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CHARACTERIZATION OF THE MOLLUSCICIDAL ACTIVITY OF *Bauhinia variegata* AND *Mimusops elengi* PLANT EXTRACTS AGAINST THE *Fasciola* VECTOR *Lymnaea acuminata*

Kanchan Lata SINGH, D.K. SINGH & Vinay Kumar SINGH

SUMMARY

The molluscicidal activity of *Bauhinia variegata* leaf and *Mimusops elengi* bark was studied against vector snail *Lymnaea acuminata*. The toxicity of both plants was time and concentration-dependent. Among organic extracts, ethanol extracts of both plants were more toxic. Toxicity of *B. variegata* leaf ethanolic extract (96h LC_{50}- 14.4 mg/L) was more pronounced than *M. elengi* bark ethanolic extract (96h LC_{50}-15.0 mg/L). The 24h LC_{50} of column purified fraction of *B. variegata* and *M. elengi* bark were 20.3 mg/L and 18.3 mg/L, respectively. Saponin and quercetin were characterized and identified as active molluscicidal component. Co-migration of saponin (R*f* 0.48) and quercetin (R*f* 0.52) with column purified bark of *M. elengi* and leaf of *B. variegata* on thin layer chromatography demonstrate same R*f* value i.e. 0.48 and 0.52, respectively. The present study clearly indicates the possibility of using *M. elengi* and/or *B. variegata* as potent molluscicide.

KEYWORDS: Molluscicidal activity; Plant species; Extracts; Lymnaeid vector; *Fasciola*; Snail; *Lymnaea acuminata*; *Bauhinia variegata*; *Mimusops elengi*.

INTRODUCTION

Fasciolosis is one of the most debilitating zoonotic diseases caused by the liver flukes *Fasciola hepatica* and *Fasciola gigantica*[14-16,36]. Incidence of fasciolosis is very common in the cattle of eastern region of the state of Uttar Pradesh in India[24]. The fresh water snail *Lymnaea acuminata* is the intermediate host of the *F. gigantica*[29]. This disease is at present emerging or re-emerging in many parts of the world such as Latin America, Europe, Africa and Asia[16]. Due to more attention on SARS, AIDS, malaria and research in immunological approaches to worm control, a little interest is focused on snail control to minimize the fasciolosis/ schistosomiasis[11]. One of the possible solutions to control fasciolosis is to disrupt the life cycle of *Fasciola* by killing the vector snail[1,9-11,27,35]. The continuous and indiscriminate use of synthetic molluscicides for the control of vector snails has created a long detrimental effect on the aquatic environment[22]. Therefore, there is a need to develop a safe and eco-friendly counterpart of synthetic molluscicides. Molluscicides of plant origin are now gaining special importance because they are more effective, cheaper and safer to non-target organisms and culturally more acceptable[8,27]. The present study describes the molluscicidal activity of *Bauhinia variegata* (Order: Fabales; Family: Fabaceae) and *Mimusops elengi* (Order: Ericales; Family: Sapotaceae) against vector snail *Lymnaea acuminata.* Earlier it has been reported that organic (ether, chloroform and ethanol) extracts of *B. variegata* and *M. elengi* leaf and bark contain tannin, saponin, glycoside, terpenoids, flavonoids etc.[5,6,8,19,25]. Although large numbers of pharmacological effects of both the plants have been noted[2,13], yet molluscicidal activity of these plants have not been reported till date.

MATERIAL AND METHODS

Plants Used: Fresh leaf of *Bauhinia variegata* and bark of *Mimusops elengi* were collected from Gorakhpur (India), washed thoroughly in running tap water and finally with sterile water, shade dried. The dried part of *B. variegata* leaf and bark of *M. elengi* were pulverized separately in the electric grinder and the crude powders were obtained, were then sieved with the help of fine mesh cloth. This fine powder was then used separately for toxicity experiments. The specimens were identified and authenticated by department of Botany DDU Gorakhpur University, Gorakhpur, India.

