



## RESEARCH PAPER

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**Bioassay of different extract of *clerodendrum infortunatum* on some vegetables seeds with their chemical investigation**

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**Abstract**

An experiment was conducted on bioassay of different extracts of *Clerodendrum infortunatum* leaves for each vegetable crop such as, swamp cabbage, ladies finger, and yard long bean with the attempt for chemical investigation on effective plant extract. The chloroform extract of *Clerodendrum infortunatum* has significantly increased and enhanced the germination, growth of shoot length and root length of yard long bean and swamp cabbage whereas, reduced and delayed the germination, growth of shoot length and root length of lady's finger seeds compared with control. The study revealed that different extract of *Clerodendrum infortunatum* contains growth regulatory active compounds. Among the extracts, chloroform extracts showed better performances in terms of percent germination and growth of shoot and root length of yard long bean and swamp cabbage. To find out effective compound from chloroform extracts, thin layer chromatography was done which showed five distinct compounds. These compounds were separated by preparative Thin Layer Chromatography. Further study is needed to determine structure for finding growth regulatory compound in Agricultural usage.

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## Introduction

There are different types of plants such as medicinal plants, fruit trees, herbal plants, flower plants etc. *Clerodendrum infortunatum* (common name Bhat; synonyms *Clerodendrum viscosum* Vent. and *Volkameria infortunata* Roxb.) is a perennialshrub belonging to the family Lamiaceae, also sometimes classified under Verbenaceae. The major compounds are sterols, sugars, flavonoids and saponins. *Clerodendrum infortunatum* leaves on preliminary chemical analysis are found to contain saponin, clerodin (a bitter diterpene) (Chopra *et al.*, 1998). An Experiment was conducted by Roy *et al.* (2006) on naturally occurring growth substances in aqueous extracts of some common weeds viz. Bothua (*Chenopodium album*), Bijlighas (*Strigadensiflora*), Shetdrone (*Leucusaspera*), Mutha (*Cyperus rotundus*), Chapra (*Eleusine indica*) and Khudeanguli (*Digitaria ischaemum*) with the attempt for chemical investigation on effective extracts. Boiled and unboiled extracts of all the weed species under test significantly reduced and delayed germination of wheat and jute seeds compared with control. The effect of boiled and unboiled extracts of Bothua (*Chenopodium album*) sowed the lowest germination in seeds of wheat. The root and shoot length of wheat and jute were also decreased in presence of above mentioned weed extracts.

Traditionally the leaf and root are widely used as antidandruff, antipyretic, ascaricide, laxative, vermifuge, and in treatments of convulsion, diabetes, gravel, malaria, scabies, skin diseases, sore, spasm, scorpion sting, snake bite and tumor (Sharma, 2001; Rahman and Zaman 1989). In Thai medicine the leaves and root are known to be diuretic; and used for treatment of intestinal infections and kidneydisfunction; when boiled or ground with water, it is taking to increase milk secretion for post-labor. In many traditional practices the leaves and root are widely used as anti hyperglycemic (Modi, 2010). The use of herbal medicine has become increasingly popular worldwide and medicinal plants are believed to be an important source of new chemical substances with potential therapeutic effects (Estakhret *al.*,

2011). Various parts of the plant have been used by tribes in colic, scorpion sting, snake bite, tumor and certain skin diseases (Nadkarniet *al.*, 2002) also used in Indian folk medicine as in the treatment of bronchitis, asthma, fever, diseases of the blood, inflammation, burning sensation and epilepsy (Kapoor, 2001). Fresh leaves are given for diarrhoea, liver disorders and headache (Duke, 2010). Fresh juice of the leaves has been used as vermifuge and in treatment of malaria (Goswami, 1998).

Now a days it is clearly observed that plant extract especially *C. infortunatum* having biological activity are relatively safe to the user, non-target organisms and environment. These stuffs are cheaper as well as eco-friendly for environment. A research has been under taken to isolate the compounds from different extract of *Clerodendrum infortunatum* considering the hypothesis of their effects on some vegetable seeds along with their chemical investigation” to assess the following objectives-

Investigation on the bioassay of different extract of *Clerodendrum infortunatum* on some vegetable seeds.

