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E-mail: bjsir07@gmail.com

Screening of Phytochemical and Biological Potential of *Clerodendron viscosum* Leaves Extracts

M. Zahurul Haque[a], M. A. Rouf, M. A. Jalil, M. Badrul Islam[b] and M. Ruhul Islam[b]

[a]*Drugs & Toxin Research Division, BCSIR Laboratories, Rajshahi-6206, Bangladesh and* [b]*Department of Chemistry, Rajshahi University, Rajshahi- 6205*

Abstract

Attempts were made to investigate different phytochemicals (secondary metabolites) of *Clerodendron viscosum* leaves extracts with different solvents e.g. petroleum ether (40-60^{o} C), ethyl acetate and chloroform. The extracts were found to contain alkaloids, carbohydrates, flavonoids, glycosides, tannins and steroids. The extracts were then subjected to bioactivity against some stored grain insect such as *Tribolium castaneus*, *Sitophiulus oryzae*, *Rhizopertha dominica* using the method of residual film technique. Among the extracts, the petroleum ether extract showed higher toxicity against *Sitophiulus oryzae* and *Rhizopertha dominica* but moderate toxicity against *Tribolium castaneus*. The ethyl acetate and chloroform extracts showed moderate toxicity against *Sitophiulus oryzae*. The effectiveness of the order of toxicity of the extracts was found to vary with different intervals of treatments (24, 48 and 72 hours).

Key words: *Clerodendron viscosum*, Phyto-chemical, Bioactivity, Stored grain insect, Probit mortality

Introduction

Clerodendron viscosum Linn. common name Ghetu, Bhat is a very common herb of verbenaceae family. It grows commonly in waste places and graveyards in all districts in Bangladesh (Ghani, 1998). It also grows commonly in waste places of all areas of India and Burma. It is well known as a medicinal plant because of its wide therapeutic uses. The plant is useful as an excellent laxative cholagogue, anthelmintic, ascarides, antiperiodic, febrifuge, in malarial fever, in torpidity of the liver, in dysentery etc. (Ghani, 1998 and Khatry *et al.*, 2006). The plant also possesses repellent properties (Husain *et al.,* 2006).The plant as a hole is useful in cure for coughs and rheumatism, ulcer, scabies, snake bites, asthma, eruption of skin etc. The crude methanol extract of *Clerodendron viscosum* Vent. (Verbenaceae) leaves were evaluated for its anti-inflammatory, antinociceptive, and neuropharmacological activities (Ahmed *et al.*, 2007) and pharmacognostical study of the root and leaf (Richard, 2006). So, the plant may be a good source of bioactive compound and thus may serve as an important raw material for drug production.

Hence attempts were made to investigate the phytochemical and biological potential of *Clerodendron viscosum* leaves.

Materials and Methods

Plant materials: For the present study fresh leaves were collected from the roadside ledges of the Rajshahi-Natore Road near Rajshahi University campus and BCSIR campus, Rajshahi, during February-March period. It was then dried in an air-circulating oven at 40^{o}C ± 2^{o}C. The dried materials were crushed into powder by a crushing machine.

Extraction of plant materials with methanol

The crushed and dried material (powder, 1.00 kg) was extracted with methanol using a soxhlet apparatus. The material was put into a thimble made of cotton cloth, placed into a soxhlet apparatus. The material was immersed in methanol and kept for 24 hours. Heat was applied to the R.B. flask using a water bath and the solvent was allowed to drop into the soxhlet apparatus after condensing into the condenser. Twenty two such cycles afforded a greenish black extract. The extract was filtered. The solid obtained was separated out and designated as SA, The filtrate was evaporated to a minimum volume using a rotary-vapor. During this process, the temperature was not allowed to rise above 60°C. The residue (145g) was preserved in a refrigerator. The concentrated extract was allowed to stand for 7 days in the refriger-

* Corresponding author: E-mail: mdzahurulhaque@yahoo.com

ator, when a dark greenish solid mass was settled down. It was then separated out by filtration and the solid was mixed with previous SA and the filtrate was again evaporated to a minimum volume and was denoted as S. This was referred to as the mother liquor. Water of about two times of the volume of the viscous mass, S. (mother liquor) was added to it. Rest of the solution was then stored for the subsequent triturating and studies.

