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CYTOTOXIC AND ANTIMICROBIAL ACTITVITY OF PARTIALLY PURIFIED LECTIN FROM BITTER GOURD SEEDS *(MOMORDICA CHARANTIA*L.).

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ABSTRACT
Lectin was partially purified from *Momordica charantia* (Bitter gourd) seeds by ion-exchange chromatography on DEAE cellulose column. The molecular mass of the partially purified Lectin was 115-35 kDa, as estimated by both in native and denatured SDS-PAGE. The Lectin agglutinated both O^+ (ve) human red blood cells as well as chicken red blood cells. The Lectin Showed poor antibacterial activity against *Shigella sonnei* and *Salmonella typhi* out of nine organisms tested. The partially purified bitter gourd seed Lectins do not show any antifungal activity against the seven fungi tested. The Dose-mortality test of the partially purified *Momordica charantia* seed Lectins was carried out against red flour beetle adults (*T. castaneum*) throughresidual film bioassay method. The result showed promising insecticidal activity against these beetles with LD50 values of 40.43 to 23.61 mg/cm2 after 12 to 48 hrs of exposure. The Lectin showed slight toxicity to brine shrimp nauplii, the calculated LC50 value was 259.5. *In vivo* cytotoxicity study of this Lectin in Swiss albino mice showed a moderate toxic effect.

KEYWORDS: Antibacterial, Chromatography, Cytotoxicity, Bioassay.

INTRODUCTION
Bangladesh is the heaven of vegetables. In Bangladesh, nearly 100 types of vegetables grow. *Momordica charantia* is one of them. The local name of *Momordica charantia* is Korolla. It is available in different land areas. It is found mainly in the land areas of Khulna, Rajshahi, Jessore, Jhenaidah, Pabna, Rangpur, Natore, Sylhet, and Magura etc.

Momordica charantia is called Bitter melon or Bitter gourd in English. Bitter melon has been used in various Asian and African traditional medicine systems for a long time.[1] Chloroform and Butanol extracts of *Momordica charantia* was reported to contain antibacterial activity against a number of bacteria including *Escherichia coli, Shigella shiga, Shigella flexneri, pseudomonas aeruginosa, Bacillus subtilis, Staphylococcus aureus, Vibrio cholera inaba,* and *Vibrio cholera ogawa.*[2]

Although animal kingdom is the main source of protein but plant kingdom also possesses a lot of protein. Green leaves, barks, roots, stems etc contain small amount of protein; while seeds are the main sources of protein in plants. Pulses contained about 18-26% and oil seeds contain approximately 30-55% protein. In addition, wheat, barley, rice, bran, maize etc. also contained significant amount of protein. Some plant seed proteins are toxic and some are nontoxic.

Lectin are a class of proteins that bind sugar specifically and reversibly and that agglutinate red blood cells. Lectins were first described in 1880 by Stillmark working in castor bean extracts. The term Lectin was proposed by Boyd[3] because of their unique carbohydrate binding properties. Lectins were widely distributed in plants kingdoms, particularly among the legumes and to a lesser extent among the cereal grains. More than 90 Lectins from leguminous plants belonging to different suborders and tribes have been isolated, mostly from seeds, and characterized to varying degree. Lectins are also found in animals, insects, and microorganism.[4] Moreover, it has become clear that Lectins are present in some invertebrate animals such as snails.[5]

A Lectin isolated from *Momordica charantia* seed as a B cell activator and partial characterization was done butdetailed information about *Momordica charantia* seed Lectin are still unknown. So, for obtaining more information about *Momordica charantia* seed Lectin, in this study, the *Momordica charantia* produced in Bangladesh are selected for the first time for research purposes.

METHODS AND MATERIALS

Sample Collection

During the summer seasons, *Momordica charantia* seeds were collected from Nouhata, Rajshahi. After collection, *Momordica charantia* seeds were cleaned, dried and stored at room temperature.