Isolation of the different bioactive compounds from chloroform extract of *Clerodendrum infortunatum*

## Materials and methods

### Experimental Site

The experiment was conducted at research laboratory, Department of Biochemistry and Molecular Biology, Hajee Mohammad Danesh Science and Technology University, Dinajpur.

### Collection of *Clerodendrum infortunatum*

*Clerodendrum infortunatum* leaves were collected from Village of Knoukhair, Upazila-Chirirbandar, District- Dinajpur. The plant was identified by the Bangladesh National Herbarium, and a herbarium was preserved at the Laboratory of the Department of Biochemistry and Molecular Biology, Hajee Mohammad Danesh Science & Technology University, Dinajpur, Bangladesh.

#### *Selection and Collection of Summer Vegetables*

Yard long bean (*Vigna unguiculata*), Swamp cabbage (*Impoeca aquatic*) and Lady's finger (*Hibiscus esculentus*) seeds were selected due to their short life and growth period and also available in sub-tropical countries. The seeds of these vegetables were collected from the Dinajpur seed market. The purity percentages and germination percentages of these seeds were 95 and 90, respectively.

#### *Preparation of Water extract of Clerodendrum infortunatum leaves*

Newly grown, fresh, green leaves of *Clerodendrum infortunatum* were collected for preparing the water extract. The leaves were weighed 4kg and they were cut into small pieces and water was added. The mixture was homogenized with mortar & Blender and paste and 2.5 kg leaf- paste were obtained. The paste was suspended in 2 liter of water and then filtered through a filter paper. Finally, a total of 3.75 Liter of extract was obtained.

#### *Preparation of chloroform extract of Clerodendrum infortunatum leaves*

6 kg of fresh & green leaves was collected. The leaves were clean and dried it at first sun light 7 days, then it was dried 48 hours at 70 ° c by oven. The leaves were then making powder by Blender Machine and obtained 1.5 kg leaves powder. The powder then dissolved in 4 liter absolute chloroform (96%) and wait 72 hours. These suspensions were filtered with thin and clean cloth and filtered by filter paper. The suspensions were dried by BUCHI Rota vapor R-114, connected with BUCHI water bath B-480 at 70 ° C. The dried extract was weighed by Digital balance. The total weight of leaves chloroform extract obtained 185gm.

#### *Preparation of ethanol extract of Clerodendrum infortunatum leaves*

6 kg of fresh & green leaves were collected. The leaves were clean and dried it at first sun light 7 days, then it was dried 48 hours at 70 ° c by oven. The leaves were then making powder by Blender Machine and obtained 1.5 kg leaves powder. The powder dissolved

in 4 liter absolute ethanol (96%) and wait 72 hours. These suspensions filtered with thin and clean cloth and filtered by filter paper. The suspensions were dried by BUCHI Rota vapor R-114, connected with BUCHI water bath B-480 at 70 ° C. The dried extract was weighed by Digital balance. The total weight of leaves ethanol extract obtained 105gm.

The individual stored herbal plant extracts were investigated as following sequential treatments:

- a) Water or control T<sub>c</sub>
- b) Aqueous extract of *C.infortunatum*T<sub>1</sub>
- c) Chloroform extract of *C.infortunatum*T<sub>2</sub>
- d) Ethanol extract of *C.infortunatum*T<sub>3</sub>

#### *Set up for the Investigation of Vegetable Crop Seeds*

Petridish experiment was done for lady's finger, swamp cabbage and Yard long bean seeds for the observation of germination percentage; shoot growth and root growth, plant height etc. For this experiment, clean petridish with two sheets filter papers were used. For the investigation of germination percentage, growth and development of vegetable seeds, fifteen ml of each aqueous extract was put in each petridish. In control, only distilled water was used and amount of distilled water was also same. Then twenty five seeds of each vegetable crop were kept in each petridish and each treatment was replicated into three times. The petridishes were kept in natural diffused light under laboratory conditions at 29±2°C temperature and relative humidity of 85±5% after placing. 5 ml of water was used per day per petridish to keep constant moisture. In control, only water was added if necessary per day per petridish.

