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Molluscicidal activity of *Morus nigra* against the freshwater snail *Lymnaea acuminata*

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ABSTRACT

The molluscicidal activity of *Morus nigra* fruit, bark and leaf powder against the snail *Lymnaea acuminata* was time and concentration dependent. Toxicity of fruit powder (96h LC_{50}: 166.92 mg/L) was more pronounced in comparison to bark powder (96h LC_{50}: 173.17 mg/L) and leaf powder (96h LC_{50}: 173.69 mg/L). Ethanolic extracts of *M. nigra* fruit, bark and leaf was more toxic than their other organic solvent extracts. The molluscicidal activity of ethanolic extract of *M. nigra* fruit powder (24h LC_{50}: 116.23 mg/L) was more effective than the ethanolic extract of bark powder (24h LC_{50}: 154.41 mg/L) and leaf powder (24h LC_{50}: 139.80 mg/L). The 96h LC_{50} of column-purified fraction of *M. nigra* fruit powder was, 10.03 mg/L whereas that of bark and leaf powder was 8.69 mg/L and 4.97 mg/L, respectively. Column and thin layer chromatography analysis demonstrates that the active molluscicidal component in *M. nigra* is quercetin (96h LC_{50}: 1.11 mg/L), apigenin (96h LC_{50}: 1.92 mg/L) and morusin (96h LC_{50}: 2.12 mg/L), respectively. Co-migration of quercetin (R_f 0.49), apigenin (R_f 0.51) and morusin (R_f 0.52) with column-purified fruit, bark and leaf of *M. nigra* on thin layer chromatography demonstrates same Rf value. The present study indicates that *M. nigra* may be used as potent source of molluscicides against the snail *Lymnaea acuminata*.

Key words: *Lymnaea acuminata*, *Morus nigra*, fasciolosis, plant molluscicide.

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INTRODUCTION

Fasciolosis is an important helminth disease caused by two trematodes *Fasciola hepatica* and *F. gigantica*. This disease belongs to the plant-borne trematode zoonoses. Fasciolosis is a zoonosis, i.e. a disease of animals that can be transmitted to humans. Cattle and human fasciolosis is a major public health problem in several areas of the world [1, 2]. In Europe, the Americas and Oceania only *F. hepatica* is a concern, but the distributions of both species overlap in many areas of Africa and Asia [3]. The definitive host range is very broad and includes many herbivorous mammals, including humans. Its life cycle includes freshwater snails (*Lymnaeidae*) as an intermediate host [4]. The worldwide losses in animal productivity due to fasciolosis were conservatively estimated at over US$ 3.2 billion per annum [5]. In addition, fasciolosis is now regarded as an emerging human disease: the World Health Organization (WHO) has estimated that 2.4 million people are infected with *Fasciola*, and a further 180 million are at risk of infection [6].

Studies carried out in recent years have shown human fasciolosis to be an important public health problem [7]. Human cases of fasciolosis have also been reported from India [8]. Human fasciolosis has been reported from countries in Europe, America, Asia, Africa, and Oceania. Humans are infected by ingestion of aquatic plants that contain the infected metacercariae [9]. Because *F. hepatica* cercariae also encyst on water surface, humans can be infected by drinking of fresh untreated water containing metacercariae [7].

The control of snail population is one of the major tools to reduce the incidence of fasciolosis in cattle as well as human being [1, 3, 10]. One of the most efficient methods for preventing the spread of fasciolosis is the use of molluscicides [1, 3]. The molluscicides of plant origin are gaining special importance in comparison to synthetic counterpart, because they are more effective, cheaper and safer to non-target organisms and culturally acceptable [1, 11]. Many plant products have been found to have a high molluscicidal potential [12, 13].

Morus nigra L. (*Moraceae*) belongs to the genus *Morus* and is found in Africa, South America and in Asia. *M. nigra* has been used in Unani medicine as antitussive, diuretic, expectorant and hypotensive. It has wide range of medicinal uses and can be used either as single drug or compound drugs to treat different ailments. The phenolic compounds of *M. nigra* have anti-oxidant and anti-bacterial activities. The bark of *M. nigra* has been used as antihelmintic and its extracts have antibacterial and fungicidal activity [14]. In the present study the molluscicidal activity of the fruit, bark and leaf of *Morus nigra* against the target snail *Lymnaea acuminata* has been evaluated to explore its full potential in control of fasciolosis.