Preparation of crude extract

50g of Bitter gourd seed was cut into small pieces and then 100ml of pure water was added and the sample was homogenized by a homogenizer. The homogenate was occasionally stirring and then filtered through a muslin cloth. The filtrate was collected and clarified further by centrifugation at 8000 rpm at 4°C for 15 minutes. The clear supernatant was collected and dialyzed against cold distilled water for 2h. After centrifugation, the clear supernatant was used as crude protein extract and also preserved in the deep freeze for experimental purposes.

Purification of Lectin

DEAE-CELLULOSE Column Chromatography used for Protein Purification.

Test of Purity

The protein pattern of the selected fraction was determined 10% SDS-PAGE according to the method of Laemmli (1970)[6] as modified by smith (1995).[7]

DETERMINATION OF PROTEIN CONCENTRATION

The protein concentration was determined by the Method of Lowry et al., 1951.[8]

HEMAGLUTINATION ASSAY

The hemagglutination assay was performed in 96-well microtiter U-bottomed plates in a final volume of 100 µl containing 50 µl of protein solution serially diluted with an equal amount of hemagglutination buffer (20 mM Tris-HCl buffer, pH 7.8 containing 0.9% NaCl and 10 mM CaCl2) and 50 µl of 2% suspension of O+ (ve) erythrocytes previously washed with 0.15 M NaCl. After gently shaking, the plate was kept at room temperature for 30 min. The visual agglutination titer of the maximum dilution giving positive agglutination was recorded.

DETERMINATION OF CYTOTOXICITY O THE PURIFIED PROTEIN BY BRINE SHIRMP BIOASSAY

This bioassay can be used as a convenient monitor for screening and fractionation in the discovery and monitoring of bioactive natural products.[7]

Preparation of simulated seawater

38 g of sea-salt (non ionized NaCl) was weighed accurately, dissolved in one liter of sterilized distilled water and then filtered off to get clear solution. The pH of the seawater was maintained between 8 and 9 by using NaHCO3 solution.

Hatching of brine shrimp

Artemia salina leach (brine shrimp eggs) collected from the pet shop was used as the test organism. Simulated sea water was taken in the small tank and the shrimp eggs (1.5 g/l) were added to one side of the tank and this side was covered. The shrimps were allowed for one days to hatch and immature as nauplii (larvae.). Constant oxygen supply was carried out and constant temperature (around 37˚C) was maintained during the hatching time. The hatched shrimps were attracted to the lamp on the other side of the divided tank through dam. These nauplii were taken for this bioassay.

Preparation of sample

The test sample contained 6.16 mg/ml Lectin. This solution was used as a stock solution.

Application of the test sample and brine shrimp nauplii to the vials

Twelve clean vials were taken for the sample in four concentrations (Three vials for each concentration) and another three vials were also taken for control. Then the concentration of every three vials was 20, 50, 100 and 200µg/ml respectively. Lectins solution containing sample were added to the each three vials gradually and finally marked up to 3 ml by seawater. With the help of a Pasteur pipette 20 living shrimps were taken to each sample vials and control vial respectively.

Counting of nauplii

After 24-hour of incubation, the vials were observed using a magnifying glass and the number of survivors in each vial were counted and noted. From this, the nauplii were counted averagely of each three vials, which contained same conc. of sample the percent (%) mortality was calculated for each dilution. The concentration-mortality data were analyzed by using Probit analysis.[8]

Table-2: In vitro antibacterial activity of partial purified *Momordica charantia* seed lectins.

Test Bacteria	Dose (µg/disc)			Solvent Control (µg/disc)	Kanamycin (µg/disc)
	74.8	149.6	224.8	20	30
	Zone of inhibition (diameter in mm)				
Gram Positive					
Bacillus subtilis	-	-	-	-	33

Bacillus megaterium	-	-	-	-	27
Sarcina lutea	-	-	-	-	32
Bacillus cereus	-	-	-	-	32
Gram Negative					
Escherichia coli	-	-	-	-	26
Shigella dysenteriae	-	-	-	-	28
Salmonella typhi	-	05	06	-	27
Shigella sonnei	-	05	06	-	28
Shigella shiga	-	-	-	-	28

"-"No sensitivity

Brine Shrimp Bioassay

The partially purified *Momordica charantia* seeds Lectin showed positive results in Brine shrimp lethality assay. The median lethal concentration (LC50) of Brine shrimp lethality was found to be 259.54 µg/ml, obtained by probit analysis. The data and result of tested compound (partially purified lectins solution) and LC50 of standard Bleomycin and gallic acid were given in the **Table-3** and **Table-4**.