Trituration with petroleum ether (40-60^{o}c)

The methanolic extract was subjected to trituration with petroleum ether (40^o-60^{o}C). About 200 ml of pet. ether was added into the R.B. flask containing the extract and shaken vigorously for about 1-1.5 hours. The petroleum ether layer was then separated out and was called petroleum ether triturate, PET. Trituration was repeated for another 16 times. The petroleum ether triturates obtained were then combined together and were taken in another R.B. flaks and it was evaporated to a minimum volume using a rotary-vapor. During the evaporation, the temperature was kept below 40^{o}C. The residue, PET (80g.) was preserved in a refrigerator.

Trituration with chloroform ($CHCl_3$)

The pH of the aqueous solution was made 3 with acetic acid. The solution obtained was reddish black in colour and was triturated with chloroform. The solution was at first treated with 200 ml of chloroform. The mixture was then transferred to a 1L of separating funnel and shaken vigorously. The mixture was allowed to stand for a few minutes. When the mixture was obviously divided into two different layers, the chloroform layer was separated out. This process was repeated 12 times and the 13 fractions of chloroform layer were mixed together. The acidic aqueous solution was preserved. The chloroform layer was greenish in colour and was evaporated to a minimum volume using a rotary-vapor. The residue was designated as ACT (20g), and preserved in a refrigerator.

Basification and trituration with chloroform ($CHCl_3$)

The acidic aqueous solution was basified to pH 10 with NH_4OH solution and the basic aqueous solution obtained was triturated with chloroform. The solution was at first treated with 200 ml. of chloroform and the mixture was then transferred to a 1L separating funnel and shaken vigorously. The mixture was allowed to stand for a few minutes. When the mixture obviously divided into two different layers, the chloroform layer was separated out. This process was repeated 5 times. All the five fractions were combined together. The basic aqueous solution was preserved in a refrigerator. The chloroform layer was colourless and was evaporated to a minimum volume using a rotary-vapor. The residue was very small in amount. The amount of this substance was so poor that no further study could be made with it.

Trituration with ethyl acetate (EtOAc)

The basic aqueous solution was then subjected to triturate with ethyl acetate. The solution was at first treated with 200 ml of ethyl acetate. The mixture was then transferred to a 1L separating funnel and shaken vigorously over and over again. Then the mixture was allowed to stand for a few minutes, when the mixture was obviously divided into two different layers. The ethyl acetate layer was separated out which was reddish in colour. This process was repeated 8 times. Eight fractions of ethyl acetate were mixed together and were evaporated to a minimum volume using a rotary-vapor. The ethyl acetate residue was denoted as EtOAcT(25g) and was preserved in a refrigerator. The basic aqueous solution was also preserved in a refrigerator.

Phyto-chemical features of the extracts

For identifying the presence of possible classes of chemical components a phytochemical screening of the extracts was done following the standard methods A brief description of the methodology is given below.