MATERIALS AND METHODS

Morus nigra fruit, bark and leaf were collected from Gorakhpur (India) and were dried separately.

Preparation of fruit, bark and leaf powder

Dried fruit, bark and leaf were pulverized separately in the electric grinder and the crude powders obtained were then used separately for toxicity experiments.

Organic solvent extracts

Five gram of fruit, bark and leaf powder of *Morus nigra* were extracted separately with 100 mL of each solvent viz. ethanol, ether, carbon tetrachloride, acetone and chloroform at room temperature for 24h. Each preparation was filtered separately through sterilized Whatmann No.1 filter paper [15] and the filtered extracts were subsequently evaporated at 40°C under vacuum. The residues thus obtained were used for the determination of molluscicidal activity. Fruit crude powder of *M. nigra* yielded 320 mg of ethanol extract, 310 mg of ether extract, 290 mg of carbon tetrachloride extract, 380 mg of acetone extract, and 335 mg of chloroform extract. Bark crude powder of *M. nigra* yielded 300 mg of ethanol extract, 325 mg of ether extract, 340 mg of carbon tetrachloride extract, 360 mg of acetone extract and 295 mg of chloroform extract. Leaf crude powder of *M. nigra* yielded 305 mg of ethanol extract, 315 mg of ether extract, 345 mg of carbon tetrachloride extract, 400 mg of acetone extract and 350 mg of chloroform extract.

Column chromatography

The ethanolic extract of *M. nigra* fruit, bark and leaf powder was obtained by dissolving 1000 mg of each in 25 mL of ethanol separately. Further the ethanolic extract of *M. nigra* fruit, bark and leaf

powder was subjected to silica gel (60-120 mesh, Qualigens glass, Precious Electrochemindus Private Limited, Mumbai, India) chromatography through a 5×45 cm column. 10 milliliters fractions of 32 elutents (fruit powder), 35 elutents (bark powder), 37 elutents (leaf powder) were eluted with 95% ethanol for each column preparation. Ethanol was evaporated under vacuum and the remaining solids obtained from all the 10 mL elutents were used for the determination of molluscicidal activity.

Pure compounds

Quercetin (3,3,4,5,7-pentahydroxyflavone) was purchased from Sigma Chemical Co. USA. Apigenin (4´,5,7-trihydroxyflavone) was isolated by the method of Liu et al. [16] and morusin 2-(2,4-dihydroxyphenyl)-5-hydroxy-8,8-dimethyl-3-(3-methyl-2-butenyl)-4H, 8H-benzo[1,2-b:3,4-b´] dipyran-4-one was isolated by the method of Tati et al. [17].

Thin layer chromatography

Thin Layer Chromatography (TLC) was performed by the method of Jaiswal and Singh [18] to identify the active molluscicidal component present in the fruit, bark and leaf powders of M. nigra. TLC was done on 20×20 cm pre-coated silica gel (Precious Electrochemical Industry, Pvt. Ltd., Mumbai, India) using benzene/ethyl acetate (9:1, v:v) as the mobile phase. Spots of column-purified fractions of *M. nigra* fruit, bark and leaf along with their respective active components were applied on TLC plates with a micropipette. Further, the TLC plates were developed by I_2 vapor. Copies of chromatogram were made by tracing the plates immediately and retardation factors (R_f) were calculated.

Collection of snails

The adult freshwater snails, *L. acuminata* (2.25 ± 0.20 cm in length) were collected locally from different ponds of Gorakhpur. The collected snails were acclimatized for 72h in the laboratory condition. They were kept in a glass aquarium containing de-chlorinated tap water at 22-24°C. The pH of the water was 7.1-7.3 and dissolved oxygen, free carbon dioxide and bicarbonate alkalinity were 6.5-7.2, 5.2-6.3 and 102-105 mg/L, respectively.

Treatment protocol for concentration-response relationship

The toxicity experiments were performed by the method of Singh and Agarwal [19]. Ten experimental animals were kept in a glass aquarium containing 3L of de-chlorinated tap water. Snails were exposed continuously for 96h to different concentrations of plant products separately (Table 1). Six aquaria were set up for each concentration. The control animals were kept in the equal volume

Table.1. Concentration of different preparations of fruit, bark and leaf powder and active components of *Morus nigra* used in toxicity trial against *Lymnaea acuminata*.