The test compound of partial pure lectins solution was found to show significant activity against Brine shrimp nauplii. In this assay, the mortality rate of Brine shrimp was found to be increased with the increase in concentration of the test sample. So it was observed that there is a positive correlation between Brine shrimp toxicity and cytotoxicity. The high value of LC50 (259.54 µg/ml), indicates the low cytotoxic effect of partially purified lectins solution. Standard cytotoxic agent Bleomycin and gallic acid were found to exhibited higher cytotoxicity giving lower LC50 values of 0.41 and 4.53 ppm respectively.

Dose µg/ml	Log dose	Number	Kill	% Kill	Corr %	Emp probit	Expt probit	Wrk probit	Weight	Final probit
60	1.778	10	2	20	20	4.16	4.219	4.150	5.03	4.214
80	1.903	10	3	30	30	4.48	4.369	4.490	5.32	4.368
200	2.301	10	4	40	40	4.75	4.846	4.760	6.27	4.860
400	2.602	10	6	60	60	5.25	5.206	5.280	6.27	5.232

Y = 2.015755 + 1.236117 X
CHI-SQUARED IS 0.1765344 WITH 2 DEGREES OF FREEDOM
NO SIG HETEROGENEITY
LOG LD-50 IS 2.41421
LD-50 IS 259.5433
95% CONF LIMITS ARE 100.8485 TO 667.9594

Table-4: LC50 of partially purified lectins solutions, standard Bleomycin and gallic acid.

Compounds	LC_{50} (µg/ml)	95% Confidence Limit (µg/ml) Lower-Upper		Regression equation	Chi-Squared	df
Partially purified lectins solutions	259.54	100.8485	667.9594	Y = 2.015755 + 1.236117 X	0.17653	2
Bleomycin	0.41	0.27	0.62	Y = 3.16 + 2.99X	0.62	2
Gallic acid	4.53	3.33	6.15	Y = 3.93 + 1.62 X	1.25	2

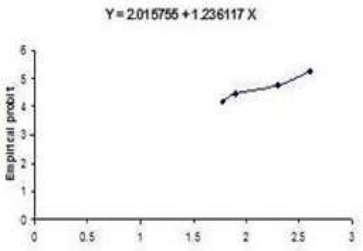

Fig.-5: Regression line of log dose of partially purified *Momordica charantia* lectins against Brine shrimp nauplii after 24 h of exposure.

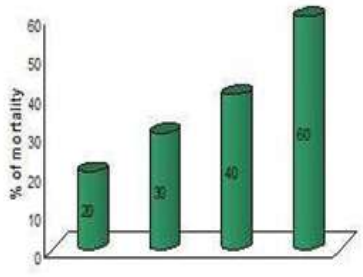

Fig.-6: Percent mortality of Brine shrimp treated with partially purified *Momordica charantia* lectins after 24 h exposure.

Antifungal Activity Test

The antifungal activity of partially purified *Momordica charantia* seed lectins was performed against seven pathogenic fungi. The doses of 78.4µg/disc, 149.6µg/disc and 224.8µg/disc were used. Standard antibiotic disc of Fluconazole 100µg/disc was used for comparison. The results of antifungal activity (zone of inhibition) of partially purified *Momordica charantia* seed lectins against respective fungi are shown in Table-5. It was found that partially purified *Momordica charantia* seed lectins has no antifungal activity against *Penicillium sp, Aspergillus flavus, Mucor, Fusarium species, Aspergillus niger, Aspergillus fumigatius* and *Candida albicans.* So, it can conclude that partially purified *Momordica charantia* seed lectins might not be an antifungal agent.

Table-5: *In vitro* antifungal activities of partially purified *Momordica charantia* seed lectins.