Test of Alkaloids

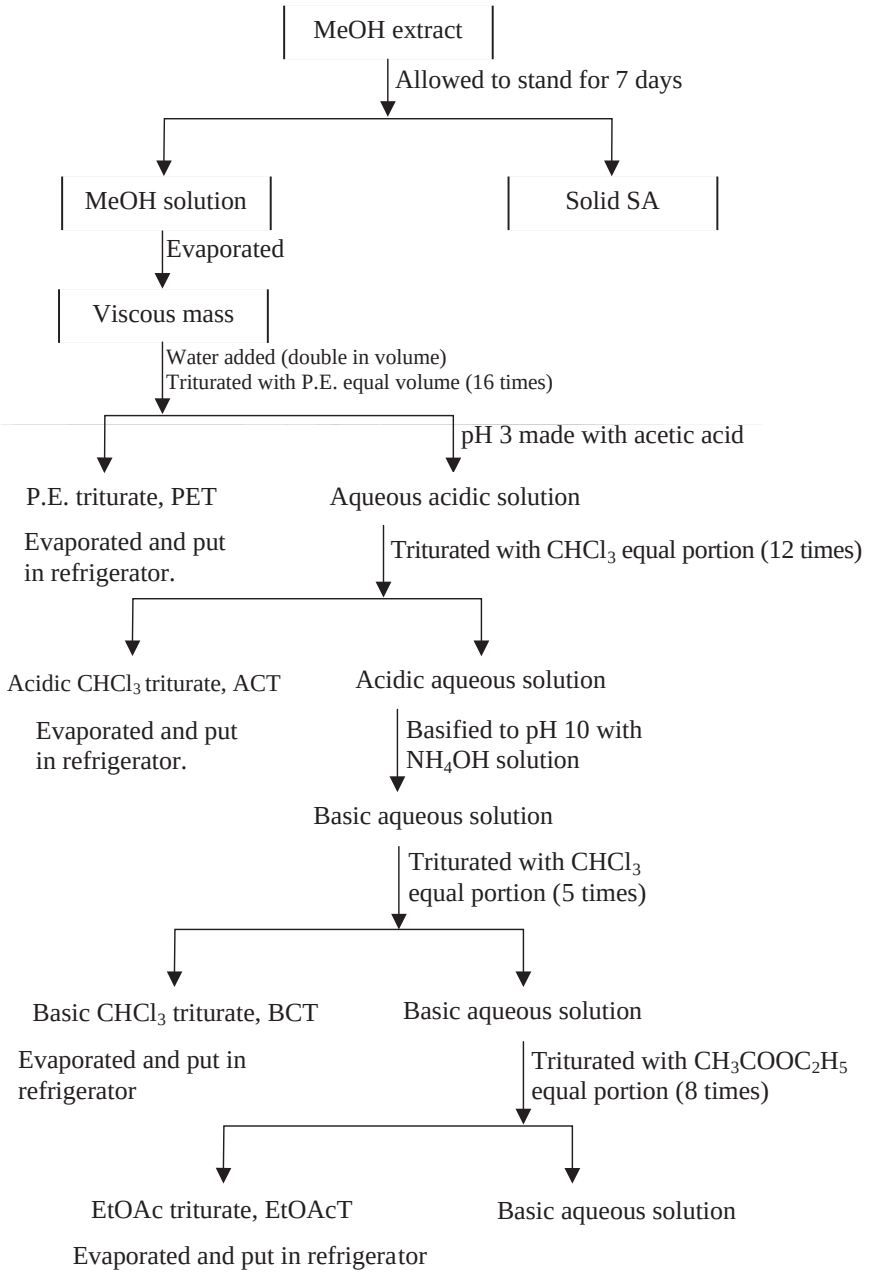
A few drop of Mayer's reagent was added to 1 ml of acetic aq. extract Formation of white or pale yellow precipitate was due to the presence of alkaloids.

Test of Carbohydrates

0.5 ml of aq. extract was added to 5 ml of benedict's solution and boiled for 5 min. Formation of coloured precipitate was due to the presence of carbohydrates.

Test of Flavonoids

0.5 ml of alcoholic extract was added to 5-10 drops of dill HCl followed by a small piece of zinc. Formation of pink or radish pink colour precipitate indicated the presence of flavonoids.



Flow Diagram of trituration process from MeOH extract.

Test of Glycosides

Small amount of alcoholic extract in 1 ml water was added to aqueous NaOH. Formation of pale yellow precipitate was due to the presence of glycosides.

Test of Resins

Small amount of alcoholic extract in 5 ml acetic anhydride was added to 0.05 ml of H_2SO_2. Formation of bright purplish red colour indicated the presence of resins.

Test of Saponins

A drop of $NaHCO_3$ was added to 5 ml of aqueous extract and shaken vigorously and left for three minutes. No honeycomb like froth was formed indicated the absence of saponins.

Test of Steroids

1 ml extract was added to 2 ml acetic anhydride and 1 ml H_2SO_4 . A Greenish colour was developed which turned to blue.

Test of Tannins

1-2 ml aq. extract was added to a few drops of $FeCl_3$. Formation of bluish black colour indicated the presence of tannins.

All the results of the qualitative analysis of the fractions are tabulated in the Table I.

Bioactivity of the three extracts of *Clerodendron viscosum* leaves against some stored grain insect such as *Tribolium castaneus*, *Sitophiulus oryzae*, *Rhizopertha dominica* was done using the method of residual film technique. The results are shown in Table II, III and IV. From Table II, it is evident that among the extracts, the petroleum ether extract showed

Table I: Phyto-chemical screening of *C. viscosum* leaves extracts

Class of compounds indicated	P.E Extract	E.A Extract	Acidic $CHCl_3$ Extract
Alkaloids	Negative	Positive	Negative
Carbohydrates	Positive	Positive	Positive
Flavonoids	Positive	Negative	Negative
Glycosides	Positive	Positive	Positive
Phenols	Negative	Negative	Negative
Proteins	Negative	Negative	Negative
Resins	Positive	Negative	Negative
Saponins	Negative	Negative	Negative
Tanins	Negative	Positive	Negative
Steroids	Positive	Positive	Positive

