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Original Article

Photodynamic Toxicity of Chlorophyllin against *Fasciola gigantica* Carrier Snail *Indoplanorbis exustus* in Visible Spectral Band

Divya Chaturvedi, Dinesh Kumar Singh and Vinay Kumar Singh*

ABSTRACT

Background: Fasciolosis is one of the most debilitating diseases caused by liver flukes *Fasciola hepatica* and *F. gigantica*. Snail Lymnaeidae and Planorbidae is the intermediate host of these flukes. Snail population management is a good tool to control fasciolosis because gastropods represent the weakest link in the life-cycle of trematode. Aim of the present study is to explore the molluscicidal activity of chlorophyllin in visible spectral band against *Fasciola gigantica* carrier snail *Indoplanorbis exustus*. **Methods:** Chlorophyll was transformed into water-soluble chlorophyllin in 100% ethanol by using different types of chemicals. Ten snails *Indoplanorbis exustus* were placed in a glass aquarium containing 3 L of dechlorinated tap water. These snails were treated with different concentrations of chlorophyllin in sunlight as well as exposed to different visible spectral band of light. **Results:** Pure chlorophyllin (96 h LC_{50} 6.54 mg/l) in sunlight was more toxic than extracted chlorophyllin (96 h LC_{50} 939.65 mg/l). There was a significant variation in the toxicity of chlorophyllin with snails, exposed to visible spectral band of light. The highest and lowest toxicity of chlorophyllin against *I. exustus* was noted in yellow light (96 h LC_{50} 2016.79 mg/l) and green light (96 h LC_{50} 2433.16 mg/l). High performance liquid chromatography (HPLC) study reveals that the active molluscicidal component extracted in spinach leaves is chlorophyllin. **Conclusion:** Due to the photodynamic nature of chlorophyllin, it has the potential to control the population of vector snails and ultimately fasciolosis in developing countries.

Key words: Fasciolosis, Photodynamic Product, Chlorophyllin, *Indoplanorbis exustus*, Visible light band.

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INTRODUCTION

Fasciolosis is water and food borne zoonotic disease caused by two trematode species *Fasciola hepatica* and *F. gigantica*.[1,2] As being linked with fasciolosis it cause dramatic losses of economic prosperity of any country and classified as re-emerging human disease.[3] It caused low fertility, reduced meat and milk yield in infected cattle population.[3] In early eighties, Singh and Agarwal[4] noted that 94% of buffaloes slaughtered at the abattoirs in Gorakhpur district of eastern Uttar Pradesh (India), are infected by *Fasciola gigantica*, which still persisted as well.[5,6] The increase in number of human fasciolosis and its outbreaks in the last two decades have changed the status of fasciolosis from zoonoses to an emerging health problem.[7] Snails belonging to families Planorbidae and Lymnaeidae are the secondary host of *Fasciola gigantica*.[5] Planorbidae snail *Indoplanorbis exustus* is acknowledged intermediate host of *Fasciola gigantica* in Gorakhpur, India.[8]

Logical approach for the fasciolosis control is to devastate the carrier snails and thus eliminate an essential link in the life cycle of the *Fasciola*. Use of bioactive plant molluscicides is a valuable tool in controlling the vector population as they are easily formulated, ecologically safe and culturally more acceptable than their synthetic counterpart.[9,10] Chlorophyllin has natural photodynamic properties and simply extracted from various plant resources (e.g. spinach, grass, dandelion, green cabbage, water hyacinth, algae etc.).[11,12] Erzinger[13] has reported that photosensitive chlorophyllin show highly toxic effects against mosquito larvae in sunlight. Earlier research revealed the molluscicidal activity of chlorophyllin against *L. stagnalis, Biomphalaria* spp. and *Physa marmorata*.[14] In the presence of light chlorophyllin becomes more effective and toxic.[11,15] In recent times, it has been reported that chlorophyllin is a potent cercaricide against *F. gigantica* cercaria larva in sunlight.[16] Previously, it has been observed that different spectral bands of the visible light stimulate the orientation and locomotion of snails towards the light source.[17] It have been noted that snail *Lymnaea acuminata* monitor the intensity variation of visible light.[5,18] Photodynamic killing of snails is one of the newly developed methods for controlling vector-borne diseases. This approach can be effectively utilized as a part of integrated approach for controlling and eliminating fasciolosis. The objective of the present study is to assess the potential efficacy of

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photodynamic chlorophyllin against the snail *Indoplanorbis exustus* in visible spectral bands of light.