Test fungi	*Momordica charantia* seed lectins			Fluconazole (100µg/disc
	(78.4µg/disc)	(149.6µg/ disc)	(224.8µg/disc)	
	Zone of inhibition (diameter in mm)			
Penicilium sp	-	-	-	25
Aspergillus flavus	-	-	-	28
Candida albicans	-	-	-	23
Fusarium species	-	-	-	26
Mucor	-	-	-	25
Aspergillus fumigatius	-	-	-	-
Aspergillusniger	-	-	-	26

"-"No Sensitivity

Insecticidal Activity Test

The dose-mortality test of the partially purified *Momordica charantia* seed lectins was carried out through residual film bioassay against the red flour beetle *T. castaneum*. The results are presented in **Table 6-9**.

The lectins showed promising activity against *T. castaneum* adults to show LD50 values of 40.43, 38.90, 28.25 and 23.61 mg/cm2 after 12, 24, 36 and 48 hrs of exposure respectively. From this experiment, it was found that the mortality rate of *T. castaneum* adults increased with the increase of concentration of the sample and the increase of the exposure time as well. So, these lectins might be a potent insecticidal agent.

Table-6: Dose-mortality effects of *Momordica charantia* seed lectins against *T. castaneum* adults after 12 hours exposure.

Dose µg/ml	Log dose	Number	Kill	% Kill	Corr %	Emp probit	Expt probit	Wrk probit	Weight	Final probit
29.650	1.472	30	10	33.33	33	4.56	4.354	4.586	15.96	4.358
25.936	1.414	30	3	10	10	3.72	4.077	3.750	13.17	4.082
22.216	1.347	30	3	10	10	3.72	3.757	3.720	10.08	3.761
19.515	1.290	30	2	6.666	7	3.52	3.489	3.540	7.14	3.493
14.828	1.171	30	1	3.333	3	3.12	2.921	3.172	3.30	2.925

Y = -2.651565 + 4.766211 X
CHI-SQUARED IS 2.509564 WITH 3 DEGREES OF FREEDOM
NO SIG HETEROGENEITY
LOG LD-50 IS 1.606759
LD-50 IS 40.43518
95% CONF LIMITS ARE 27.80623 TO 58.79994.

Table-7: Dose-mortality effects of *Momordica charantia* seed lectins against *T. castaneum* adults after 24 hours exposure.

Dose µg/ml	Log dose	Number	Kill	% Kill	Corr %	Emp probit	Expt probit	Wrk probit	Weight	Final probit
29.650	1.472	30	12	40.00	40	4.75	4.508	4.740	17.43	4.472
25.936	1.414	30	5	16.66	17	4.05	4.237	4.048	15.09	4.212
22.216	1.347	30	2	6.667	7	3.52	3.923	3.602	12.15	3.912
19.515	1.290	30	2	6.667	7	3.52	3.660	3.529	9.06	3.660
14.828	1.171	30	2	6.667	7	3.52	3.103	3.724	4.62	3.126

Y = -2.111693 + 4.472805 X
CHI-SQUARED IS 4.626931 WITH 3 DEGREES OF FREEDOM

NO SIG HETEROGENEITY
LOG LD-50 IS 1.589985
LD-50 IS 38.90318
95% CONF LIMITS ARE 27.60566 TO 54.82417.

Table-8: Dose-mortality effects of *Momordica charantia* seed lectins against *T. castaneum* adults after 36 hours exposure.

Dose µg/ml	Log dose	Number	Kill	% Kill	Corr %	Emp probit	Expt probit	Wrk probit	Weight	Final probit
29.650	1.472	30	20	66.66	67	5.44	5.095	5.425	19.11	5.093
25.936	1.414	30	12	40.00	40	4.75	4.838	4.760	18.81	4.836
22.216	1.347	30	7	23.33	23	4.26	4.541	4.264	17.43	4.540
19.515	1.290	30	5	16.66	17	4.05	4.292	4.084	15.09	4.291
14.828	1.171	30	4	13.33	13	3.87	3.765	3.894	10.08	3.765
11.108	1.046	30	2	6.667	7	3.52	3.211	3.629	5.40	3.212

Y = -1.400778 + 4.411201 X
CHI-SQUARED IS 5.54617 WITH 4 DEGREES OF FREEDOM
NO SIG HETEROGENEITY
LOG LD-50 IS 1.451028
LD-50 IS 28.25064
95% CONF LIMITS ARE 24.29877 TO 32.84524.