Table II: Bioactivity of *C. viscosum* leaves against some stored grain insect

Name of the insect	Time (hr.)	P.E Extract (Mortality %)			E.A Extract (Mortality %)			Acetic chloroform extract (Mortality %)			Control (Mortality %)
		D_1	D_2	D_3	D_1	D_2	D_3	D_1	D_2	D_3	
Tribolium castaneus	24	20	40	60	0	0	0	0	0	0	0
	48	40	70	80	0	0	0	0	0	0	0
	72	70	80	90	0	0	0	0	0	0	0
Sitophiulus oryzae	24	70	70	80	20	20	30	2	30	40	0
	48	90	94	94	20	30	40	4	30	40	0
	72	95	98	98	40	40	44	10	40	60	0
Rhizopertha dominica	24	70	70	80	0	0	0	0	0	0	0
	48	90	94	96	0	0	0	0	0	0	0
	72	94	98	98	0	0	0	0	0	0	0

D1= 157.19µg/cm², D_2= 314.38µg/cm², D_3=471.57µg/cm²

Determination of biological activity

Bioactivity of the three extracts of *Clerodendron viscosum* leaf against some stored grain insect such as *Tribolium castaneus*, *Sitophiulus oryzae*, *Rhizopertha dominica* was done using the method of residual film technique (Finney, 1947 and Busvine, 1971). The results are shown in the Table II, III and IV.

Results and Discussion

The qualitative chemical analysis of *C. viscosum* leaves extracts (PET, ACT and EtOAcT) was tested and the results are summarized in Table I. From Table I, it is observed that the extracts showed the presence of alkaloids, carbohydrates, flavonoids, glycosides, resins, tannins and steroids.

higher toxicity against *Sitophiulus oryzae* and *Rhizopertha dominica* but moderate toxicity against *Tribolium castaneus*. The ethyl acetate extracts showed moderate toxicity, whereas the acetic chloroform extracts lowest toxicity against *Sitophiulus oryzae*. Extracts of ethyl acetate and acetic chloroform showed no toxic effect against the insects T*ribolium castaneus* and *Rhizopertha dominica*. For comparison of the toxicity LD_{50} values, regression equation and relative toxicity of the test materials were determined (Table III and Table IV). The probit analysis of percent mortalities (Finney, 1947 and Busvine, 1971) in all the cases gave c^2 values, which indicated the absence of any significant heterogeneity.The regression lines of tested extracts are presented in Fig. 1-5. The regression analysis of probit mortality gave linear line

Table III: Relative toxicity of pet. ether extract of *C. viscosum* leaves against some stored grain insects

Name of insects	Time (hours)	c^2 values for heterogeneity	Regression Equation	LD_{50} mg/cm^2	95% Confidence limits		Relative toxicity
					Lower	Upper	
Tribolium castaneus	24	0.2413	y= 0.3990+0.4345x	372.573	294.727	470.978	1.00
	48	0.1913	y= 0.1017+0.4398x	199.891	153.615	260.107	1.86
	72	0.1294	y= -1.6022+0.6915x	71.682	23.7495	216.354	5.20
Sitophiulus oryzae	24	0.6653	y= 4.1769+0.5876x	25.172	0.2847	2224.265	1.00
	48	0.1692	y= 4.8517+0.6494x	1.692	1.6179	176877.4	14.88
	72	6.998	y= 4.6501+0.9095x	2.4248	9.633	61038.12	10.38
Rhizopertha dominica	24	0.6653	y= 4.1769+0.5876x	25.172	0.2849	2224.265	1.00
	48	0.2098	y= 3.0251+1.4482x	23.1065	1.5589	342.500	1.09
	72	0.1209	y= -3.4550+0.8615x	7.2805	0.0207	2556.927	3.46

Table IV: Relative toxicity of different extracts of *C. viscosum* leaf against *Sitophiulus oryzae* stored grain insect

Name of insects	Time (hours)	c^2 values for heterogeneity	Regression Equation	LD_{50} mg/cm^2	95% Confidence limits		Relative toxicity
					Lower	Upper	
Ethyl acetate	24	0.8138	y= 2.7753+0.6088x	4509.304	23.4297	867865.4	1.00
	48	1.5676	y= 1.1503+1.3289x	788.767	319.075	1949.863	1.86
	72	8.6532	y= 4.271+0.1945x	5598.094	6.18449	5.06728	5.20
Acidic chloroform	24	2.1567	y= -4.3610+3.4541x	512.973	405.244	649.3398	1.00
	48	1.2462	y= 1.062+0.3316x	529.668	400.559	700.3901	14.88
	72	9.1385	y= -3.9795+3.4634x	391.459	331.979	461.596	10.38

expresses the response of different dosages. The result is satisfactory and conforms to other related works reported earlier (Haque *et al.*, 2008). The repellent response of