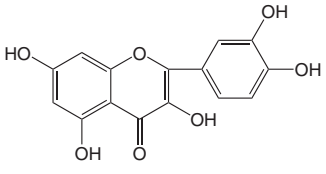
Solvent Extracts: Fifty grams of the leaf of *B. variegata* and bark of *M. elengi* were extracted separately with 100 mL of each solvent viz. ethanol (95%), acetone (99%), ether (99.5%) and chloroform (99%) at room temperature for 24 h. Each preparation was filtered separately through sterilized whatman No.1 filter paper[9] and the filtered extracts were subsequently evaporated under vacuum. The residues, thus obtained, were used for the determination of molluscicidal activity. The leaf of *B. variegata* yielded 220 mg chloroform extract, 250 mg acetone extract, 180 mg ether extract, 300 mg ethanol extract *M. elengi* bark powder yielded 180 mg chloroform extract, 175 mg ether extract, 190 mg acetone extract, 210 mg ethanol extract.

Malacology Laboratory, Department of Zoology, DDU Gorakhpur University, Gorakhpur-273 009 UP, India. Phone-+91-9415855488 (Mobile). Email: drvksingh_gpu@yahoo.in; vinaygkpuniv@gmail.com
Correspondence to: Dr. Vinay Kumar Singh. E-mail: vinaygkpuniv@gmail.com.

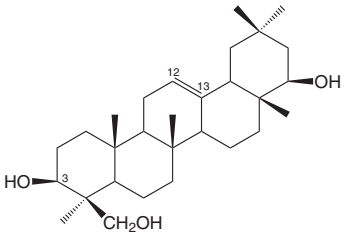
Column purification: One hundred milliliters of *B. variegata* leaf ethanol extract and *M. elengie* bark ethanol extract were subjected to silica gel (60-120 mesh, Qualigens glass, Precious Electro Chemindus Private Limited, Mumbai, India) chromatography through 95×45cm column. Seventy five fractions of five milliliters were eluted with ethanol (95%). Ethanol was evaporated under vacuum and the remaining solids obtained were used for the determination of molluscicidal activity of each fraction.

Thin Layer Chromatography: Thin layer chromatography (TLC) was performed by method of SINGH & SINGH[31] as modified by JAISWAL & SINGH[9] to identify the active component present in *B. variegata* leaf extract and *M. elengi* bark extract. TLC was done on 20×20 cm precoated silica gel (Precious Electrochemical Industry. Pvt , Ltd, Mumbai, India). The solvent benzene/ ethyl acetate (9:1 v:v) was used as the mobile phase. Spots of column purified fractions of *B. variegata* leaf extract and *M. elengi* bark extract along with their respective active components quercetin and saponin were applied on TLC plates with the help of micropipette. Further, the TLC plates were developed by iodine vapor. Copies of the chromatogram were made by tracing the plates immediately and the retardation factor (R*f*) was calculated.

Pure Compound: Quercetin (3,3,4,5,7–penta hydroxyflavone) and saponin (Sapogenin~10-20%) were procured from Sigma Chemical Co. USA.



Chemical structure of Quercetin



Chemical structure of Saponin

Collection of test animals: The adult fresh water snails, *L. acuminata* (2.25 ± 0.20 cm in length) were collected locally from different ponds, lakes and low lying submerged fields in Gorakhpur and were used as test animals. The collected snails were acclimatized for 72 h in the laboratory condition. Experimental animals kept in the glass aquaria containing dechlorinated tap water at 23 ± 1 °C. The pH, dissolved oxygen, free carbon dioxide and bicarbonate alkalinity were 7.1-7.3, 6.5-7.3 mg/L, 5.2-6.3 mg/L and 102-105 mg/L, respectively. Dead animals were removed to avoid any spoilage of the aquaria water.