Table-10: Dose-mortality effects of *Momordica charantia* seed lectins against *T. castaneum* adults after 48 hours exposure.

Dose µg/ml	Log dose	Number	Kill	% Kill	Corr %	Emp probit	Expt probit	Wrk probit	Weight	Final probit
29.650	1.472	30	26	86.667	87	6.13	5.564	5.976	17.43	5.525
25.936	1.414	30	16	53.333	53	5.08	5.262	5.098	18.81	5.216
22.216	1.347	30	10	33.333	33	4.56	4.913	4.565	19.02	4.859
19.515	1.290	30	7	23.333	23	4.26	4.621	4.281	18.03	4.561
14.828	1.171	30	6	20.000	20	4.16	4.001	4.160	13.17	3.928
11.108	1.046	30	2	6.667	7	3.52	3.350	3.572	6.24	3.262

Y = -2.287703 + 5.307299 X
CHI-SQUARED IS 8.183545 WITH 4 DEGREES OF FREEDOM
NO SIG HETEROGENEITY
LOG LD-50 IS 1.373147
LD-50 IS 23.61279
95% CONF LIMITS ARE 21.4867 TO 25.94928.

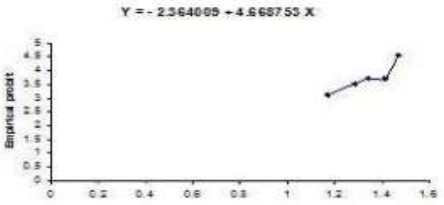

Fig.-7: Probit mortality (regression) line of the *Momordica charantia* seed lectins against *T. castaneum* adults after 12 hours exposure.

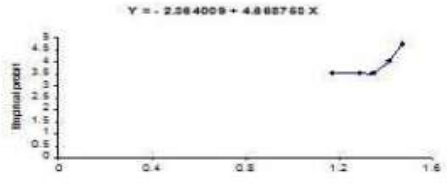

Fig.-8: Probit mortality (regression) line of the *Momordica charantia* seed lectins against *T. castaneum* adults after 24 hours exposure.

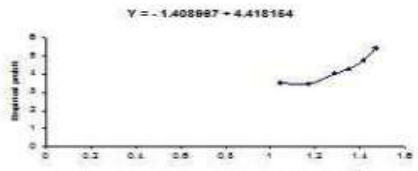

Fig.-9: Probit mortality (regression) line of the *Momordica charantia* seed lectins against *T. castaneum* adults after 36 hours exposure.

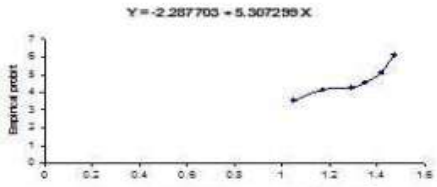

Fig.-10: Probit mortality (regression) line of the *Momordica charantia* seed lectins against *T. castaneum* adults after 48 hours exposure.

***In vivo* cytotoxicity study of the partially purified *Momordica charantia* seed lectins**

In vivo cytotoxicity study of the partially purified lectins from *Momordica charantia* seed was performed in Swiss albino mice. In this study, in group-1, 25% mice were died at the dose of 72.58 µg/ml at 96 hours time period and other mice's were gradually died within 6 days. In case of group-2, 50% mice's were died at the dose of 145.16µg/ml at 48 hours time period and other mice's were gradually died within 5 days. In case of group-3, 50% mice's were died at the dose of 290.32µg/ml at 24 hours time period and other mice's were also gradually died within 2 days. In control group, no mouse was died.

