Ravendra Kumar, Om Prakash, Ganesh Panday, AK Pant and RM Srivastava

Abstract
Present research was undertaken to investigate the chemical composition, total phenolics assay, *in vitro* antioxidant, anti-inflammatory and insect antifeeding activity in chloroform extract of *Limnophila indica* (L.) Druce. GC-MS analysis revealed the occurrence of 4,5-Dimethyl-1,2,3,6,7,8,8a,8b-octahydrobiphenylene (18.3%) as a major compound. Total phenolic content, flavanoid content and orthodihydric phenol in the extract were 150.55±0.33 mg/g GAE, 40.72±0.69 mg/g CNE and 56.66±0.93 mg/g CLE, respectively. *In-vitro* antioxidant activity estimation through NO radical scavenging activity, DPPH radical scavenging activity, metal chelating activity of Fe^{+3} and reducing power activity of Fe^{+3} to Fe^{+2} resulted its potential result with IC_{50} values of 35.85±3.12, 28.52±1.32, 49.29±0.15 and 39.17±0.65 respectively. Anti-inflammatory activity determined using inhibition of protein denaturation method and extract showed significant activity (IB_{50}=27.96±1.14). Chloroform extract of *L. indica* at the dose levels of 20% and 25% also exhibited significant antifeeding activity against *Spilosoma oblique.*

Keywords: *Limnophila indica*, antioxidant, anti-inflammatory, antifeeding, *Spilosoma obliqua*

1. Introduction
The plant *Limnophila indica* (L.) Druce, belongs to the angiosperm family Plantaginaceae, earlier it was included in the angiosperm family Scrophulariaceae which proved as erroneous on account of the recent splitting of this polyphyletic family and reorganization of the taxa in the order Lamiales. The family Plantaginaceae consists of about 87-120 genera and 1614-2106 species [1-4]. *Limnophila indica* is one of the common aquatic or marshy perennial herbs in India. Outside India, it is known in Indomalaysia, China, Taiwan, South Korea, Japan, Nepal, Pakistan, Sri Lanka, Iraq, Africa, Madagascar, Australia and USA. This species is known by various local names like 'Ambulia', 'Ambuli', 'Amagandhi Bon', 'Amargandhi', 'Amragandhah', 'Bagga Patti', 'Gulabi', 'Kerlata', 'Karpur', 'Kutra', 'Mangannari', 'Nitti Ganeru', 'Papri', 'Purti', 'Turati', 'Thario', Tareti, etc, and by English name 'Indian Marsh weed' in different parts of India [5,6,7]. It is reported as antiseptic and febrifuge. An infusion of the leaves is used in the treatment of dysentery, diarrhoea and dyspepsia. The juice of the plant, combined with cumin (*Cuminum cyminum* L.) and other aromatic plants is used in the treatment of dysentery. The juice of the plant is rubbed on the body as a remedy for strong fevers. A liniment is made from the plant, combined with coconut oil, and is used in elephantiasis. In addition to these, plant is also reported as anthelminitic, appetized, brain tonic and edible [5, 6, 8].

Phytochemicals components phenolics, flavonoids, alkaloids, terpenoids and fatty acid esters were present in the various extracts of the *L. indica* [9, 10] 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 [10]. Shubhadra *et al.*, (2011) [9] reported limonene as a major component in the essential oil of the *L. indica*, whereas Kumar *et al.*, (2019) [11] reported *epi*-cyclocolorenone rich essential oil of *L. indica* with antioxidant, anti-inflammatory and antifeeding activity. We also have been reported chemical analysis, antioxidant, anti-inflammatory and antifeeding activities in methanolic and hexane extracts of *Limnophila indica* [12, 13]. In continuation to

research programme on *L. indica*, in present investigation the chemical analysis, antioxidant, anti-inflammatory and antifeeding activity of chloroform extracts from aerial parts of this aquatic medicinal herb is being presented.

2. Material and Methods

2.1 Collection of Plant material

The plant material was collected from the Tarai region of Uttarakhand, India in the month of December 2017. The identification and authentication of the plant was made by Dr. D.S. Rawat, Assistant Professor and plant taxonomist, Department of Biological Sciences, G.B. Pant University of Agriculture and Technology, Pantnagar. The voucher specimen has been deposited in the Department of Biological Sciences, GBPUAT, Pantnagar for future reference.