Toxicity Experiment

Concentration-response relationship: The toxicity experiments were performed by the method of SINGH & AGARWAl[28]. Ten experimental animals were kept in a glass aquarium containing 3l of dechlorinated tap water. Snails were exposed continuously for 96h to different concentrations of *B. variegata* leaf extract and *M. elengi* bark extract (Table-1). Six aquaria were set up for each concentration. The control animals were kept in equal volumes of water under similar conditions without treatment. Mortality of snails was recorded at intervals of 24h up to 96h. 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. The LC values lower and upper confidence limits (LCL and UCL), slope values, t- ratio, g-values and heterogeneity factor were calculated by using polo computer software of ROBERTSON *et al*.[18] (2007). The regression coefficient between exposure time and different values of LC_{50} was determined by the method of SOKAL & ROHLF[32].

Table 1
Concentration of different plants products and their active components used for the toxicity determination against *L. acuminata*

Parts of plants and their extracts	Concentration (mg/L)
B. variegata leaf powder	100, 150, 200, 250
Chloroform extract	20, 30, 50, 70
Ether extract	20, 30, 50, 70
Acetone extract	20, 30, 50, 70
Ethanol extract	20, 30, 50, 70
Column purified	10, 15, 20, 30
Quercetin	7, 9, 12, 15
M. elengi Bark powder	30, 50, 70, 120
Chloroform extract	20, 30, 40, 50
Ether extract	20, 30, 40, 50
Acetone extract	20, 30, 50, 70
Ethanol extract	20, 30, 50, 70
Column purified	9, 12, 15, 25
Saponin	3, 5, 7, 20

RESULTS

The toxicity of different organic solvent extracts of leaf powder of *B. variegata* and bark *M. elengi* was time and concentration dependent. The 24h LC_{50} of the leaf powder of *B. variegata* and bark powder of *M. elengi* was 244.70 mg/L and 91.19 mg/L, respectively (Table-2 & 3). There was a significant ($p < 0.05$) negative correlation between the LC_{50} and exposure time. *B. variegata* leaf ethanol extract (24h LC_{50}- 38.42 mg/L) and *M. elengi* bark ethanol extract (24h LC_{50}- 44.61 mg/L) were more toxic in comparison to other organic solvents (Table-2 & 3). The column purified fraction of *B. variegata* and *M. elengi* were highly toxic. The maximum molluscicidal activity of column purified *B. variegata* leaf and *M. elengi* were noted in the 20-30 and 16-26 of the 5 mL Si-gel eluted fractions, respectively. The 96h LC_{50} of column purified fraction of *B. variegata* (5.28 mg/L) was higher than the *M. elengi* (7.20 mg/L) (Table- 2 & 3). The 96 LC_{50} of quercetin and saponin was 5.28 mg/L and 1.30 mg/L, respectively. Thin layer chromatography analysis demonstrated that the R*f* values of quercetin (0.52) was equivalent to the R*f* value of column purified fraction of *B. variegata* (0.52) and saponin (0.48) was equivalent to the R*f* values of the column purified fractions of *M. elengi* (0.48).

The slope values were steep and separate estimates of LC based on each of the six replicates were found to be within 95% confidence limit 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 the probability levels (90, 95, 99). There was significant negative regression ($p < 0.05$) between exposure time and LC_{50} of the treatments (Table- 2 & 3).

Table 2

Toxicity of *Bauhinia variegata* leaf powder, organic solvent extracts, column purified fraction and quercetin (active component) against snail *Lymnaea acuminata* at different exposure periods