Plant material used	Concentration (mg/L)
Morus nigra fruit powder	150, 200, 250, 300
Ethanol extract	30, 50, 70, 90
Ether extract	30, 50, 70, 90
Carbon tetrachloride extract	30, 50, 70, 90
Acetone extract	50, 70, 90, 110
Chloroform extract	30, 50, 70, 90
Column purified	7, 10, 15, 20
Quercetin	1, 3, 5, 7
Morus nigra bark powder	150, 200, 250, 300
Ethanol extract	30, 50, 70, 90
Ether extract	30, 50, 70, 90
Carbon tetrachloride extract	30, 50, 70, 90
Acetone extract	50, 70, 90, 110
Chloroform extract	30, 50, 70, 90
Column purified	9, 15, 25, 35
Apigenin	1, 3, 5, 7
Morus nigra leaf powder	100, 150, 200, 250
Ethanol extract	30, 50, 70, 90
Ether extract	30, 50, 70, 90
Carbon tetrachloride extract	30, 50, 70, 90
Acetone extract	50, 70, 90, 110
Chloroform extract	30, 50, 70, 90
Column purified	7, 10, 30, 50
Morusin	1, 3, 5, 7

of water under similar conditions without treatment. Mortality of snails was recorded at the interval of 24h each; upto 96h. The dead animals were removed immediately to avoid 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. The LC values, lower and upper confidence limits (LCL and UCL), slope values, t-ratio, g-values and heterogeneity factors were calculated by using POLO computer software of Robertson et al. [20].

The regression coefficient between exposure time and different values of LC_{50} was determined by the method of Sokal and Rohlf [21].

RESULTS

The toxicity of fruit, bark and leaf powder of *M. nigra* and their different organic solvent extracts was time and concentration dependent. The 24h LC_{50} of the fruit, bark and leaf powder were 353.21, 325.19 and 377.90 mg/L, respectively. At 96h, LC_{50} values were 166.92, 173.17 and 173.69 mg/L, respectively (Table 2, 3 and 4). Maximum toxicity among different organic solvent extracts of fruit,

Table 2. Toxicity of *Morus nigra* fruit powder, its different organic solvent extracts, column-purified fractions and active component quercetin against the snail *Lymnaea acuminata* at different exposure periods.

Exposure Period	Tested Materials	LC_{50} mg/l	Limits LCL-UCL	Slope Value	t-ratio	g-value	Heterogeneity
	Fruit Powder	353.21	302.52-505.35	4.05±.92	4.40	0.20	0.23
	Ethanol Extract	116.23	92.12-196.92	2.67±.60	4.47	0.19	0.24
	Ether Extract	168.12	115.27-559.99	2.38±.67	3.58	0.30	0.33
24h	CCl_4 Extract	151.38	106.05-457.94	2.15±.60	3.61	0.30	0.20
	Acetone Extract	148.14	117.17-292.33	2.94±.79	3.74	0.27	0.15
	Chloroform Extract	135.53	113.58-204.58	3.73±.85	4.40	0.20	0.26
	Column Purified	26.81	20.75-49.16	2.57±.59	4.35	0.20	0.21
	Quercetin	10.34	7.10-23.85	1.52±.34	4.46	0.19	0.26
	Fruit Powder	310.62	265.47-462.65	2.96±.79	3.77	0.27	0.12
	Ethanol Extract	101.89	81.26-169.69	2.25±.53	4.28	0.21	0.16
	Ether Extract	109.63	81.22-279.96	1.69±.50	3.39	0.34	0.13
48h	CCl_4 Extract	109.18	84.04-212.91	2.04±.52	3.92	0.25	0.22
	Acetone Extract	105.35	91.03-142.36	2.92±.69	4.23	0.22	0.13
	Chloroform Extract	109.06	93.02-155.100	2.77±.69	4.01	0.24	0.10
	Column Purified	23.18	17.59-50.46	1.85±.51	3.62	0.29	0.13
	Quercetin	6.73	4.56-16.03	1.05±.28	3.82	0.26	0.19
	Fruit Powder	222.92	198.50-252.46	3.46±.75	4.59	0.18	0.19
	Ethanol Extract	59.84	49.66-74.33	2.10±.48	4.43	0.20	0.14
	Ether Extract	75.70	62.96-104.85	2.11±.49	4.33	0.20	0.11
72h	CCl_4 Extract	86.62	64.71-244.93	1.35±.47	2.87	0.47	0.12
	Acetone Extract	74.55	62.15-87.64	2.53±.65	3.87	0.26	0.10
	Chloroform Extract	75.27	62.69-89.04	2.45±.65	3.80	0.27	0.11
	Column Purified	13.36	10.99-17.17	1.98±.49	4.08	0.23	0.11
	Quercetin	2.77	1.70-4.08	1.01±.26	3.88	0.26	0.17
	Fruit Powder	166.92	144.33-183.08	4.86±.82	5.89	0.11	0.29
	Ethanol Extract	36.95	27.74-43.75	2.54±.49	5.18	0.14	0.24
	Ether Extract	50.57	36.21-64.81	1.60±.46	3.45	0.32	0.09
96h	CCl_4 Extract	42.65	34.26-49.52	2.64±.49	5.43	0.13	0.19
	Acetone Extract	53.49	38.76-62.37	2.94±.68	4.32	0.20	0.24
	Chloroform Extract	50.23	36.94-58.46	3.32±.70	4.71	0.17	0.25
	Column Purified	10.03	8.11-11.70	2.47±.50	4.96	0.16	0.30
	Quercetin	1.11	0.49-1.65	1.25±.27	4.61	0.18	0.20