2.2 Preparation of extract

The aerial plant part of *L. indica* was chopped, shade dried, powdered and extracted in chloroform solvent using Soxhlet apparatus. The extract obtained was filtered and concentrated using a rotary evaporator. After drying the yield of the extract was recorded to be 1.1% and stored at 4^0C for further analysis.

2.3 GC-MS Analysis

The plant extract was analyzed on GCMS-QP2010 Ultra Rtx-5MS column (30m×0.25mm and film thickness 0.25µm). The column temperature was programmed for 50-210 °C at the rate of 3°C/min and then again upto 280^0C at the rate of 8°C/min. Helium gas at the rate of 1.21 ml/min column flow and 69.0 kPa pressure was used as the carrier gas at the injector temperature at 260 °C. MS were recorded under EI condition (70 ev) with an injection volume of 0.1 µL with split mode of 1:120. Identification of the constituents of the essential oil done by comparing their mass spectra fragmentation pattern and their retention indices with that of MS library (NIST14.lib, FFNSC2.lib, WILEY8.LIB) and comparing the spectra with literature data [14].

2.4 Phytochemical assay

2.4.1 Total phenolic assay

The total phenol content estimation of the extract was done using Folin-Ciocalteu reagent (FCR) **[15]** with minor adjustments. Reaction mixture prepared by mixing 0.5 ml of the extract, 1ml Folin -Ciocalteu reagent, 1 ml (7%) Na_2CO_3, 5 ml distilled water and allowed to stand for 30 min. Absorbance was recorded at 765 nm. Total phenol content expressed as gallic acid equivalent (GAE) in mg/g.

2.4.2 Total flavonoids assay

Method developed by Choi *et al.,* (2006) [16] was adopted for total flavonoids assay. In this method 1ml of plant extract was mixed with 1.25 ml of distilled water and 75µl (5%) $NaNO_3$ and incubated for 5 min. 150µl (10%) $AlCl_3$, 500 µl of 1M NaOH and 275 µl were added and mixed after incubation. Absorbance was then measured at 510 nm. Total flavonoid content was expressed as catechin equivalent (CNE) in mg/g.

2.4.3 Ortho-dihydric phenol content estimation

1 ml of extract mixed with 1ml (0.5N) HCl, 1ml Arnow's reagent, 2ml (1N) NaOH and 4.5 ml of distilled water. Absorbance measured at 515 nm. Total ortho-dihydric phenol content was expressed with catechol equivalent (CLE) in mg/g [17].

2.5 Evaluation of antioxidant activity

2.5.1 NO radical scavenging activity

The NO radical scavenging activity was screened following the developed protocol generally being practiced with slight modifications [18]. Briefly the reaction mixture consisting of plant extracts (50-250 µg/ml) mixed with 0.5mL of 10mM sodium Nitroprusside in phosphate buffered saline and incubated at 25^0C for 180 min. Griess reagent was added in the reaction mixture and absorbance was taken at 546 nm. Ascorbic acid was used as the standard antioxidant. The% NO radical scavenging activity was calculated as per the formula:

$$\% \text{ Inhibition} = 100 \times (V_t / V_c - 1)$$

Where, V_t = absorbance of sample, V_c = absorbance of control

2.5.2 DPPH (2, 2-diphenyl-2-picrylhydrazyl) free radical scavenging activity

Standard protocol for the free radical scavenging activity of the plant extracts was followed [19]. Various concentrations plant extracts (50-250 µg/ml) were mixed with 5mL of 0.004% methanol solution of DPPH. The reaction mixture was kept in dark for half an hour for incubation and absorbance was taken at 517nm. BHT was used as standard antioxidant. The% inhibition of DPPH free radical was calculated by using the formula:

$$\% \text{ Inhibition} = 100 \times (V_t / V_c - 1)$$

Where, V_t = absorbance of sample, V_c = absorbance of control

2.5.3 Metal chelating activity

The metal chelating activity of Fe^{2+} of plant extracts was screened by the method adopted by Kumar *et al.,* (2012) [19]. Reaction mixture consisting of 0.1 ml (2mM) $FeCl_2.4H_2O$, 0.2 ml (5mM) ferrozine and methanol was added and made up the final volume upto 5 ml with various concentrations of plant extracts (50-250 µg/ml) and was incubated for half an hour. The absorbance was taken at 562nm. Citric acid was used as the standard antioxidant. Percent metal chelating activity was evaluated using the formula:

$$\% \text{ Inhibition} = 100 \times (V_t / V_c - 1)$$

Where, V_t = absorbance of sample, V_c = absorbance of control

2.5.4 Reducing power activity

The reducing power activity of plant extracts was done as per the developed protocol by Parki *et al.,* (2017) [20]. Various concentrations of plant extracts (50-250 µg/ml) added to 2.5 ml of phosphate buffer (200 mM, pH= 6.6), 2.5 ml of 1% $K_3Fe(CN)_6$ and kept for 20 min incubation at 50 ± 1^0C and the added 2.5ml of trichloroacetic acid, and centrifuged at 650 RPM for 10 min. 1 ml of supernatant, mixed with 5 ml distilled water and 1 ml of ferric chloride (0.1%). Absorbance was recorded at 700nm. Catechin was used as the standard antioxidant. The% reducing power of the essential oil was calculated using the formula:

$$\% \text{ Inhibition} = 100 \times (V_t / V_c - 1)$$

Where, V_t = absorbance of sample, V_c = absorbance of control

2.6 Evaluation of In-vitro anti-inflammatory activity

In-vitro anti-inflammatory activity was screened as per the developed protocol by Kar *et al.*, (2012) [21]. The reaction mixture consisting of plant extracts (50-250 µg/ml), 100 ppm (200µL) fresh albumin protein, 2.8 ml of freshly prepared phosphate buffered saline (PBS) of pH 6.4 and made up the final volume to 5ml and then incubated at 37^0C for 15min and then at 70^0C for 5min. After cooling the absorbance was measured at 660nm. Diclofenac sodium was used as standard. The percent inhibition was calculated by the formula:

$$\% \text{ Inhibition} = 100 \times (V_t / V_c - 1)$$

Where, V_t = absorbance of sample, V_c = absorbance of control

2.7 Evaluation of antifeeding activity

2.7.1 Test insect

The Bihar hairy caterpillar, *Spilosoma obliqua* is a key pest of pulse crop in India. The damage is mostly due to the third and its onward instars. The pest is a polyphagous, infests by defoliation causing serious damage to several pulse crops [22].

2.7.2 Collection of larvae and maintenance

Larvae of the insect were collected from the Soyabean (*Glycine max*) field at Crop Research Center, G.B. Pant University of Agriculture and Technology, Pantnagar, Uttarakhand, India during the month of July. The insects were reared in the laboratory at 27^0C temperature and 75-80% relative humidity in a jar covered with muslin cloth. The larvae were specially fed on fresh leaves of Soyabean on daily basis. The full grown fourth instar larvae previously kept for 24 hours of starvation were used to investigate the antifeeding activity.

2.7.3 Experimental procedure

The experiment was carried out as per the developed protocol [23]. Leaves of known area of 25 cm^2 were taken and dipped in various concentrations of plant extracts (5-25%) for 1 min. These were then air dried and transferred to the petri plates for feeding the insects. The 24 hours starved fourth instar test insect larvae were released as one insect per petri plate. Readings were taken after 12 hours inteval at 12, 24, 36, 48 hours. Graph paper method was used to measure the leaf area consumed by the insects. And then calculating percent antifeeding activity of the plant extract as per the formula:

$$\text{Percent antifeeding: } \frac{\text{Leaf area consumed in control} - \text{leaf area consumed in treatment}}{\text{Leaf area consumed in control} + \text{leaf area consumed in treatment}} \times 100$$

2.8 Statistical analysis

All the experiments were conducted in triplicates and the data expressed as mean ± standard deviation. Data illustrated in the graph were subjected to ANOVA ($p<0.01$) for in-vitro antioxidant and anti-inflammatory activity while ANOVA ($p<0.05$) for insect antifeeding activity with two factor analysis with replication via. SPSS software. Data analyzed were found to be significantly different at the respective level of significance. Regression line method was used to calculate $IC_{50,}$ RP_{50} and IB_{50}.