Exposure period	Tested materials	LC_{50} (mg/L)	Limits		Slope value	t-ratio	g-value	Heterogeneity
			LCL	UCL				
24h	*B. variegata* leaf powder	244.70	216.22	302.00	3.78±0.68	5.51	0.12	0.42
	Ethanol extract	38.42	31.91	52.45	2.44±0.45	5.42	0.13	0.17
	Ether extract	57.19	40.90	134.30	1.77±0.44	4.02	0.23	0.18
	Acetone extract	38.64	32.78	50.35	2.84±0.49	5.76	0.11	0.56
	Chloroform extract	43.32	35.21	63.47	2.42±0.47	5.16	0.14	0.34
	Column purified	20.30	16.99	27.41	2.72±0.48	4.16	0.12	0.28
	Quercetin	12.13	10.42	24.73	2.19±0.62	3.59	0.32	0.16
48h	*B. variegata* leaf powder	203.45	179.12	245.20	3.03± 0.60	5.08	0.14	0.37
	Ethanol extract	28.74	24.00	36.37	2.12±0.39	5.35	0.13	0.15
	Ether extract	34.78	27.91	51.28	1.84±0.39	4.63	0.17	0.25
	Acetone extract	30.09	25.41	37.95	2.31±0.41	5.64	0.12	0.40
	Chloroform extract	35.35	28.08	54.00	1.76±0.40	4.46	0.19	0.29
	Column purified	16.03	12.90	22.95	1.79±0.47	3.16	0.14	0.32
	Quercetin	9.86	8.45	13.75	2.16±0.60	3.60	0.29	0.13
72h	*B. variegata* leaf powder	155.94	137.78	174.18	3.52 ±0.58	5.97	0.10	0.40
	Ethanol extract	20.94	17.15	25.13	2.14±0.38	5.58	0.12	0.18
	Ether extract	20.74	17.13	24.66	2.25±0.38	5.82	0.11	0.34
	Acetone extract	21.47	17.75	25.70	2.20±0.39	5.69	0.11	0.40
	Chloroform extract	22.54	18.56	27.40	2.09±0.38	5.43	0.13	0.31
	Column purified	10.13	7.73	17.75	3.82±0.37	3.60	0.14	0.28
	Quercetin	6.82	4.79	9.93	4.75±0.60	3.39	0.25	0.10
96h	*B. variegata* leaf powder	126.70	110.93	139.72	4.35±0.63	6.85	0.08	0.57
	Ethanol extract	14.42	11.93	17.51	2.61±0.40	6.49	0.09	0.66
	Ether extract	15.03	12.00	17.67	2.57±0.40	6.43	0.09	0.64
	Acetone extract	15.50	12.52	18.00	2.70±0.40	6.67	0.08	0.73
	Chloroform extract	15.22	12.06	18.10	2.47±0.39	6.22	0.10	0.76
	Column purified	5.98	4.08	9.47	2.42±0.39	5.16	0.13	0.33
	Quercetin	5.39	4.81	8.41	3.96±0.64	4.55	0.12	0.24

Mortality was determined at every 24 h up to 96 h. Each set of experiment was replicated six times. Abbreviation: *B. variegata* leaf powder = *Bauhinia variegata* leaf powder; 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 – *B. variegata* leaf powder – 16.79+; Ethanol extract – 15.91+; Ether extract – 10.18++; Acetone extract – 17.30+; Chloroform extract. – 13.16+; column purified – 20.37+; quercetin – 6.55+. +: linear regression between x and y; ++: non-linear regression between log x and log y.

DISCUSSION

The present study clearly demonstrates that *B. variegata* leaf extract and *M. elengi* bark extract are the potent molluscicides. Mortality caused by all the plant preparations was time- and concentration dependent and there was a negative regression between exposure time and LC values. Toxicity of crude/ purified preparations of both plants against *L. acuminata* is in the range of potent molluscicide. Thus, high molluscicidal activity, the LC_{50} being less than 100 ppm[8,27]. The 96h LC_{50} of crude preparations of both plants are approximately 100 ppm, whereas all organic extracts LC_{50} are less than 100 ppm. Among all the organic solvent extracts, the higher toxicity of ethanol extract of *B. variegata* leaf and *M. elengi* bark powder indicate that the active molluscicidal component present in the leaf and bark of both plants are more soluble in ethanol than other organic solvents. Molluscicidal activity of *B. variegata* leaf and *M. elengi* bark is due to the presence of quercetin and saponin as evident from the individual toxicity and identification by TLC. Earlier, it has been reported that saponins are potent molluscicides[20,26,27,33]. Pharmacological and biological effects of saponin as antibacterial[7], antihelmintic[13], antigastric ulcer[23] and hypotensive[4] have been noted. Methanol extract of *Mimusops elengi* bark and seed shows significant antifungal activity[21]. Bark extract of *M. elengi* showed moderate inhibitory activity against HIV type-1 protease[12].