MATERIALS AND METHODS

Pure Compound

Chlorophyllin is purchased from sigma chemical Co.USA.

Experimental animal

Adult *Indoplanorbis exustus* (0.95 ± 0.026 cm in length) were collected locally from Ramgarh lake and low-lying submerged fields. The animals were acclimatized for 72 h in laboratory condition. Experimental animals were kept in glass aquaria containing 3l of dechlorinated tap water maintained at room temperature (22-25ºC). The pH of the water was 7.1-7.3 and dissolved oxygen, free carbon dioxide and bicarbonate alkalinity were 6.5-7.1, 5.2-6.2 and 102-104 mg/l, respectively. Dead animals were removed immediately from the aquaria to avoid any contamination.

Preparation of chlorophyllin

Preparation of chlorophyllin was done according to the method of Wohllebe *et al.*[19] as modified by Singh and Singh.[16] Chlorophyll was isolated from spinach (*Spinacia oleracea*) using 100% ethanol (for about 2 h at 55ºC). Then, $CaCO_3$ (about 1mg/g plant material) was added as a buffer, it prevents the transformation of chlorophyll into pheophytin. The extract was subsequently filtered using Whatman qualitative filter papers (Whatman International Ltd, UK) and 50 ml petroleum benzene was added. After shaking the mixture, the chlorophyll moved into the lipophilic benzene phase. The two phases were separated in separatory funnel and about 1.0 ml methanolic KOH was added to 50 ml of the benzene phase. Upon agitation, the chlorophyll came into contact with the methanolic KOH and was transformed into water-soluble chlorophyllin (This process occurs due to the breakage of the ester bond between the chlorophyllin and the phytol tail by saponification). After separation of the methanolic KOH phase and the benzene phase most of the chlorophyllin was found in KOH phase. The extract was stored in a dark flask at room temperature. However, only fresh chemicals were used in the course of these experiments.

Design of photo toxicity experiments

Light experiment was set up according to the method of Tripathi *et al.*[5] Xenon arc lamp (500 W) was used as visible light source. Interference colored filters was used to perform the spectral response between 400 nm to 650 nm. Exposure of visible light at different wavelengths and fix intensity (500 W/m^2) was used against the chlorophyllin treated snails to observe their mortality. Toxicity experiments were done at normal room temperature (22-25ºC).

Thin layer chromatography

Thin layer chromatography (TLC) was performed according to the method of Barone and Tansey[20] as modified by Upadhyay and Singh.[21] Thin layer chromatography was carried out on 20×20 cm precoated silica gel (Merck Specialities Private Limited, Mumbai, India) using benzene/ethyl acetate (9:1, w/v) as the mobile phase. The loading of extracted chlorophyllin with pure chlorophyllin were applied on TLC plates with a micropipette. TLC plates were developed with iodine vapour. Copies of chromatogram were made by tracing the plates immediately and R*f* value were calculated.

High performance liquid chromatography

Identification of active component present in chlorophyllin was done by HPLC.

Sample preparation

The sample of extracted chlorophyllin was prepared by weighing 50 mg and then dissolving in 20 ml of acetonitrile. The sample was properly vortexed to ensure dissolution. Prior to injected 20 µl sample, the solutions was passed through a Millipore filter (ultra filter disc 3K 43 mm 10 pk, Cole Parmer, Germany) to remove any undissolved particles.

Preparation of standard solution

Pure standard solution of chlorophyllin was prepared by diluting 10 mg chlorophyllin in 20 ml of acetonitrile. The mixture was vortexed to ensure proper dissolution of pure compound. The solution, thus obtained, was passed through Millipore filter (ultra filter disc 3K 43 mm 10 pk, Cole Parmer, Germany).

Instrumentation

The HPLC system was equipped with two LC- 10ATVP pumps, a Cecil CE 4201 UV- variable detector and a Microliter®#702 (Hamilton-Bonaduz, Schweiz) syringe with a loop size of 20 µl. Reverse-phase chromatographic analysis was carried out under isocratic conditions using a reverse-phase Luna 5 µ C_{18} Phenomenex column (250×4.6 mm) at 27ºC. Acetonitrile (HPLC grade) was used as the mobile phase solvent under a pressure of 260-270 Kgf/cm^2 and run time of 15 min. The analysis was carried out a flow rate of one ml/min., the extracted chlorophyllin effluent being monitored at 220 nm. Data acquisition were done with Power Stream™ software.