3. Results and Discussion

3.1 Chemical composition

The combination of GC-MS analysis of chloroform extract of *L. indica* reveals the presence of thirty-five compounds together accounts for 72.9% of the total extract composition. The results revealed the chloroform extract to be mainly dominated by 4,5-Dimethyl-1,2,3,6,7,8,8a,8b-octahydrobiphenylene (18.3%), 4-(3,3-dimethyl-1-butynyl)-4-hydroxy-2,6,6-trimethyl-2-cyclohexen-1-one (6.7%), nootkaton-11,12-epoxide (5.9%), valerenal (5.0%), 5,7-dihydroxy-3,6,8-trimethoxyflavone (3.7%), β-eudesmol (3.6%), 3-oxo-α-ionol (3.1%), ethylene glycol monosalicylate (2.7%), 5-hydroxycalamenene (2.6%), hexadecanoic acid (2.6%), retusine (2.3%), 5-o-desmethylnobiletin (2.3%), 5,5,8a-trimethyl-3,5,6,7,8,8a-hexahydro-2h-chromene (1.8%), isovelleral (1.7%), khusimyl methyl ether (1.3%), 5,6-dihydroxy-3',4',7,8-tetramethoxyflavone (1.0%), besides other components having individual concentration less than 1.0%. Complete identification of the extract composition is tabulated in Table 1.

Table 1: Chemical composition of chloroform extract of *L. indica*

S. N.	Retention Indices	Compounds	% Peak Area
1	1097	dimethyl(3-methylbut-2-enyloxy)isobutoxy- silane	0.5
2	-	ginkgol	0.3
3	1153	1,4-cyclohexanediol	0.9
4	1309	5,5,8a-trimethyl-3,5,6,7,8,8a-hexahydro-2h-chromene	1.8
5	1443	4,5-dimethyl-1,2,3,6,7,8,8a,8b-octahydrobiphenylene	18.3
6	1550	elemicin	0.5
7	1555	6-epi-shyobunol	0.5
8	-	incensol oxide	0.2
9	-	valerenal	5.0
10	-	dihydroactinolide	0.8
11	-	(-)-loliolide	0.2
12	1609	Z-7-tetradecenal	0.4
13	1623	ethylene glycolmonosalicylate	2.7
14	1627	3-oxo-α-ionol	3.1
15	1734	isovelleral	1.7
16	-	10,10-dimethyl-tricyclo[7.1.1.0]undec-2-en-4-one	0.3
17	-	4-(3,3-dimethyl-1-butynyl)-4-hydroxy-2,6,6-trimethyl-2-cyclohexen-1-one	6.7
18	1742	nootkaton-11,12-epoxide	5.9
19	1757	5-hydroxycalamenene	2.6

20	-	neophytadiene	0.6
21	1968	hexadecanoic acid	2.6
22	2045	phytol	0.2
23	2077	octadecanoic acid methyl ester	0.3
24	-	widdrol	0.7
25	2167	octadecanoic acid	0.1
26	2183	linoleic	0.3
27	-	stigmasta-5,22-dien-3-ol	0.6
28	2885	1,1,6-trimethyl-3-methylene-2-(3,6,9,13-tetramethyl -6-ethenye-10,14-dimethylene-pentadec-4-enyl)cyclohexane	0.4
29	2925	retusine	2.3
30	2957	5,7-dihydroxy-3,6,8-trimethoxyflavone	3.7
31	2981	cirsilineol	0.5
32	-	5-o-desmethylnobiletin	2.3
33	-	β-eudesmol	3.6
34	-	khusimyl methyl ether	1.3
35	3170	5,6-dihydroxy-3',4',7,8-tetramethoxyflavone	1.0
Total			72.9

3.2 Quantitative phytochemical analysis

3.2.1 Total phenol content (TPC)

TPC estimation using FCR resulted in a significant amount of total phenols in the extract as expressed in terms of mg/gm of Gallic Acid Equivalent (mg GAE/gm)) and the results are displayed in Table 2. Chloroform extract comprises of significantly higher phenol content that is 150.55±0.33 mg/g GAE.

