ISSN 0974 – 5211

Journal of Natural Products

Volume 8 (2015)

www.JournalofNaturalProducts.Com

Research Paper

Effect of different extract of gulancha (*Tinospora cordifolia*) on some vegetable seeds with their chemical investigation

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(Received 05 May 2015; Revised 20 May- June 2015; Accepted 24 June 2015)

ABSTRACT

Gulancha (*Tinospora cordifolia)* is an herb known as medicinal plant in different countries of the World. A bioassay study of gulancha extracts was conducted on swamp cabbage (*Impoea aquatica*), lady's finger (*Hibiscus esculentus*) and tomato (*Lycopersicon esculentus*) with their chemical investigation. The ethanol extract of gulancha significantly enhanced germination percentage, growth of shoot and root of swamp cabbage, lady's finger and tomato seeds compared with control respectively. Likewise, aqueous extract of guluncha reduced and delayed germination, growth of shoot length and root length of this vegetable crop. However, the thin layer chromatography (TLC) of ethanol extracts of gulancha detected five compounds at Hexane: Ethyl acetate (10:1, v/v). This study enhanced our knowledge on understanding of chemical investigation of medicinal plant (gulancha) and their effects on crop production.

Key words: Bioassay; Chemical Investigation; Extract; Vegetables.

INTRODUCTION

Plants are the natural resources of different organic and bio-organic compounds essential for other living resources in the World. Different types of naturally occurring organic, bio-organic compound have been isolated from plants. Most of them have effective medicinal values, growth regulatory effects, herbicidal and pesticidal effects and also toxic values. The attention is being needed to the importance of rotation in medicinal plant or between medicinal herbs and other crops due to strong allelopathic effects of medicinal plants (Rohan et al., 2005; Guo et al., 2006; Nazir et al., 2007). Plant extracts play an important role for increasing crop yield influencing the germination and growth performance (Roy el al., 2012; Sayed et al., 2012). The availability of medicinal plants demands the isolation, separation, purification and characterization of physiologically active principles which are actually useful in the

field of agriculture. The herb 'Gulancha' (*Tinospora cordifolia*) belongs to the family Menispermaceae and is a glabrous climbing shrub found throughout Bangladesh, India, Myanmar and Sri Lanka. Typically, gulancha grows in deciduous and dry forests (Uddin et al., 2011). It is reported that gulancha has anti-spasmodic, anti-inflammatory, anti-allergic and anti-oxidant properties (Singh et al., 2003). The stem is commonly used in dyspepsia, fevers, and urinary diseases. The root and stem of gulancha are prescribed in combination with other drugs as an anti-dote to snake bite and scorpion sting (Zhao et al., 1991). Premanath and Lakshmidevi (2010) examined the anti-oxidant effects of leaves of gulancha extracted with hexane, chloroform, methanol, ethanol and water. The water soluble fraction of gulancha leaves fraction has immune-stimulatory and disease resistance properties and potentially be used as an immune-prophylactic agent (Alexander et al., 2010). Gulancha is widely used in Indian ayurvedic medicine for treating diabetes mellitus (Stanely et al., 2001). It is reported that the daily administration of either alcoholic or aqueous extract of gulancha reduces the blood glucose level and increases glucose tolerance in rodents (Grover et al., 2001). First report showed that various types of extracts of gulancha having different bioactive compounds influence the germination and growth rate of crops (Aktar et al., 2012). Little is known about the growth regulatory activity of gulancha, therefore we investigated second time its growth regulatory activities on seed germination of some vegetables.

The aim of the study is the investigation of growth regulatory effects of different gulancha extracts on some vegetables and isolation of different bioactive compounds from ethanol extracts of gulancha.

MATERIALS AND METHODS

Experimental site and design: The experiment was conducted in the laboratory of Agricultural Chemistry, Hajee Mohammad Danesh Science and Technology University, Dinajpur, Bangladesh. The aqueous, chloroform and ethanol extracts of gulancha were prepared from stem of gulancha plant. The isolated extracts were then tested on germination and growth of three vegetables plant seeds such as tomato*(Lycopersicon esculentus)*, swamp cabbage*(Impoea aquatic)* and lady's finger*(Hibiscus esculentus).* Chemical investigation was also carried out to identify the effective extract. There were four treatments; a) water or control (T_c), b) aqueous extract (T_1), c) ethanol extract (T_2) and d) chloroform extract (T_3) were set up to conduct the experiment.