In this experiment, all subsequent observations were recorded and it was started from 31<sup>th</sup> January, 2012. After setting the experiment, the germination percentages, shoot length, root length and completion of germination were recorded. Effects of different treatments on morphology of seedlings were also recorded. The data were subjected to analyze the coefficient of variance and means were compared by the

DMRT method.

#### *Technique for Shoot and Root Growth Measurement*

Ten healthy seedlings were taken from each replication of all treatments for measurement of shoot and root length. Each replication of individual treatments was averaged the root and shoot lengths measured individual treatment finally.

#### *Identification of Effective Extracts*

The collected data were analyzed statistically using Duncan's New Multiple Range Test (DMRT) compared the difference means. After analyses these data, it was observed that the aqueous extract of Bhat inhibits the germination, shoot length and root length, whereas Chloroform extract enhance germination, shoot length and root length of these vegetable crops.

#### *Chemical investigation of effective extract of Clerodendrum infortunatum*

Thin Layer Chromatography (TLC) is one of the most important techniques, by which we are able to detect or identify the presence the number of compounds or number of components present in a crude extract or crude compound in which  $R_f$  value of each component was calculated by using this formula:

$$R_f = \frac{\text{Distance traveled by the component}}{\text{Distance traveled by the solvent front}}$$

Five different compounds were detected by Thin

Layer Chromatography (TLC) with different  $R_f$  value (Fig. 4) and separated by preparative TLC (Fig. 5)

#### *Tests for Sterol of different purified compound*

After purification of different crude compounds, the isolated and purified compounds were subjected to test for sterol by following reaction.

#### *Salkowaski reaction*

A small amount of compound was taken and dissolved in chloroform and a few drops of concentrated sulfuric acid were added to it. A radish color developed indicating the presence of sterol.

#### *Lieberman-Burchard Reaction*

A small amount of compound was taken and dissolved in chloroform and a few drops of concentrated sulfuric acid were added to it followed by 2-3 drops of acetic anhydride. A slightly greenish color developed indicating the presence of sterol.

### **Results and discussion**

The results showed that growth regulatory activity in different extracts of *Clerodendrum infortunatum* Plant fresh and clean leaf for germination, root and shoot growth of three vegetables viz Yard long bean, Swamp cabbage and lady's finger. The result of the present study has been presented in tables and Figures along with adequate discussion in this chapter.

**Table 1.** Effects of *Clerodendrum infortunatum* (bhat) leaf extract on percent germination of yard long bean, swamp cabbage and lady's finger seeds.

Treatments	Germination %								
	yard long bean			swamp cabbage			lady's finger		
	2 <sup>nd</sup> day	4 <sup>th</sup> day	7 <sup>th</sup> day	2 <sup>st</sup> day	4 <sup>rd</sup> day	7 <sup>th</sup> day	2 <sup>st</sup> day	4 <sup>rd</sup> day	7 <sup>th</sup> day
T <sub>c</sub>	29.33ab	58.67 a	80.00a	36.00a	53.33a	76.00ab	36.00a	50.67 a	61.33a
T <sub>1</sub>	22.67bc	38.67 b	66.67b	13.33b	33.33b	61.33b	12.00b	29.33c	44.00b
T <sub>2</sub>	30.67 a	54.67a	81.33a	32.00a	52.00a	80.00a	16.00b	41.33b	64.00a
T <sub>3</sub>	20.00 c	46.67ab	66.67b	17.33b	37.33b	62.67ab	29.33a	49.33ab	58.67a
LSD(0.05)	7.173	13.84	10.98	12.49	11.99	17.67	10.32	8.935	6.388

Means followed by the same letter(s) did not differ significantly at 5% level by DMRT.