3.2.2 Total flavonoid content (TFC)

Total flavonoid content of the extract were determined by Catechin calibration curve and expressed in terms of mg of Catechin Equivalent per gm (mg CNE/gm) (Table 2). Significantly higher flavonoid content reported in choloroform extract that is 40.72±0.69 mg/g CNE.

3.2.3 Total ortho-dihydric phenol (ODP) content

Catechol calibration curve drawn to estimate total ortho-dihydric phenol content in both the extract and expressed in terms of mg of Catechol Equivalent per gm (mg CLE/gm) (Table 2). The result reports the chloroform extract to possess significantly higher ortho-dihydric phenol content that is 56.66±0.93 mg/g CLE.

Table 2: Phytochemical assay of the chloroform extract of *L. indica*

Phytochemical assay	LICE
TPC (mg/g GAE)	150.55±0.33
TFC (mg/g CNE)	40.72±0.69
ODP (mg/g CLE)	56.66±0.93

Notes: LICE-Chloroform extract of *L. indica*, TPC-Total phenolic assay, TFC-Total flavonoids assay, ODP-Ortho-dihydric phenol GAE- gallic acid equivalent, CNE- Catechin equivalent, CLE-Catechol equivalent.

3.3 Antioxidant activity

3.3.1 NO radical scavenging activity

NO radical scavenging effect of both the fractions at various dose levels of 50-250 µg/ml was found to be significant and substantial when compared to standard antioxidant ascorbic acid (Table 3). Chloroform extract possesses significantly higher antioxidant property having lower IC_{50} of the dose level of 35.85±3.12µg/ml.

3.3.2 DPPH radical scavenging activity

The radical scavenging activity of the extract of *L. indica* was assessed resulting its antioxidant activity in a sequential dose dependent manner. At all concentrations (50-250 µg/ml) the extract exhibited profound inhibition effect on DPPH radical formed which was analyzed to be significantly different ($p<0.01$) with the varied range of inhibition signifying its potent antioxidant activity validated with its IC_{50} value compared to that of the standard antioxidant BHT (Table 3). Chloroform extract possesses considerably higher antioxidant activity with a significantly lower IC_{50} value of 28.52±1.32 µg/ml.

3.3.3 Metal chelating activity of Fe^{+3}

Increased disruption of complex formation of Fe^{+2} by ferrozine with increasing concentration reports the metal chelating activity at all concentrations (50-250 µg/ml) of chloroform extract of *L. indica* in a concentration dependent manner. Percent metal chelation at various concentrations resulted a broad range of inhibition of ferric ion chelation indicating its potent antioxidant activity. IC_{50} value of standard antioxidant citric acid 22.77±0.11 µg/ml and that of extract 49.29±0.15 µg/ml signifies the metal chelation property (Table 3).

Table 3: Antioxidant activity of chloroform extract of *L. indica* represented in terms of IC_{50} values

Sample and standards	NO radical scavenging activity	DPPH radical scavenging activity	Metal chelating activity of Fe^{+2}	Reducing power activity
**LICE	35.85±3.12	28.52±1.32	49.29±0.15	39.17±0.65
*Ascorbic acid	7.82±0.15	-	-	-
*BHT	-	9.28±0.09	-	-
*Citric acid	-	-	11.13±0.17	-
*Catechin	-	-	-	18.12±0.01

Notes: **samples, *standard antioxidant, LICE- Chloroform extract of *L. indica*, BHT- Butylated Hydroxyl Toluene.

3.3.4 Reducing power activity of Fe^{+3} to Fe^{+2}

At all the concentrations of 50-250 µg/ml, the extract displayed significant reducing power activity of Fe^{+3} to Fe^{+2} but substantial compared to standard antioxidant catechin ($p<0.01$). Regression analysis reveals chloroform extract to possess higher antioxidant activity as revealed by lower RP_{50}

value of 39.17±0.65 µg/ml (Table 3).