Treatment protocol for concentration-response relationship

Toxicity experiments were done according to the method of Singh and Agarwal.[22] A total of 10 snails were placed in a glass aquarium containing 3 l of dechlorinated tap water. Snails were treated with different concentrations of extracted and pure chlorophyllin and incubated for 4 h in darkness. Thereafter, in I set of experiment the extracted and pure chlorophyllin treated snails were exposed to sunlight. In II set of experiment extracted chlorophyllin treated snails were exposed to different spectral band of monochromatic visible light. The control animals were kept in the equal volume of water under similar conditions without treatment. In control group, I snails were exposed to sunlight without any treatment. In control group II snails were exposed to monochromatic visible lights without any treatment. Each experiment was replicated 6 times. Mortality of snails was recorded at 24 h up to 96 h. The dead animals were removed immediately to avoid any contamination in aquarium water. The mortality of snails was established by the contraction of body within the shell, no response to needle probe was taken as evidence of death.

Concentration mortality data for each group of snails were analyzed using the probit log analysis program, POLO-PC (LeOra Software) Robertson *et al.*[23] to estimate the LC_{50} of extracted chlorophyllin and the 95% confidence intervals for these concentrations. The slope of the probit lines was also estimated. This program ran chi-square test for goodness of fit of the data to the probit model. If the model fits, the calculated value of chi-square is less than the chi-square table value for the appropriate degrees of freedom. If the model does not fit, the LC_{50} value for the particular population may not be reliably estimated and is adjusted with the heterogeneity factor (Observed chi-square values divided degrees of freedom). This program uses heterogeneity factor as a correlation factor when the value of Pearson's chi-square statistic is significant as $P < 0.05$. The index of significance for potency estimation (g-value) was used to calculate 95% confidence intervals for potency (relative potency is equivalent to tolerance ratio). Parallelism of the probit regression lines implies a constant relative potency at all levels of response. POLO-PC was used to test equality and parallelism of

the slope of the probit lines Robertson *et al.*[23] The regression coefficient between exposure time and different values of LC_{50} was determined by the method of Sokal and Rohlf.[24]

RESULTS

The molluscicidal activity of extracted/pure chlorophyllin was tested at different time of exposure to various light spectra and chlorophyllin concentration against the pest *Indoplanorbis exustus* (Table 1). A significant ($p < 0.05$) negative regression was noted in between the exposure time and LC_{50} of the treatments (Table 2). The LC_{50} at 96 h of pure and extracted chlorophyllin was 6.54 mg/l and 939.65 mg/l in sunlight (Table 2). Toxicity was noted in the presence of visible spectral band of lights at fix intensity (500 W/m^2). The highest toxicity was noted in yellow light (LC_{50} at 96 h: 2016.79 mg/l) and lowest in green light (LC_{50} at 96 h: 2433.16 mg/l) (Table 3).

The thin layer chromatography analysis demonstrated that the R*f* value of extracted chlorophyllin (0.50) was nearly equivalent to the R*f* value of pure chlorophyllin (0.48). The identification of active components was done by comparing the retention time (R_t) and chromatographic peaks of extracted chlorophyllin and pure chlorophyllin. The HPLC fingerprint profile of the extracted chlorophyllin showed major peaks at the retention time of 10.89 min. (Figure 2) whereas the pure standard solutions of chlorophyllin showed major peaks at the retention time of 1.74 min. (Figure 3).

The slope values given in Tables 2 and 3 were steep and separate estimates of LC based on each of six replicates were found to be within the 95% confidence limits of LC_{50}. The t-ratio was higher than 1.96 and heterogeneity factor was less than 1.0. The g-value was less than 0.5 at all probability levels (90, 95 and 99 respectively) (Tables 2 and 3). There was significant negative regression ($p < 0.05$) between the exposure time and LC_{50} of the treatments (Tables 2 and 3).

DISCUSSION

The data given above indicate that chlorophyllin pure/extracted from spinach is very effective and efficient molluscicide. Toxicity against the snail *Indoplanorbis exustus* is time and concentration dependent as evident by the negative regression between exposure period and LC_{50} values of the different treatments. Toxicity of photodynamic chlorophyllin is noted against *I. exustus* in the occurrence of different visible spectral bands of lights at a fix intensity of 500 W/m^2. All the monochromatic visible lights have adequate energy to elicit the response of photodynamic chlorophyllin. Yellow light caused higher toxicity of chlorophyllin against snails than other monochromatic visible lights. Significant variation in toxicity of chlorophyllin exposed to same intensity of visible monochromatic light is evident from different LC_{50} of chlorophyllin. All lights have different wavelengths and it represents that variation in wavelength of light has significant effect on mortality, as evident from highest toxicity of chlorophyllin was observed in yellow light (LC_{50} at 96 h: 2016.79 mg/l) and lowest in green light (LC_{50} at 96 h: 2433.16 mg/l).