Preparation of aqueous extracts of gulancha: The gulancha stems were collected from Chirirbandar under Dinajpur dristict. Different extracts were prepared as described previously (Aktar et al., 2012). In brief, about 50g of fresh and clean stems were taken and cut into smaller pieces as well as washed by tap water to remove debris. The cut pieces were mixed with 1000ml of water and then blended to prepare the aqueous extract. The aqueous mixture was transferred to a 1000 ml reagent bottle. The sample of aqueous mixture was then kept for 72hours at room temperature of 29±2°C and relative humidity of 85±5% with regular interval of stirring. After 72h incubation of aqueous solution, the brownish and dark solutions were filtered through three layers of filter paper (Whatman no. 1). These were transferred to another 1000ml bottle. The filtrates of individual plant extract were stored at room temperature and used for treating the seeds of vegetable crops along with water as a control and other comprehensive study.

Preparation of chloroform and ethanol extract of gulancha: Five kilogram green and fresh stem was cleaned and chopped into small pieces. The small pieces of stem were sun dried for 7days, and then the stem was dried by oven at 70°C for 48 hours. For making powder the dried stem (2.6kg) grinding by grinding machine and obtained 2kg stem powder. The powder then dissolved in 5Liter absolute chloroform (96%) and wait 72 hours for a suspension. These suspensions were filtered with thin and clean cloth and finally filtered by filter paper. The suspension was 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. Then 5g crude was dissolved in 100ml water and used for treating the seeds of vegetable crops along with water as a control and other comprehensive study. For preparation of ethanol extract same procedure was applied except using ethanol instead of chloroform.

Experimental setup of vegetable crop seed germination and growth: A series of petridish for different treatments were used to conduct this experiment. 15ml of aqueous, ethanol and chloroform extract were poured into each clean petridish with two sheets filter papers. Similarly, 15ml distilled water was used for control treatment. 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. 5ml of distilled water was used per day per petridish to keep constant moisture. In the treatment control (Tc), only water was added if necessary per day per petridish. All subsequent observations were recorded. After setting the experiment, the germination percentages, shoot length, root length and completion of germination were recorded. Three randomly selected seedlings were taken from each replication of all treatments for measurement of shoot and root length. Finally, the root and shoot length data of each replication for individual treatments was averaged**.**

Chemical investigation on effective extracts: Thin Layer Chromatography (TLC) is one of the most important techniques to detect or identify the number of compounds present in a crude extract or crude compound. Thin Layer Chromatography (TLC) was carried on glass plates (slides) coated with silica gel G type 60 (BDH, England).The detected compounds in iodine tank were used to calculate the R_f value were calculated by using the formula (Furniss et al., 1989).

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

Separation of compounds by preparative Thin Layer Chromatography: Fractions were individually sseparated by preparative TLC of solvent system (hexane: ethylacetate 10:1 v/v). 20×20cm 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 Rf 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.

Test for sterols of different purified compound: After purification of different crude compounds, the isolated, purified compounds were subjected to test for sterol by following reactions **(**Salkowaski and Liebermann-Burchard reactions**)**. A small amount of compound was taken and dissolved in chloroform and a few drops of concentrate sulphuric acid were added to the solution. The color of the solution was observed and recorded. A little amount of compound was dissolved in chloroform and a few drops of concentrated sulphuric acid were added to it followed by 2-3 drops of acetic anhydride.

Statistical analysis: The collected data were statistically analyses by using Duncan's New Multiple Range Test (DMRT).

RESULTS

Effect of different extracts of gulancha on Lady's finger:

Germination percentage: The germination percentage was counted in 2^{nd}, 4^{th} and 7^{th} days presented in (Table1). In 2^{nd} and 4^{th} day germination percentage were varies from each other. Interestingly, in 7^{th} day, the highest germination percentage was found in T_2 (66.67%) while the lowest in T_1 (44.0%).

Table-1: Effects of different extracts of gulancha on seed germination of lady's finger, swamp cabbage and tomato seeds.

Germination (%)									
Treatments	Lady's finger			Swamp cabbage			Tomato		
	2nd day	4th day	7th day	2nd day	4th day	7th day	2nd day	4th day	7th day
T_c	36.00 a	50.67 a	61.33 a	36.00 a	53.33 a	76.00 ab	29.33 ab	58.67 a	80.00 a
T_1	12.00 b	29.33 c	44.00 b	13.33 b	33.33b	61.33 b	22.67 bc	38.67 b	66.67 b
T_2	16.00 b	41.33 b	66.67 b	32.00 a	52.00a	80.00 a	30.67 a	54.67a	81.33 a
T_3	29.33 a	46.67 ab	64.00 a	17.33 b	37.33b	62.67 ab	20.00 c	46.67 ab	66.67 b
LSD (0.05)	10.32	8.935	6.388	12.49	11.99	17.67	7.173	13.84	10.98

Shoot length: Shoot length of lady's finger at different days after sowing was significantly influenced by different gulancha extract (Table 2). The highest shoot length of lady's finger seedling was found in (3.667cm) at 8 DAS and lowest in T_1. At 11 DAS the highest shoot length was recorded in T_2 (5.737cm) and the lowest was found in T_1 (0cm), respectively. The shoot growth performance of ladies finger was gradually increased with time being. This is possibly due to the effects and presence of growth regulator or other bioactive substances in the ethanol extract of gulancha.