*Effect of Different extracts of Germination percentage*

*Clerodendrum infortunatum on Yard long bean*

The germination percentage was counted in 2<sup>st</sup>, 4<sup>rd</sup>

and 7<sup>th</sup> days presented in table 1. In 2<sup>st</sup> day, the height germination percentage was found in T<sub>2</sub> (30.67%) which was followed by T<sub>c</sub> and T<sub>3</sub> and the lowest germination percentage was recorded in T<sub>1</sub> (22.67%), respectively. In 4<sup>rd</sup> day, the height germination percentage was found in T<sub>c</sub> (58.67%) which was

followed by T<sub>2</sub> and T<sub>3</sub> and the lowest germination percentage was recorded in T<sub>1</sub> (38.67%), respectively. In 7<sup>th</sup> day, the height germination percentage was found in T<sub>2</sub> (81.33%) and the lowest germination percentage was recorded in T<sub>1</sub> (66.67%), respectively.

**Table 2.** Effects of *Clerodendrum infortunatum* (bhat) leaf extract on shoot length of yard long bean, swamp cabbage and lady's finger seeds.

Treatments	Shoot length of (cm)								
	yard long bean			swamp cabbage			lady's finger		
	5 <sup>th</sup> day	8 <sup>th</sup> day	11 <sup>th</sup> day	5 <sup>th</sup> day	8 <sup>th</sup> day	11 <sup>th</sup> day	5 <sup>th</sup> day	8 <sup>th</sup> day	11 <sup>th</sup> day
T <sub>c</sub>	1.363bc	1.687b	4.533a	1.493ab	2.400a	2.840a	1.637a	3.667a	5.737a
T <sub>1</sub>	1.640 b	2.393b	2.527a	1.233 b	2.250a	3.107b	1.467b	1.750b	0.000d
T <sub>2</sub>	2.603 a	3.573a	3.513a	1.653 a	2.267a	4.583b	1.617a	3.563a	5.380b
T <sub>3</sub>	1.023 c	2.030b	2.970a	1.460ab	1.790a	1.503c	1.527b	3.280a	5.163c
LSD(0.05)	0.4853	0.9371	2.247	0.3222	1.003	0.8640	0.0893	0.6656	0.1548

Means followed by the same letter(s) did not differ significantly at 5% level by DMRT.

#### Shoot length

Shoot length of long yard bean at different days after sowing influenced significantly by the effects of different leaf extract (Table 2). At 5 Days after sowing (DAS) with the Chloroform extract of *Clerodendrum infortunatum* was the highest shoot length (2.603 cm) whereas the lowest shoot length (1.023 cm) was recorded in T<sub>3</sub> treatment. Other treatments showed more or less moderate statistical results at the same time. The highest shoot length of yard long bean seedling was found in T<sub>2</sub> i.e. Chloroform leaf extract of *Clerodendrum infortunatum* (3.573 cm) at 8 DAS that was statistically similar to others. At 11 DAS the highest shoot length was recorded in T<sub>c</sub> (4.533 cm) and the lowest was found in T<sub>1</sub> (2.527 cm), respectively. At 14 DAS, The highest shoot length o was found in T<sub>c</sub> i.e. Chloroform leaf extract of *Clerodendrum infortunatum* (4.580cm) followed by T<sub>1</sub> and T<sub>3</sub>. On the other hand the lowest shoot length was recorded in T<sub>2</sub> (2.883 cm). Possibly due to presence of growth regulator or other bioactive substances in the chloroform extract of *Clerodendrum infortunatum* shoot growth of long yard bean was gradually increasing.

#### Root length

Root length of long yard bean at different days after sowing influenced significantly by the effects of different stem extract (Table 3). At 5 Days after sowing (DAS) with the Chloroform leaf extract of *Clerodendrum infortunatum* was the highest root length (1.983 cm) which was similar to T<sub>c</sub> and T<sub>3</sub> treatments, whereas the lowest root length (1.14 cm) was recorded in T<sub>1</sub> treatment. The highest root length of yard long bean seedling was found in T<sub>2</sub> i.e. Chloroform leaf extract of *Clerodendrum infortunatum* (2.847 cm) at 8 DAS that was statistically similar to others. At 11 DAS the highest root length was recorded in T<sub>2</sub> (4.437 cm) and the lowest was found in T<sub>1</sub> (1.417 cm), respectively. At 14 DAS, The highest root length of long yard bean was found in T<sub>2</sub> i.e. Chloroform leaf extract of *Clerodendrum infortunatum* (4.967 cm) followed by T<sub>c</sub> and T<sub>3</sub>. On the other hand the lowest root length was recorded in T<sub>1</sub> (1.873 cm). Similarly due to presence of some bioactive substances in the chloroform extracts of *Clerodendrum infortunatum* root growth was also enhanced.