Recently, Singh and Singh[16] demonstrated the larvicidal activity of chlorophyllin against *F. gigantica* at the fix intensity of 300 W/m^2. The effectiveness of chlorophyllin depends on light attenuation in the water body.[25] Relating to attenuation it was tested earlier that about 36 W/m^2 of visible day light are sufficient to stimulate photodynamic destruction of *Chaoborus crystallinus* larvae.[26] The time-dependent toxic effect of tested plant products may be due to the uptake of active compound by the snails, which progressively accumulated in the body with an increase exposure period. It is also possible that the active compound could change into more toxic forms in the aquarium water or in the snail's body in visible band spectra of light.

When sunlight penetrates water at a marked angle then longer visible wavelength are absorbed more by water than shorter visible wavelengths during penetration.[27,16] The toxicity experiments clearly demonstrated that pure chlorophyllin is more toxic than extracted chlorophyllin and chlorophyllin become more effective in sunlight than different spectral band of monochromatic visible lights. It may be due to higher solubilized atom of chlorophyllin in sunlight transferred its excitation energy to oxygen, which generate singlet oxygen and other reactive oxygen species (ROS), which have the potential to kill the vector organism.[28,29,15] ROS caused strong oxidative stress to the cells which damage the cell membrane, protein, DNA and other cell structures.[30,31] Photodynamic chlorophyllin was capable to kill mosquito larvae and other small animals within a few hours in sunlight.[11] Recent research on chlorophyllin has been advocated by researchers. As Erzinger *et al.*[32] demonstrated that photodynamic chlorophyllin was able to kill four different species, a small crustacean (*Daphnia similis*), a unicellular alga (*Euglena gracilis*) and two species of fish (*Astyanax bimaculatus* and *Cyprynus carpio*) which are the vector of parasitic diseases. Earlier, Kumar and Singh[33] reported that chlorophyllin show toxic effects against *Lymnaea acuminata* in the presence of red visible light and sunlight. Recently, Hader *et al.*[15] reported the toxicity of photodynamically active chlorophyllin against fish ectoparasite *Ichthiophthirius multifiliis, Ichtyobodo, Dactylogyrus, Trichodina, Argulus.*

The steep slope indicates that a small increase in the concentration of molluscicide caused higher mortality. The t-ratio value greater than 1.96 indicates that the regression is significant ($p< 0.05$). The heterogeneity factor value less than 1.0 denotes that in the replicate test of random samples; the concentration response is limited and thus the model fits the data adequately. The index of significance of the potency estimation g indicates that the value of the mean is within the limit at all probability levels (90, 95 and 99 respectively) since it is less than 0.5.

Thin layer chromatography (TLC) study demonstrates the preliminary identification of the active components in extracted and pure chlorophyllin. The co-migration of extracted and pure chlorophyllin on TLC plate show nearly equivalent R*f* value of extracted (0.50) and pure (0.48) chlorophyllin. The stationary phase, silica gel can be considered polar while the organic solvent used as the mobile phase is non-polar.[34] Components of mixture differ in polarity and have different tendencies to absorb onto the silica gel or dissolve in the organic solvent.[35] The more polar components have a stronger interaction with the silica gel and absorb on the silica gel strongly, therefore, less distance it can travel up the plate and show lower R*f* value. In contrast, non-polar components move higher up the plate and show higher R*f* value.[34,35]

High performance liquid chromatography (HPLC) has already been considered to be the simplest and most reproducible technique for analyzing complex mixtures of pigments in food and other sources. HPLC fingerprinting is the best way for chemical characterization.[36]

Willstatter and Escher[37] discovered that chlorophyll was a mixture of two compounds which were designated as chlorophyll a and chlorophyll b. Chlorophyll a contains $-CH_3$ group and chlorophyll b contain –CHO group, respectively and after removal of phytol tail chlorophyll converts into chlorophyllin (Figure 1).[38] It was reported by Lim,[39] that the replace-

Table 1: Concentration of extracted and pure chlorophyllin used in toxicity experiment against *Indoplanorbis exustus*

Experimental condition	Chemical	Concentration (mg/l)
Sunlight	Ext Chl	900, 1000, 1100, 1200
	Pure Chl	10, 20, 30, 40
Different spectra of light	Ext Chl	1900, 2100, 2300, 2500