3.4 *In-vitro* anti-inflammatory activity

Graded concentrations (50-250 µg/ml) of both the plant extracts were assessed inhibition of protein denaturation as a measure of anti-inflammatory activity. And the results were found to be satisfactory and also significant at all concentrations and even substantial with that of standard diclofenac sodium ($p<0.01$). On assessment chloroform extract was observed to possess significantly higher anti-inflammatory activity with IB_{50} value of 27.96±1.14µg/ml (Table 4).

Table 4: *In-vitro* anti-inflammatory activity of chloroform extract of *L. indica* represented in terms of IB_{50} values

Sample and standards	IB_{50} Value
**LICE	27.96±1.14
*Diclofenac sodium	10.15±0.10

Notes: **samples, *standard antioxidant, LICE- Chloroform extract of *L. indica*

3.5 Insect antifeeding activity

In present study the antifeeding activity of chloroform extract of *L. indica* was investigated against *S. obliqua*, and results exhibited the significant antifeeding activity in the extract in a sequentially dose dependent manner. At lowest concentration of 5% it was 32.98, 1.59, 2.21, 0.00% at the consecutive time intervals of 12, 24, 36, 48 hours respectively. However, at 10% it was found to be 87.95, 88.18, 77.53, 37.41% respectively. At 15% it was 91.74, 90.46, 79.85, 70.64%. At 20% it was 93.31, 92.82, 89.89, 79.61% while at 25%, 100.00, 100.00, 95.66, 94.14% antifeeding activity was observed in the same time frame (Table 5). The present research confirms the antifeedant activity of the chloroform extract of *L. indica* exhibited significant antifeeding activity at the dose levels of 20% and 25% and the results are statistically analyzed to be significantly different ($p<0.05$) at all the concentrations (5-25%) and at all-time intervals in sequentially dose and time dependent manner.

Table 5: Insect antifeeding activity of the chloroform extract of *L. indica*

DOSES (%)	After 12 hours		After 24 hours		After 36 hours		After 48 hours	
	Leaf area consumed	Antifeeding activity	Leaf area consumed	Antifeeding activity	Leaf area consumed	Antifeeding activity	Leaf area consumed	Antifeeding activity
5	7.91±1.33	32.98	23.08±1.28	1.59	23.91±1.87	2.21	25.00±0.00	0.00
10	1.00±0.84	87.95	1.49±1.02	88.18	3.16±1.87	77.53	11.38±1.83	37.41
15	0.67±1.17	91.74	1.19±1.03	90.46	2.80±1.52	79.85	4.30±2.28	70.64
20	0.54±0.94	93.31	0.88±0.77	92.82	1.33±1.11	89.89	2.83±1.91	79.61
25	0.00±0.00	100.00	0.00±0.00	100.00	0.55±0.57	95.66	0.75±0.82	94.14
Control	15.71±2.79	-	23.83±2.02	-	25.00±0.00	-	25±0.00	-

4. Discussion

In previous, β-monolinolein and aristolone reported as the major compound in the extracts (methanol and hexane) of *L. indica* [12, 13]. Flavonoids (5,7-dihydroxy-3,6,8-trimethoxyflavone and 5,6-dihydroxy-3',4',7,8-tetramethoxyflavone) identified and quantified in the chloroform extract of *L. indica*, which also were isolated and identified in extracts of *L. indica* and other species of *Limnophila* [11]. Chloroform extract of *L. indica* also possessed significant amount of TPC, TFC and ODP than methanol and hexane extract [12, 13]. Chloroform extract of *L. indica* showed higher antioxidant, anti-inflammatory and antifeeding activity as compared to methanolic and hexane extracts [12, 13], may be due to the presence of significant amount of phenolics and flavonoids compounds or major components [4,5-Dimethyl-1,2,3,6,7,8,8a,8b-octahydrobiphenylene (18.3%), 4-(3,3-dimethyl-1-butynyl)-4-hydroxy-2,6,6-trimethyl-2-cyclohexen-1-one (6.7%), nootkaton-11,12-epoxide (5.9%) and valerenal (5.0%)] of the extract, which were not found in methanol and hexane extract [12, 13] of the plant. The other species of *Limnophila* (*L. aromatica*, *L. conferta* and *L. geoffrayi*) have also been reported as significant antioxidant and anti-inflammatory agent [24, 25, 26]. Extracts of various plant species constituting active compounds like sesquiterpenes, diterpenoids, triterpenes, lactones, quinolene, phenolics, fatty acids, saponins, alkaloids, flavonoids exhibited potent antifeeding, insecticidal and growth inhibitory properties [27-29]. The results of the present research are in support of the previous investigations, suggesting the significant antioxidant, anti-inflammatory and antifeeding activity in the methanolic and hexane extract of the aerial plant part of *L. indica* [12, 13].