*Effect of different extract of Clerodendrum infortunatum on Swamp cabbage*

*Germination percentage*

The germination percentage was counted in 2<sup>st</sup>, 4<sup>rd</sup> and 7<sup>th</sup> days presented in table 1. In 2<sup>st</sup> day, the height germination percentage was found in T<sub>1</sub> (36.0%) which was followed by T<sub>2</sub> and T<sub>3</sub> and the lowest germination percentage was recorded in T<sub>1</sub> (13.33%), respectively. In 4<sup>rd</sup> day, the height germination

percentage was found in T<sub>c</sub> (53.33%) which was followed by T<sub>2</sub> and T<sub>3</sub> and the lowest germination percentage was recorded in T<sub>1</sub> (33.33%), respectively. In 7<sup>th</sup> day, the height germination percentage was found in T<sub>2</sub> (80.0%) and the lowest germination percentage was recorded in T<sub>1</sub> (61.33%), respectively.

**Table 3.** Effects of *Clerodendrum infortunatum* (bhat) leaf extract on root length length of yard long bean, swamp cabbage and lady's finger seeds.

Treatment s	Root length (cm)								
	yard long bean			swamp cabbage			lady's finger		
	5 <sup>th</sup> day	8 <sup>th</sup> day	11 <sup>th</sup> day	5 <sup>th</sup> day	8 <sup>th</sup> day	11 <sup>th</sup> day	5 <sup>th</sup> day	8 <sup>th</sup> day	11 <sup>th</sup> day
T <sub>c</sub>	1.147 b	2.770ab	3.507a	1.273a	2.793a	4.380 b	1.35a	3.017a	5.300a
T <sub>1</sub>	1.140 b	1.600 c	1.417b	0.766a	0.863b	0.963c	1.257a	1.330b	0.000d
T <sub>2</sub>	1.517 b	2.847a	4.437a	1.247a	3.057a	5.500a	1.340a	3.177a	5.033b
T <sub>3</sub>	1.983 a	1.693bc	1.770b	1.047a	2.837a	4.417 b	1.303a	2.617a	4.733c
LSD(0.05)	0.4377	1.111	1.092	0.6158	0.4476	0.6286	0.6318	0.7120	0.2364

Means followed by the same letter(s) did not differ significantly at 5% level by DMRT.

*Shoot length*

Shoot length of swamp cabbage at different days after sowing influenced significantly by the effects of different leaf extract (Table 2). At 5 Days after sowing (DAS) with the Chloroform extract of *Clerodendrum infortunatum* was the highest shoot length (1.653 cm) whereas the lowest shoot length (1.233 cm) was recorded in T<sub>1</sub> treatment. Other treatments showed more or less moderate statistical results at the same time. The highest shoot length of yard long bean

seedling was found in T<sub>2</sub> i.e. Chloroform leaf extract of *Clerodendrum infortunatum* (2.267 cm) at 8 DAS that was statistically similar to others. At 11 DAS the highest shoot length was recorded in T<sub>c</sub> (4.583 cm) and the lowest was found in T<sub>1</sub> (1.503 cm), respectively. Possibly due to presence of growth regulator or other bioactive substances in the chloroform extract of *Clerodendrum infortunatum* shoot growth of swamp cabbage was gradually increasing.

**Table 4.** R<sub>f</sub> values of detected components of *Clerodendrum infortunatum*

Name of the Plant Species	Ratio(hexane:ethylacetate)	Detected component	R <sub>f</sub> value
<i>Clerodendrum infortunatum</i>	5:1	R <sub>1</sub>	0.86
		R <sub>2</sub>	0.71
		R <sub>3</sub>	0.52
		R <sub>4</sub>	0.49
		R <sub>5</sub>	0.43