Abbreviation: Ext- Extracted, Chl- Chlorophyllin

Table 2: Toxicity of extracted and pure chlorophyllin in sunlight against *Indoplanorbis exustus*

Exposure Period	Treatment	LC_{50} mg/l (w/v)	LCL	UCL	Slope value	t-ratio	g-value	Heterogeneity
24 h	Ext Chl	1371.59	1232.31	2020.21	6.68 ± 2.03	3.28	0.35	0.15
	Pure Chl	37.22	29.69	56.54	1.87±0.40	4.64	0.17	0.23
48 h	Ext Chl	1187.43	1118.12	1364.55	7.28 ± 1.85	3.91	0.25	0.16
	Pure Chl	22.57	16.97	30.24	1.48±0.37	3.99	0.24	0.25
72 h	Ext Chl	1028.96	964.45	1088.74	7.13 ± 1.79	3.97	0.24	0.27
	Pure Chl	15.70	11.03	19.57	1.75±0.37	4.65	0.17	0.64
96 h	Ext Chl	939.65	883.78	976.62	11.35 ± 1.99	5.69	0.11	0.44
	Pure Chl	6.54	2.54	9.66	1.85±0.43	4.28	0.20	0.35

Six batches of ten snails were exposed to different concentration. Mortality was determined at 24 h to 96 h. Concentrations given are the final concentration (w/v) in the glass aquarium water. Abbreviation: Ext- Extracted, Chl- Chlorophyllin, LCL- Lower confidence limit, UCL-Upper confidence limit. Significant negative regression ($p < 0.05$) was observed between exposure time and LC_{50} treatments. Ts – testing significant of the regression coefficient- Ext Chl -8.804^{+} to -3.315^{+} and 102.8^{++} to 2155^{++} and Pure Chl -0.6134^{+} to -0.2108^{+} and 0.0^{++} to 90.37^{++} was observed. $^{+}$Linear regression between X and Y. $^{++}$Non- linear regression between X and Y.

Table 3: Toxicity of extracted chlorophyllin in the presence of different spectra of light against *Indoplanorbis exustus*

Exposure period	Treatment	Different spectra of light	LC_{50} mg/l (w/v)	LCL	UCL	Slope values	t-ratio	g- value	Heterogeneity
24 h	Ext Chl	Green	2844.62	2612.40	3628.83	9.99±2.60	3.83	0.26	0.38
		Violet	2739.81	2554.34	3259.69	10.43±2.46	4.23	0.21	0.32
		Blue	2592.33	2592.33	2866.12	11.66±2.34	4.98	0.15	0.26
		Orange	2548.53	2415.69	2851.25	9.67±2.10	4.59	0.18	0.29
		Red	2440.94	2330.23	2663.53	9.35±2.00	4.67	0.17	0.26
		White	2320.46	2233.79	2451.60	10.11±1.97	5.13	0.14	0.15
		Yellow	2235.96	2171.54	2309.26	13.46±2.04	6.59	0.08	0.17
48 h	Ext Chl	Green	2760.96	2520.88	3734.57	7.16±2.09	3.41	0.32	0.17
		Violet	2681.70	2480.02	3360.56	7.61±2.06	3.68	0.28	0.20
		Blue	2464.91	2351.45	2697.30	9.59±2.03	4.72	0.17	0.17
		Orange	2387.51	2291.20	2557.41	9.91±1.99	4.96	0.15	0.26
		Red	2299.50	2220.64	2409.02	11.03±1.99	5.54	0.12	0.26
		White	2178.42	2097.68	2259.46	10.95±1.95	5.60	0.12	0.20
		Yellow	2151.63	2082.41	2217.12	13.13±2.02	6.49	0.09	0.29
72 h	Ext Chl	Green	2588.50	2408.73	3189.62	7.04±1.98	3.54	0.30	0.13
		Violet	2520.12	2379.55	2870.95	8.32±2.00	4.15	0.22	0.19
		Blue	2355.48	2263.03	2509.11	9.84±1.97	4.98	0.15	0.13
		Orange	2274.73	2185.51	2398.54	9.47±1.93	4.90	0.15	0.26
		Red	2172.88	2092.68	2252.28	11.08±1.96	5.64	0.12	0.31
		White	2117.44	2034.18	2189.05	11.58±1.97	5.85	0.11	0.22
		Yellow	2075.05	2003.81	2133.92	14.33±2.09	6.84	0.08	0.33
96 h	Ext Chl	Green	2433.16	2295.35	2792.54	6.99±1.90	3.66	0.28	0.22
		Violet	2380.57	2241.28	2747.46	6.30±1.88	3.33	0.34	0.11
		Blue	2223.19	2139.50	2320.09	10.18±1.93	5.25	0.13	0.17
		Orange	2125.44	2029.61	2207.68	10.08±1.94	5.19	0.14	0.29
		Red	2089.26	2005.03	2157.85	12.00±2.00	5.99	0.10	0.23
		White	2029.92	1946.67	2092.65	13.62±2.11	6.44	0.09	0.39
		Yellow	2016.79	1950.42	2069.14	17.05±2.33	7.30	0.07	0.44