5. Conclusion

As per the results of the present research it is evident that the plant *Limnophila indica* L. (Druce) is a potent antioxidant and anti-inflammatory agent, indicating its potentiality in the field of food, pharmaceutical and cosmetic industry. The plant also possesses a substantial insect antifeeding activity predicting its possible use as a botanical insecticide acting as a substitute to synthetic insecticide and can be an important component of sustainable agriculture which is an emerging need of today in the field of agriculture.

6. Acknowledgement

The author hereby acknowledges Dr. D.S. Rawat, Dept. of Biological Sciences, C.B.S.H., G.B.P.U.A.T., Pantnagar for the identification of the plant species and Dr. R.M. Srivastava, Dept. of Entomology, College of Agriculture, G.B.P.U.A.T., Pantnagar for proper guidance of insect antifeeding activity assessment. Thanks are due to A.I.R.F., J.N.U., New Delhi for providing facility for GC-MS analysis.

7. References

1. The plant list; http://www.theplantlist.org/ <http://www.theplantlist.org/. 2013. 25 June, 2018.
2. Mabberley DJ. Mabberley's Plant-Book A portable dictionary of plants, their classification and uses. 3rd edition. Cambridge University Press, Cambridge, UK. 2017, 1102.
3. POWO. Plants of the World Online. http://plantsoftheworldonline.org/. 2017, 2019.
4. CoL. Catalogue of Life. http://www.catalogueoflife.org/col/; 2018. 28 January, 2019.
5. Jain V, Jain SK. Compendium of Indian Folk Medicine