So it seems that the partially purified lectins of *Momordica charantia* seed were moderate toxic to Swiss albino mice **(Table-10).**

Group No.	Dose (µl)	Amount of protein(µg/ml) ///////ml)	Survival Time (Hour) (hours)	Survival
1	20	72.58	96	25%
2	40	145.16	48	50%
3	80	290.32	24	50%
Control	Nil	Nil	-----	----

DISCUSSION

Lectin was partially purified from *Momordica charantia* (Korolla) seeds using ion-exchange chromatography. The partially purified Lectin migrated as multiple bands (at least three) in SDSPAGE and the apparent molecular weight was 115-35 kDa, as estimated by both in native and denatured SDS-PAGE. This report is similar to *Concanavalin-A* from *Canavalia ensiformis* yielded three major bands in SDS-PAGE.[14] To confirm the protein as a Lectin, blood cell agglutination test was performed. The Lectin agglutinated O+ (ve) human blood cells and chicken blood cells. The minimum agglutinating activity of the partial purified Lectin was found to be 5.6 µg/50µl for O+ (ve) human blood cells, 22.5 µg/50µl for chicken blood. From the agglutination value 5.6 µg/50µl and 22.5µg/50µl, it can be concluded that the protein is moderately active Lectin. The partially purified Lectin showed a low antibacterial activity against *Shigella sonnei* and *Salmonella typhi*. The report is similar to *Cassia fistula Linn. Seed lectin-2* and *Cassia fistula Linn. Seed lectin-3*, those showed slight antibacterial activity against *Shigella sonnei* bacteria. Lectins from *Momordica charantia* seed did not show any antifungal activity against *Penicilium sp, Aspergillus flavus, Mucor, Fusarium species, Aspergillus niger, Aspergillus fumigatius* and*Canadida albicans*. The result coincides with Lectin from Tuberous Rhizome of *Kaempferia rotunda* that did not show any antifungal activity when tested against *Candida albicans, Aspergillus niger, Fusarium vasinfectum* and *Mucor sp.* To find out the insecticidal activity of this Lectin, Dose-mortality was performed. The results of Dosemortality test of the partially purified *Momordica charantia* seed lectins against the red flour beetle (*T. castaneum*) was carried out by residual film bioassay method and showed a promising activity against *T. castaneum* adults with LD50 values 40.43, 38.90, 28.25 and 23.61 mg/cm2 after 12, 24, 36 and 48 hrs of exposure respectively. So, these lectins might be a potent insecticidal agent.

The Lectin showed a very low toxicity against Brineshrimp nauplii and the observed LC50 value was 259.54 µg/ml. *Cassia fistulas* Linn. seed lectins showed more toxicity against brine-shrimp nauplii than *Momordica charantia* seed Lectin and the value of LC50 of *Cassia fistula* Linn. seed lectin-1 was 6.68 µgm/ml, *Cassia fistula* Linn. Seed lectin-2 was 13.33 µgm/ml and *Cassia fistula* Linn. Seed lectins-3 was 6.31 µgm/ml.

In vivo cytotoxicity study in Swiss albino mice, the *Momordica charantia* seed lectins showed to be moderate toxic. In this experiment, mice in group 1, 2 and 3 were injected intraperitoneally of partially purified lectins at the doses of 20 µl (72.58 µg/ml), 40 µl (144.8µg/ml) and 80 µl (289.6µg/ml). Mice in group 4 served as positive control group. 25% of mice were died at the dose of 72.58 µg/ml at 96 hours time period and other were gradually died. In case of group-2, 50% mice's were died at the dose of 144.8µg/ml at 48 hours time period and others died later on. In case of group-3, 50% mice's were died at the dose of 289.6µg/ml at 24 hours time period and other mice's were also gradually died. In case of control group, no mouse was died. If the partial purified lectins were non-toxic, the mice's would survive. So, *Momordica charantia* lectins were moderate toxic against the Swiss albino mice.

CONCLUSION

Finally, it can be concluded that the partially purified protein from *Momordica charantia* (Korolla) was a Lectin with a potent insecticidal and parricidal activity. The Lectin also contains a low antibacterial activity against gram (–ve) bacteria, but no antifungal activity. The Lectin is moderately toxic for animal. Further research is required to purify the Lectin and to establish the antimicrobial, cytotoxic property as well as the structure function relationship of *Momordica charantia* lectins. Extensive work was not possible for us due to the limitation of time, chemicals and instrumental facilities.

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