Six batches of ten snails were exposed to different concentration. Mortality was determined at every 24 h up to 96 h. Concentrations given are the final concentration (w/v) in the glass aquarium water. Abbreviation: Ext- Extracted, Chl- Chlorophyllin, LCL- Lower confidence limit, UCL-Upper confidence limit. Significant negative regression ($p < 0.05$) was observed between exposure time and LC_{50} of treatments. Ts – testing significant of the regression coefficient of Green light -10.11^{+} to -2.93^{+} and 1531^{++} to 3750^{++}, Violet light -6.90^{+} to -2.10^{+} and 1805^{++} to 3350^{++}, Blue light -5.60^{+} to -4.53^{+} and 1557^{++} to 3256^{++}, Orange light -6.88^{+} to -4.63^{+} and 1365^{++} to 3297^{++}, Red light -6.60^{+} to -3.24^{+} and 1418^{++} to 3078^{++}, White light -5.94^{+} to -1.82^{+} and 1479^{++} to 2814^{++}, Yellow light -3.81^{+} to -2.30^{+} and 1589^{++} to 2627^{++} was observed. $^{+}$Linear regression between X and Y. $^{++}$Non- linear regression between X and Y.

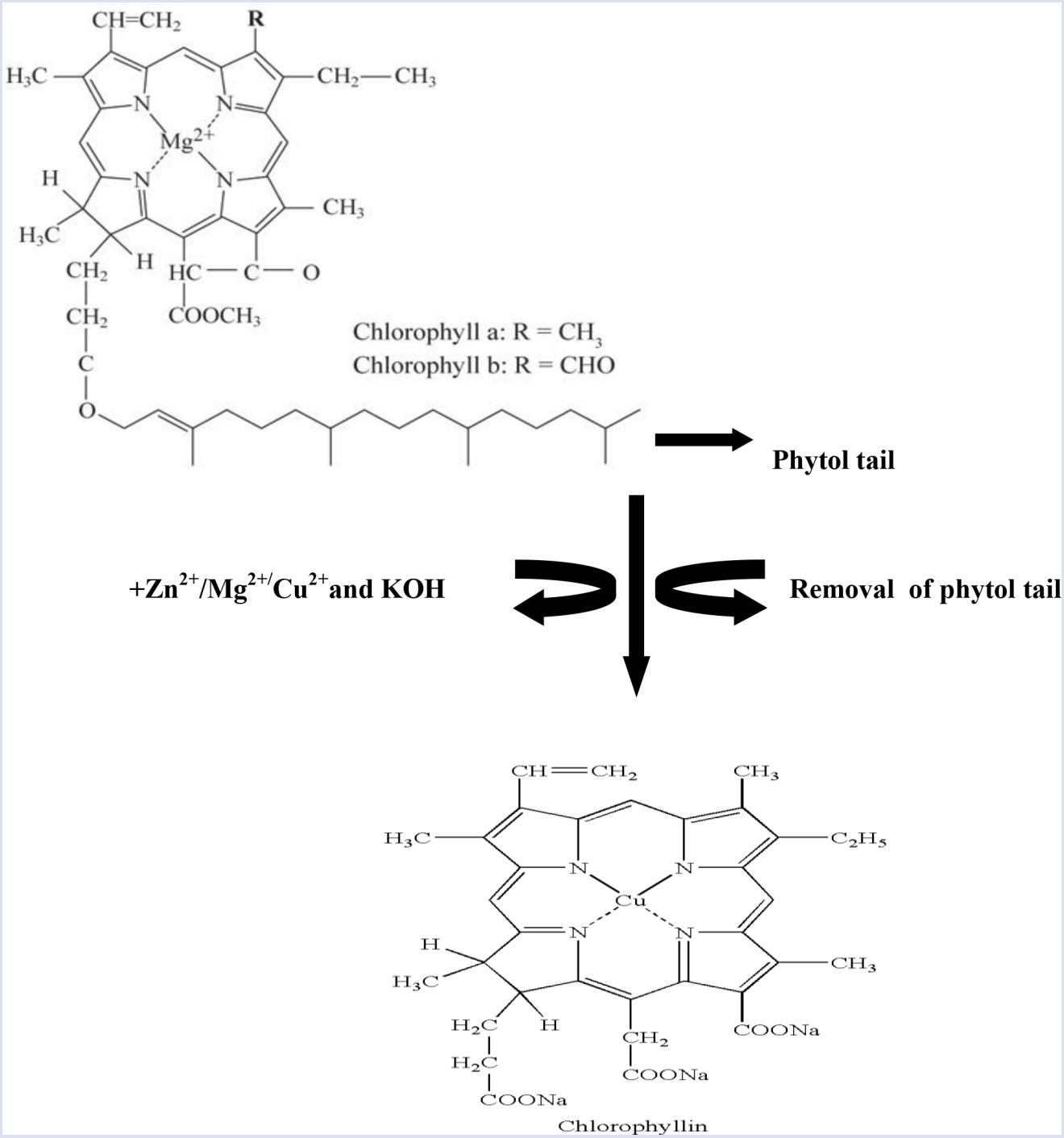
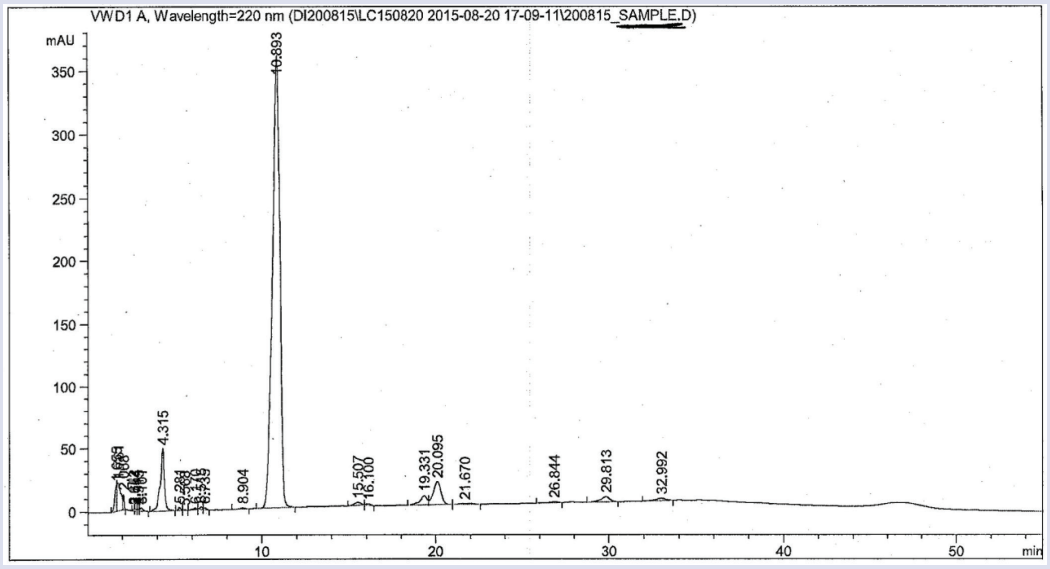
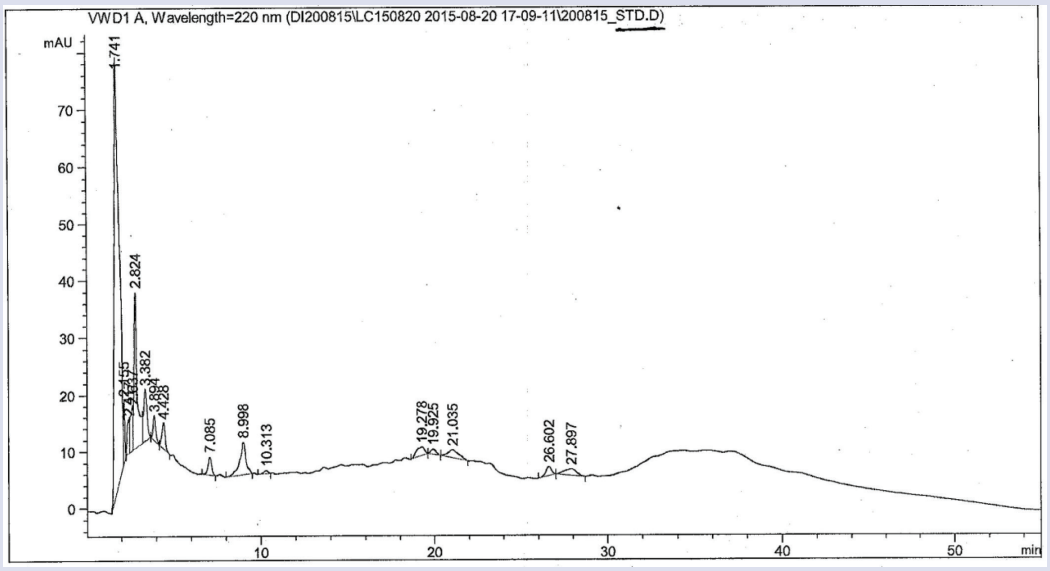