Bauhinia variegata, commonly known as 'cow paw', has great therapeutic properties, mainly due to the presence of flavonoids and other secondary metabolites include terpen, quinines and lactones[3]. Antiprotozoal, antihelmintic, antitumor, antiulcer and cytotoxic of *B. variegata* have been reported by CECHINEL FILHO[2]. Flavones and

Table 3
Toxicity of *M. elengi* bark powder, different organic solvent extracts, column purified fraction and saponin (active component) against snail *Lymnaea acuminata* at different exposure periods.

Exposure period	Tested materials	LC_{50} (mg/L)	Limits		Slope value	t-ratio	g-value	Heterogeneity
			LCL	UCL				
24h	*M. elengi* bark powder	91.19	75.77	130.60	2.50±0.53	4.72	0.17	0.20
	Ethanol extract	44.61	35.37	70.40	2.17±0.44	4.83	0.16	0.13
	Ether extract	45.09	39.93	54.67	3.49±0.63	5.48	0.12	0.13
	Acetone extract	58.29	48.79	85.22	3.25±0.69	4.65	0.17	0.30
	Chloroform extract	52.81	46.40	66.46	4.12±0.75	5.48	0.12	0.39
	Column purified	18.34	10.50	57.75	2.19±0.34	3.49	0.31	0.22
	Saponin	15.57	13.68	19.88	4.02±0.78	5.72	0.14	0.26
48h	*M. elengi* bark powder	69.65	58.04	92.84	2.06±0.48	4.29	0.20	0.25
	Ethanol extract	31.38	25.46	43.72	1.85±0.39	4.70	0.17	0.12
	Ether extract	36.90	32.49	43.20	2.99±0.58	5.13	0.14	0.15
	Acetone extract	46.26	39.67	61.75	2.71±0.60	4.52	0.18	0.22
	Chloroform extract	46.49	40.26	60.09	2.96±0.61	4.85	0.16	0.30
	Column purified	15.71	8.07	17.27	1.76±0.28	2.70	0.51	0.16
	Saponin	13.90	11.78	17.82	3.20±0.28	3.70	0.18	0.19
72h	*M. elengi* bark powder	47.24	38.46	55.18	2.44±0.48	5.09	0.14	0.42
	Ethanol extract	21.71	17.74	26.33	2.06±0.38	5.39	0.13	0.20
	Ether extract	29.38	24.68	33.60	2.83±0.57	4.94	0.15	0.18
	Acetone extract	34.09	29.57	39.63	2.86±0.57	4.89	0.16	0.23
	Chloroform extract	34.25	30.38	38.87	3.31±0.58	5.64	0.12	0.28
	Column purified	10.60	9.18	12.41	4.76±0.28	5.70	0.11	0.26
	Saponin	4.25	2.99	7.06	1.89±0.26	3.41	0.33	0.20
96h	*M. elengi* bark powder	36.37	28.59	42.43	2.87±0.50	5.66	0.12	0.72
	Ethanol extract	15.07	11.83	17.89	2.40±0.39	6.09	0.10	0.67
	Ether extract	23.49	19.77	26.33	3.93±0.63	6.23	0.09	0.49
	Acetone extract	25.78	21.72	29.00	3.43±0.59	5.75	0.11	0.56
	Chloroform extract	26.02	22.06	29.21	3.48±0.60	5.80	0.11	0.80
	Column purified	7.20	5.74	8.15	3.84±0.28	3.70	0.14	0.31
	Saponin	1.30	0.27	2.16	0.69±0.25	2.69	0.53	0.19

Mortality was determined at every 24 h up to 96 h. Each set of experiment was replicated six times. Abbreviation: *M. elengi* bark powder = *Mimusops elengi* bark powder; 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 – *M. elengi* bark powder – 9.09+; Ethanol extract – 6.68++; Ether extract – 19.54+; Acetone extract – 16.80+; Chloroform extract– 12.09+; column purified – 12.02+; Saponin – 4.96+. +: linear regression between x and y; ++: non-linear regression between log x and log y.

quercetin have been isolated from leaf of *B. variegata*[17]. Quercetin targets cysteine string proteins (CSPα) and impairs synaptic transmission[37]. The time dependent toxic effect of these plant products may be either due to the uptake of the active moiety which progressively increases the amount of active component in the snails body with increase in exposure period or it might be possible that the active compound could change into more toxic forms in the aquarium water or in the snails body.