Table-2: Effects of different extracts of gulancha on shoot length of lady's finger, swamp cabbage and tomato seedlings.

Shoot length(cm)									
Treatments	Lady's finger			Swamp cabbage			Tomato		
	5th day	8th day	11thday	5th day	8th day	11thday	5th day	8th day	11thday
T_c	1.637a	3.667a	5.380b	1.493ab	2.400a	2.840a	1.363bc	1.687b	4.533a
T_1	1.467b	1.750b	0.000d	1.23b	2.250a	3.107b	1.640 b	2.393b	2.527a
T_2	1.617a	3.563a	5.737a	1.653a	2.26a	4.583b	2.603 a	3.573a	3.513a
T_3	1.52b	3.280a	5.163c	1.460ab	1.790a	1.503c	1.023c	2.030 b	2.970a
LSD(0.05)	0.08935	0.6656	0.1548	0.3222	1.003	0.864	0.4853	0.9371	2.247

Root length: Root length of lady's finger at different days after sowing was also significantly influenced by the effects of different glancha stem extract (Table 3). At

11 DAS the highest root length was recorded in T_2 (5.30cm) and the lowest was found in T_1 (0cm), respectively. Similarly, the presence of some bioactive substances in the ethanol extracts of gulancha might enhance the root growth of lady's finger.

Table-3: Effects of different extracts of gulancha on root length of lady's finger, swamp cabbage and tomato seedlings.

Root length (cm)									
Treatments	Lady's finger			Swamp cabbage			Tomato		
	5th day	8th day	11th day	5th day	8th day	11th day	5th day	8th day	11th day
T_c	1.353 a	3.017 a	5.033 b	1.273 a	2.793 a	4.380 b	1.147 b	2.770 ab	3.507 a
T_1	1.257 a	1.330 b	0.0 d	0.7667 a	0.863 b	0.9633 c	1.140 b	1.600 c	1.417 b
T_2	1.340 a	3.177 a	5.300 a	1.247 a	3.057 a	5.500 a	1.517 b	2.847 a	4.437 a
T_3	1.303 a	2.617 a	4.733 c	1.047 a	2.837 a	4.417 b	1.983 a	1.693 bc	1.770 b
LSD (0.05)	0.6318	0.712	0.2364	0.6158	0.4476	0.6286	0.4377	1.111	1.092

Effect of different extract of gulancha on Swamp cabbage:

Germination percentage: The germination percentage of swamp cabbage seeds were counted in 2nd, 4th and 7th days presented in (Table 1). In 7th day, the height germination percentage was found in T_2 (80.0%) and the lowest germination percentage was recorded in T_1 (61.33%), respectively.

Shoot length: Shoot length of swamp cabbage at different days after sowing was significantly influenced significantly by the effects of different leaf extract (Table 2). The highest shoot length of swamp cabbage seedling was found in T_2 i.e. ethanol extract of gulancha (2.267cm) at 8 DAS that was statistically similar to others. At 11 DAS the highest shoot length was recorded in T_2 (4.583cm) and the lowest was found in T_3 (1.503cm), respectively. The increased growth of swamp cabbage root is probably due to the presence of growth regulatory or other bioactive substances in the ethanol extract of gulancha.

Root length: Root length of swamp cabbage at different days after sowing was significantly influenced by the effects of different stem extract (Table 3). At 11 DAS the highest root length was recorded in T_2 (5.500cm) and the lowest was found in T_1 (0.9633cm), respectively. Similarly, the increased root growth is performed possibly due to presence of some bioactive substances in the ethanol extracts of gulancha.

Effect of different extracts of gulancha on Tomato

Germination percentage: The germination percentage of Tomato was counted in 2nd, 4th and 7th days presented in (Table 1). In 7th day, the highest germination percentage was found in T_2 (81.33%) and the lowest germination percentage was recorded in T_1 (66.67%), respectively.

Shoot length: Shoot length of tomato at different days after sowing influenced significantly by the effects of different extracts (Table 2). The shoot growth Tomato was gradually increased in ethanol extracts of gulancha. This is possibly happened due the presence of some growth regulatory substances in the extract that enhanced the growth performance in Tomato.

Root length: Root length of Tomato at different days after sowing was significantly influenced by the effects of different stem extract (Table 3). At 5 Days after sowing (DAS) with the ethanol extract, of the highest root length was observed (1.983cm) which was similar to T_c and T_3 treatments, whereas the lowest root length (1.14 cm) was recorded in T_1 treatment. At 14 DAS, The highest root length of tomato was found in T_2 (4.967cm) i.e. ethanol extract of gulancha followed by T_c and T_3. Conversely, the lowest root length was recorded in T_1 (1.873cm). Similarly, the root

growth was also enhanced compare to other treatments. This is possibly due to presence of some bioactive substances in the ethanol extracts of gulancha.