*Root length*

Root length of swamp cabbage at different days after sowing influenced significantly by the effects of different stem extract (Table 3). At 5 Days after sowing (DAS) with the Chloroform leaf extract of *Clerodendrum infortunatum* was the highest root

length on T<sub>c</sub> (1.273 cm) which was similar to T<sub>2</sub> and T<sub>3</sub> treatments, whereas the lowest root length (0.7667 cm) was recorded in T<sub>1</sub> treatment. The highest root length of yard long bean seedling was found in T<sub>2</sub> i.e. Chloroform leaf extract of *Clerodendrum infortunatum* (3.057 cm) at 8 DAS that was

statistically similar to others. At 11 DAS the highest root length was recorded in T<sub>2</sub> (5.500 cm) and the lowest was found in T<sub>1</sub> (0.9633 cm), respectively. Similarly due to presence of some bioactive

substances in the chloroform extracts of *Clerodendrum infortunatum* root growth was also enhanced.

**Table 5.** Chemical tests for sterol for isolated fractions.

Name of fraction	Salkowski reaction	Liebermann-Burchard reaction
Fraction 1	+ve	+ve
Fraction 2	-ve	-ve
Fraction 3	-ve	-ve
Fraction 4	-ve	-ve
Fraction 5	-ve	-ve

#### *Effect of Different extracts of Clerodendrum infortunatum on lady's finger*

##### *Germination percentage*

The germination percentage was counted in 2<sup>st</sup>, 4<sup>rd</sup> and 7<sup>th</sup> days presented in table 1 In 2<sup>st</sup> day, the height germination percentage was found in T<sub>c</sub> (36.00%) which was followed by T<sub>3</sub> and T<sub>2</sub> and the lowest germination percentage was recorded in T<sub>1</sub> (12.00%), respectively. In 4<sup>rd</sup> day, the height germination percentage was found in T<sub>c</sub> (50.67%) which was followed by T<sub>3</sub> and T<sub>2</sub> and the lowest germination percentage was recorded in T<sub>1</sub> (29.33%), respectively. In 7<sup>th</sup> day, the height germination percentage was found in T<sub>2</sub> (64.00%) and the lowest germination percentage was recorded in T<sub>1</sub> (44.00%), respectively.

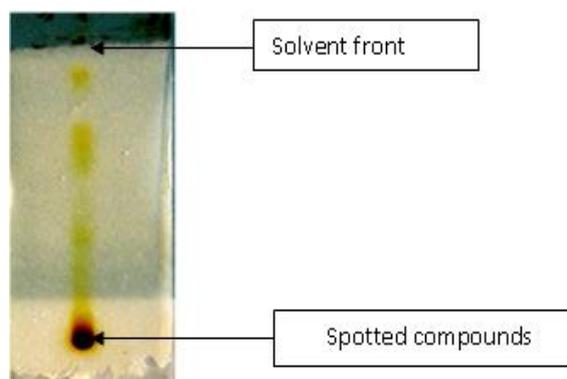


**Fig. 1.** Seeds setup for the investigation.

##### *Shoot length*

Shoot length of ladies finger at different days after sowing influenced significantly by the effects of different leaf extract (Table 2). At 5 Days after sowing (DAS) the highest shoot length on T<sub>c</sub> (1.637 cm) whereas the lowest shoot length (1.467 cm) was

recorded in T<sub>1</sub> treatment. Other treatments showed more or less moderate statistical results at the same time. The highest shoot length of yard long bean seedling was found in (3.667 cm) at 8 DAS and lowest in T<sub>1</sub>. At 11 DAS the highest shoot length was recorded in T<sub>2</sub> (5.737 cm) and the lowest was found in T<sub>1</sub> (0 cm), respectively. Possibly due to presence of growth regulator or other bioactive substances in the chloroform extract of *Clerodendrum infortunatum* shoot growth of ladies finger was gradually increasing.