A comparison of the molluscicidal activity of quercetin active component present in *B. variegata* and saponin present in *M. elengi* with synthetic molluscicides clearly demonstrates that these components are more potent against *L. acuminata*. The 96 h LC_{50} of quercetin (5.39 mg/L) and saponin (1.30 mg/L) are lower than those of synthetic molluscicides carbaryl (14.40 mg/L), phorate (15.0 mg/L), formothion (8.56 mg/L) and niclosamide (11.8 mg/L)[9,28]. 96 h LC_{50} of saponin (1.30 mg/L) and quercetin (5.39 mg/L) is even lower than active plant molluscicidal components of *Allium sativum* bulb (271.06 mg/L)[31], *Cinnamomum tamala* (830.90 mg/L)[34] *Zingiber officinale* rhizome (273.80 mg/L), *Allium cepa* bulb (253.27 mg/L); *Trachyspermum ammi* (97.59 mg/L)[30].

It is evident from the steep slope values that a small increase in the concentration of different treatment causes mortality in snails. A t-ratio value greater than 1.96 indicates that the regression is significant. Values of the heterogeneity factor less than the 1.0 denote that in the replicates lines would fall within 95% confidence limit and thus the model fits the data adequately. The index of significance of potency estimating values indicates that the value of the mean is within the limits at all probability levels (90, 95, 99) as it is less than 0.5.

In conclusion, it can be stated that *B. variegata* and *M. elengi* extracts may be used as potent plant molluscicide as their active components are more toxic than their synthetic counterparts. Both plants are found abundantly in this area, so that it is easily available, ecologically safe and culturally more acceptable among native live-stock keepers. Further studies on these plants are needed to verify, whether the extracts of both plants are toxic to other invertebrate (mollusks and aquatic insects) or vertebrate (small fishes) sharing the same habitat with vector *Lymnaea*. The outcome will certainly give an idea that both plants can be used in aquatic environment with negative ecological consequences. More studies on the mode of action of active molluscicidal components in snail body are also required to explore its full potential as molluscicide.

RESUMO

Caracterização da atividade moluscicida dos extratos das plantas *Bauhinia variegata* e *Mimusops elengi* contra o vetor da *Fasciola, Lymnaea acuminata*

A atividade moluscicida das folhas da *Bauhinia variegata* e da casca do *Mimusops elengi* foi testada contra o vetor caracol, *Limnaea acuminata.* A toxicidade de ambas as plantas é dependente do tempo e da concentração. Entre os extratos orgânicos, os extratos de etanol de ambas as plantas foi mais tóxico. A toxicidade do extrato etanólico da folha da *B. variegata* (96 h LC_{50} - 14,4 mg/L) foi mais pronunciada do que o extrato etanólico da casca do *M. elengi* (96h - LC_{50} - 15,0 mg/L). As frações purificadas em coluna durante 24 h LC_{50} do *B. variegata* e da casca do *M. elengi* foram 20,3 mg/L e 18,3 mg/L, respectivamente. A saponina e a quercentina foram caracterizadas e identificadas como os componentes ativos moluscicidas. A co-migração da saponina (Rf 0,48) e da quercentina (Rf 0,52) com a casca purificada por coluna do *M. elengi* e as folhas da *B. variegata* na cromatografia demonstraram o mesmo valor Rf isto é, 0,48 e 0,52 respectivamente. O presente estudo indica claramente a possibilidade de usar *M. elengi* e/ou *B. variegata* como moluscicidas potentes.

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