Chemical investigation of ethanol extracts of gulancha

Identification of number of compounds: Thin Layer Chromatography (TLC) of ethanol extract of gulancha showed distinctly five compounds at Hexane: Ethylacetate (10:1 v/v).

This result suggests that it may contain five distinct compounds, designated as F_1, F_2, F_3, F_4 and F_5 respectively (Fig.1). The amount of all fractions obtained after complete separation was- **Fraction-1: 17 mg; Fraction-2: 7 mg; Fraction-3: 5 mg; Fraction-4: 5.3 mg and Fraction-5: 4.1mg.**

Table- 4: R_f values of detected components of gulancha.

Detected component	R_f value
R_1	0.86
R_2	0.71
R_3	0.52
R_4	0.49
R_5	0.43

Chemical test for sterol for isolated fraction: The results of the different chemical tests were presented in tabular form (Table 5). Among the different fractions identified by chemical test, fraction 1 was showed positive (+ ve) in both Salkowaski and Liebermann-Burchard reaction (Table 5). This indicates that fraction 1 might be sterol type of compound or compounds. The development of radish coloration indicates the presence of sterol (Salkowaski reaction; Fig.). A slightly greenish color development also indicates the presence of a sterol (Libermann-Burchard reaction).

Table- 5: Chemical tests of sterol for isolated fractions

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

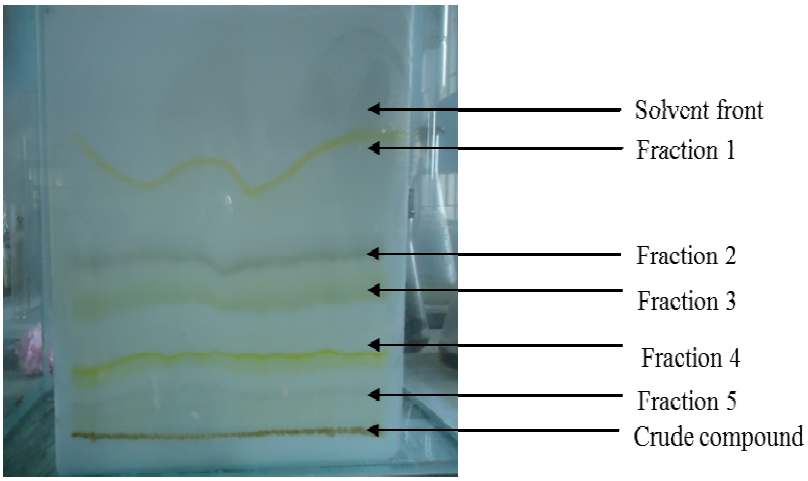

Figure 1: Preparative TLC plate showing different fractions.

Gulancha is a medicinal plant which showed antimicrobial, cytotoxic and enhanced immune system (Alexander et al., 20110; Uddin et al., 2011). Some researcher examined only its medicinal activities. On the other hand, fewer reports have been found of gulancha on seed germination and seedlings growth of crops. According to our data, ethanol extract showed stimulatory effects on seed germination and seedling growth (root and shoot) of lady's finger, swamp cabbage and tomatoes. In contrast, aqueous extract significantly delayed or reduced the germination, and seedlings growth of these vegetables. These data were closely related to the previous results conducted by Aktar et al., 2012. It was studied that different extracts of some medicinal plants enhanced the germination, and seedlings growth of some vegetables crops (Roy et al., 2012). The thin layer chromatography data suggested that some phytochemicals were presented in different extracts of gulancha. These chemicals might have stimulatory or inhibitory effect on crop growth (Rohan et al., 2005; Roy et al., 2012; Sayed et al., 2012). Some phytotoxic chemicals were available in gulancha that can be shown inhibitory effects on crop growth. In addition that, some chemicals might be influenced the crop growth.

CONCLUSION

Gulancha aqueous extract inhibits the germination, shoot length and root length while the ethanol extract enhance germination, shoot length and root length of these vegetable crops. It was also observed that ethanol extracts of gulancha enhance the percent germination, shoot and root length of swamp cabbage and lady's finger in all respects comparison with other treatments. Therefore, ethanol extracts of gulancha may contain some growth regulatory or other bio-active substances that triggered the growth performance and germination of the tested vegetables seeds. The outcomes of the study improve our understanding of chemical investigation of important herbs in the plant kingdom.

Acknowledgement: We are grateful to Departments of Agricultural Chemistry and Department of Biochemistry and Molecular Biology, Hajee Mohammad Danesh Science and Technology University (HSTU), Dinajpur, Bangladesh for laboratory support.

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