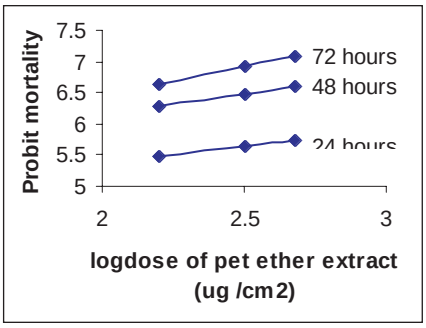


Fig. 1: Regression line of probit mortality log dose of pet-ether extract against *Sitophiddlus oryazae* after 24, 48 and 72 hours of exposure

Clerodendron viscosum leaves extract to adult and larvaeof Tribolium castaneus was studied earlier (Husain *et al.*, 2006). Results indicated that both the adults and larvae were repelled by contact with food medium treated with leaves dust of *Clerodendron viscosum* at different concentration. Results were also tested using chisquare analysis.

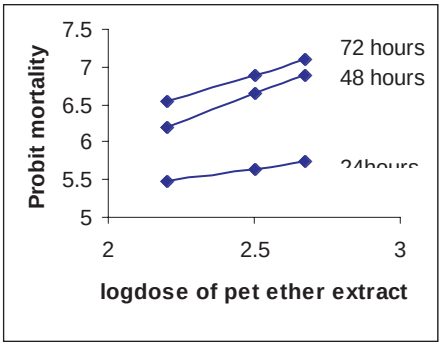


Fig. 2: Regression line of probit mortality log dose of pet-ether extract against *Rhizopertha dominica* after 24, 48 and 72 hours of exposure

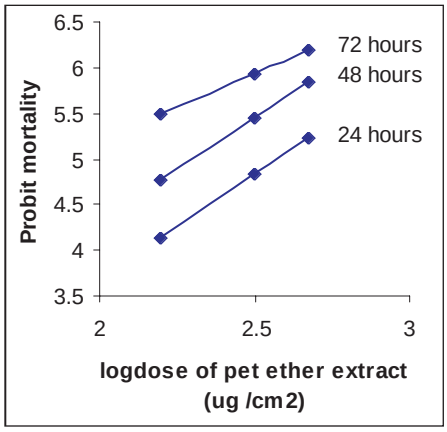


Fig. 3: Regression line of probit mortality log dose of pet-ether extract against *Tribilium castaneus* after 24, 48 and 72 hours of exposure.

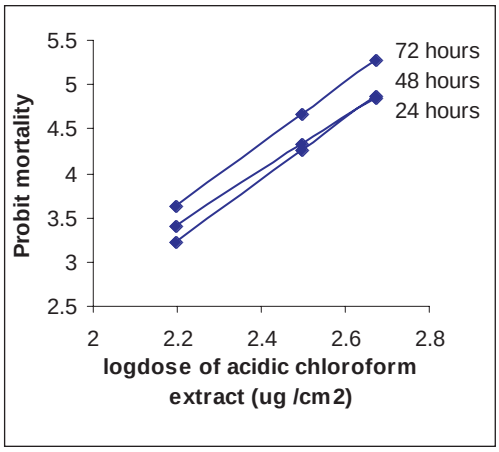


Fig. 4: Regression line of probit mortality log dose of acidic chloroform extract against *Sitophiddlus oryazae* after 24, 48 and 72 hours of exposure.

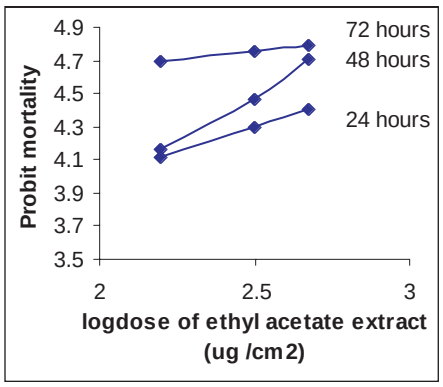


Fig. 5: Regression line of probit mortality log dose of ethyl acetate extract against *Sitophiddlus oryazae* after 24, 48 and 72 hours of exposure.

Conclusion

From the insecticidal analysis, it is observed that the petroleum ether extract of the leaves of *Clerodendron viscosum* Linn. is bioactive against some stored grain insects. So, further investigation is necessary in using this plant as crude form of insecticide and for production of important insecticide.

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