Figure 1: **Transformation of chlorophyll in chlorophyllin from spinach (*Spinacia oleracea*).**



Peak #	Peak Name	Ret. Time [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area [%]
1	Chlorophyllin b	1.76	VV	0.20	335.47	21.79	2.51
2	Chlorophyllin a	10.89	BB	0.42	1.06	359.60	79.98

Figure 2: High Performance Liquid Chromatography of extracted chlorophyllin.



Peak #	Peak Name	Ret. Time [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area [%]
1	Chlorophyllin b	1.74	VV	0.27	1527.02	76.28	56.06
2	Chlorophyllin a	10.31	BB	0.25	9.07	5.40	0.33

Figure 3: High Performance Liquid Chromatography of pure chlorophyllin.

ment of $-CH_3$ group (in chlorophyll a) with –CHO group (in chlorophyll b) at the position C-7 increases the polarity of the chlorophyll b; since chlorophyll b becomes more polar than chlorophyll a and appears on the shorter retention time. It was also reported that the retention time always decreases in the same order (pheophytin a > chlorophyll a > pheophytin b > chlorophyll b > chlorophyllide a > chlorophyllide b) and predominantly depends on the polarity of the mobile phase.[39,40] In the present study the separation of chlorophyll derivates by HPLC method clearly demonstrate the major peaks at the retention time of 10.89 min. in extracted chlorophyllin and 1.74 min. in pure standard chlorophyllin. From the above reporting, it can be state that higher retention time indicates the presence of chlorophyllin a in extracted chlorophyllin[41,42] and comparatively lower retention time indicates the presence of chlorophyllin b in pure chlorophyllin. In present observation, major peaks of the retention time of chlorophyllin a and b clearly demonstrate that these are the active components which are found more abundantly in extracted and pure chlorophyllin, respectively. Now, the HPLC data clearly define the minor differences between the *Rf* values of TLC results. It can also be concluded that the toxicity of extracted chlorophyllin is due to chlorophyllin a than chlorophyllin b.

CONCLUSION

The laboratory studies reported in this work demonstrate that photodynamic chlorophyllin is very powerful and adequate molluscicide for target vector snails. It can be avowed that molluscicidal activity of chlorophyllin is due to their active components: chlorophyllin a and b. Such type of exploratory research work by means of plant extracts can be effective approach to kill the snail population. Being economically and environmentally friendly, this approach can get high public acceptance also. Photodynamic chlorophyllin as a molluscicide show great potential of photosensitization prospective for the control of endemic fasciolosis in developing countries.

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CONFLICTS OF INTEREST

We declare that we have no conflict of interest.

ABBREVIATIONS USED

TLC: Thin Layer Chromatography; **HPLC:** High Performance Liquid Chromatography; **Ext:** Extracted; **Chl:** Chlorophyllin.

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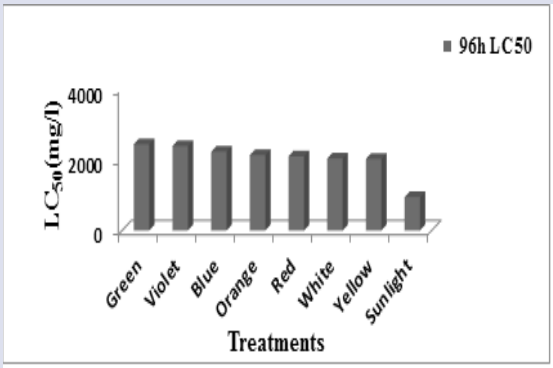
GRAPHICAL ABSTRACT



SUMMARY

- Fasciolosis is an imperative zoonotic helminthes disease throughout the world.
- Chlorophyllin is a photodynamic product and more effective in sunlight.
- HPLC study reveals active component of extracted chlorophyllin is chlorophyllin a.
- Chlorophyllin is a very promising photodynamic substance and it has the potential to control the population of vector snails and ultimately fasciolosis in developing countries.

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