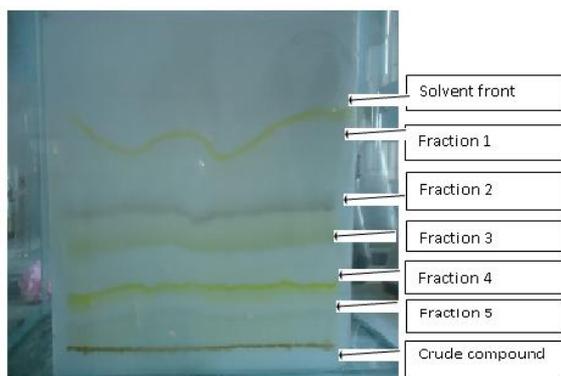


**Fig. 2.** Thin Layer Chromatographic.

##### *Root length*

Root length of ladies finger at different days after sowing influenced significantly by the effects of different stem extract (Table 3). At 5 Days after sowing (DAS) with the Chloroform leaf extract of *Clerodendrum infortunatum* was the highest root length (1.340 cm) which was similar to T<sub>c</sub> and T<sub>3</sub> treatments, whereas the lowest root length (1.257 cm) was recorded in T<sub>1</sub> treatment. The highest root length

of yard long bean seedling was found in T<sub>2</sub> i.e. Chloroform leaf extract of *Clerodendrum infortunatum* (3.177 cm) at 8 DAS that was statistically similar to others. At 11 DAS the highest root length was recorded in T<sub>2</sub> (5.300 cm) and the lowest was found in T<sub>1</sub> (0 cm), respectively. Similarly due to presence of some bioactive substances in the chloroform extracts of *Clerodendrum infortunatum* root growth was also enhanced.



**Fig. 3.** A preparative TLC plat.

#### Chemical Investigation

The results in this experiment indicates that the different extracts of *Clerodendrum infortunatum* have increasing or inhibitory activity on germination parameters like time to get fast germination, coefficient of germination, germination percentages and increasing on root and shoot length or early growth of vegetables. It is very interesting that the increasing tendency of germination parameters roots and shoots length in chloroform extracts of *Clerodendrum infortunatum*. It is a great interesting challenge for the farmers of our country as well as me that why and which compound is responsible for this type of activity. For this reason I have isolated the crude compounds from the powder of respective leave of herbal plants with different non-polar and polar solvents like chloroform and ethanol etc.

#### TLC (Thin Layer Chromatography) of Chloroform Extract of *Clerodendrum infortunatum*

The TLC (Thin Layer Chromatography) of chloroform extract of *Clerodendrum infortunatum* was showed distinctly five compounds at Hexane: Ethylacetate (5:1 v/v). This result suggested that it contained five distinct compounds, designated as R<sub>1</sub>, R<sub>2</sub>, R<sub>3</sub>, R<sub>4</sub> and

R<sub>5</sub> respectively. These compounds are detected in iodine tank and the following R<sub>f</sub> value were calculated by using the formula (Furnisset *al.*, 1989).

#### Separation of individual fractions by preparative TLC

Five fractions were individually Separationby preparative TLC of solvent system (hexane: ethyl acetate 5:1 v/v). 20X20 cm wide and 0.50mm thick Preparative TLC plate (Merck, Germany) was used for this purpose. Preparative TLC was used to separate different components of a mixture after establishing the solvent system for TLC. The solution was placed along a straight line vertically at right end to left of the plate by means of glass capillary tube. The solvent was then allowed to vertically in a large solvent (same ratio) tank containing the solvent used as the mobile phase so that the line containing the mixture stayed half inch above the solvent level in the tank. After the mobile phase moved over appreciable distance, the plates were taken out and dried in air. The appropriate zones corresponding to different R<sub>f</sub> values were detected by exposing one side of the plate in iodine vapor with the rest of the plates surfaces covered by a clean glass plates. The relevant zone/zones were significantly indicated compound were cut out from the plates and extracted separately with appropriate solvent. The solvent then removed under reduced pressure to get the desired compound.

*The amount of all fractions after complete separation was as follows-*

1. Fraction-1: 17 mg.
2. Fraction-2: 7 mg.
3. Fraction-3: 5 mg.
4. Fraction-4: 5.3 mg.
5. Fraction-5: 4.1mg.

#### Chemical test for sterol for isolated fraction

The results of the different chemical tests were presented in tabular form. Of the entire fractions only fraction 1 were showed both Salkowaski and Liebermann-Burchard reaction positive, which was indicated that fraction 1 may be sterol type of compound or compounds.

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