and Ethnobotany (1991-2015), Deep Publications, New Delhi. 2016, 195.
6. Gaur RD. Flora of the district garhwal north west himalaya. 1st edition. Trans media, Srinagar, Garhwal, India. 1999, 486-502
7. Efloraofindia. onwards. *Limnophila indica*. https://sites.google.com /site/efloraofindia/species/m---z/p/plantaginaceae/limnophila/limnophila-indica. 28 January, 2019. 2007
8. Tropical Plants Database, Ken Fern. tropical.theferns.info. <tropical.theferns.info/viewtropical.php?id=Limnophila+indica>. 2014. 10 february, 2019.
9. Subhadra S, Gade G, Ravindran VK, Emani VS, Parre S, Banji D. Microanatomical and phytoanalytical studies of *Limnophila indica* Linn. (Druce). Journal of Pharmacy Research. 2011; 4(11):4146-4150.
10. Brahmachari G. *Limnophila*: chemical and pharmacological aspects, an update. The Open Natural Products Journal. 2014; 7:1-14.
11. Kumar R, Kumar R, Prakash O, Srivastava RM, Pant AK. Chemical composition, *in vitro* antioxidant, anti inflamatory and antifeedant properties in the essential oil of asian marshweed *Limnophila indica* L. Druce. Journal of Pharmacognosy and Phytochemistry. 2019; 8(1):1689-1694.
12. Kumar R, Kumar R, Anjum B, Prakash O, Joshi A, Pant AK *et al*. Phytochemical analysis, *in-vitro* antioxidant, anti-inflammatory and insect antifeeding activity of methanolic extract of *Limnophila indica* (L.) Druce. International Journal of Chemical Studies. 2019; 7(1):1691-1696.
13. Kumar R, Kumar R, Prakash O, Srivastava RM, Pant AK. GC MS analysis of the hexane extract of *Limnophila indica* (L.) Druce, its total phenolics, *in-vitro* antioxidant, anti- inflammatory and antifeeding activity against *Spilosoma obliqua*. Journal of Entomology and Zoology Studies. 2019; 7(1):970-975.
14. Adams RP, Identification of Essential Oil Components by Gas Chromatography/ Mass Spectrometry. Allured Publ. Corp., Carol Stream, IL, 2007.
15. Singleton VL, Rossi JA. Colorimetry of total phenolics with phosphomolybdic phosphotungustic acid reagents. American Journal of Enology and Viticulture. 1965; 16:144-158.
16. Choi Y, Lee SM, Chun J, Lee HB, Lee J. Influence of heat treatment on the antioxidant activities and polyphenolic compounds of Shiitake (*Lentinus edodes*) mushroom. Food Chemistry. 2006; 99(2):381-387.
17. Mahadevan A, Sridhar R. In: Methods in physiological plant pathology. 3rded. Sivakami Publication, Chennai. 1986, 190-192.
18. Joshi A, Prakash O, Pant AK, Kumar R, Negi MS. Chemical Analysis and Antioxidant Activity of Essential Oils of Two Morphotypes of *Lippia alba* (Mill.) NE Br. ex Britton & P. Wilson (Verbenaceae). Journal of Essential Oil Bearimg Plants. 2018; 21(3):687-700.
19. Kumar R, Prakash O, Pant AK, Isidorov VA, Mathela CS. Chemical composition, antioxidant and myorelaxant activity of essential oils of *Globba sessiliflora* Sims. Journal of Essential Oil Research. 2012; 24(4):385-391.
20. Parki A, Chaubey P, Prakash O, Kumar R, Pant AK. Seasonal Variation in Essential Oil Compositions and Antioxidant Properties of *Acorus calamus* L. Accessions. Medicines. 2017; 4(4): 81.
21. Kar B, Kumar RS, Karmakar I, Dola N, Bala A, Mazumder UK *et al*. Antioxidant and *in vitro* anti-inflammatory activities of *Mimusops elengi* leaves. Asian Pacific Journal of Tropical Biomedicine. 2012; 2(2):S976-S980.
22. Warad M, Kalleshwaraswamy CM. Biology of Bihar hairy caterpillar, *Spilarctia obliqua* (Walker) (Erebidae: Lepidoptera) on field bean. 2017; 12(5):1256-1260.
23. Vattikonda SR, Sangam SR. Assessment of antifeedant potential of azadirachtin, a plant product of *Azadirachta indica* against *Papilio demoleus* L. (Lepidoptera: Papilionidae) larvae. Journal of Entomology and Zoology Studies. 2016; 4(5):956-959.
24. Do QD, Angkawijaya AE, Tran-Nguyen PL, Huynh LH, Soetaredjo FE, Ismadji S *et al*. Effect of extraction solvent on total phenol content, total flavonoid content, and antioxidant activity of *Limnophila aromatica*. Journal of Food and Drug Analysis. 2014; 22:296-302.
25. Suksamrarn A, Poomsing P, Aroonrerk N, Punjanon T, Suksamrarn S, Kongkun S. Antimycobacterial and antioxidant flavones from *Limnophila geoffrayi*. Archives of Pharmacal Research. 2003; 26(10):816-820.
26. Reddy GBS, Melkhani AB, Kalyani GA, Rao JV, Shirwaikar A, Kotian M *et al*. Chemical and pharmacological investigations of *Limnophila conferta* and *Limnophila heterophylla*. International Journal of Pharmacognosy. 1991; 29:145-153.
27. Sahayraj K. Antifeeding effect of some plant extracts on Asian armyworm *Spodoptera litura*. (Fabricus). Current Science. 1998; 74(6):523-525.
28. Arivoli S, Tennyson S. Antifeeding activity, developmental indices and morphogenetic variations of plant extracts against *Spodoptera litura* (Fab) (Lepidoptera: Noctudae). Journal of Entomology and Zoology Studies. 2013; 1(4):87-96.
29. Elsharkawy ER, Ed-Dra A, Abdallah EM, Shafa A. Antioxidant, antimicrobial and antifeedant activity of phenolic compounds accumulated in *Hyoscyamus muticus* L. African Journal of Biotechnology. 2018; 17(10):311-321.