Mortality was determined at every 24h up to 96h. Each set of experiment was replicated six times.
Abbreviation: LCL= lower confidence limit; UCL= upper confidence limit. Significant negative regression ($p < 0.05$) was observed between exposure time and LC_{50} of treatments. T_s= testing significance of the regression coefficient of Morus nigra fruit powder (11.08^{+}), ethanol extract (7.81^{+}), ether extract (11.40^{+}), carbon tetrachloride extract (12.81^{++}), acetone extract (17.76^{+}), chloroform extract (18.00^{+}), column-purified (6.81^{+}), quercetin (4.25^{+}). (+) Linear regression between x and y. (++) Non-linear regression between log x and log y.

bark and leaf powder was observed in the ethanolic extract (Table 2, 3 and 4). The column-purified fraction of *M. nigra* was highly toxic. In fruit elutent Nos. 25-30, in bark 25-30 and in leaf elutent No. 30-37 were toxic against the snail *L. acuminata*. The 24h LC_{50} of column-purified fraction of *M. nigra* fruit, bark and leaf were 26.81, 43.30 and 65.61 mg/L and at 96h, LC_{50} was found to be 10.03, 8.69 and 4.97 mg/L, respectively (Table 2,3 and 4). The LC_{50} of quercetin, apigenin and morusin at 24h was 10.34, 12.57, 13.40 mg/L, respectively (Table 2, 3 and 4). Thin layer chromatography analysis demonstrated that the R_f values of quercetin (0.50), apigenin (0.53) and morusin (0.54) were equivalent to the R_f values of the column-purified fractions of *M. nigra* (fruit, bark and leaf).

The slope values were steep and separate estimation of LC based on each of the six replicates was found to be within 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 the probability levels i.e. 90, 95, 99. There was significant negative regression ($p < 0.05$) between exposure time and LC_{50} of the treatments (Table 2, 3 and 4).

Table 3. Toxicity of *Morus nigra* bark powder, its different organic solvent extracts, column-purified fractions and active component apigenin against the snail *Lymnaea acuminata* at different exposure periods.

Exposure Period	Tested Materials	LC_{50} mg/l	Limits LCL-UCL	Slope Value	t-ratio	g-value	Heterogeneity
	Bark Powder	325.19	279.23-466.51	3.39±.82	4.13	0.23	0.15
	Ethanol Extract	154.41	122.61-281.19	2.88±.72	4.03	0.24	0.21
	Ether Extract	157.91	110.28-480.58	2.31±.64	3.63	0.29	0.23
24h	CCl_4 Extract	162.47	106.37-831.89	1.76±.55	3.19	0.38	0.09
	Acetone Extract	166.98	122.03-589.50	2.34±.75	3.12	0.40	0.14
	Chloroform Extract	159.42	124.32-315.92	2.73±.70	3.88	0.26	0.16
	Column Purified	43.30	33.39-73.80	2.22±.46	4.85	0.16	0.25
	Apigenin	12.57	8.05-38.11	1.43±.35	4.15	0.22	0.22
	Bark Powder	268.15	236.72-339.78	3.13±.77	4.08	0.23	0.10
	Ethanol Extract	86.48	70.88-129.60	2.16±.50	4.30	0.21	0.16
	Ether Extract	146.84	96.53-917.50	1.53±.51	2.97	0.44	0.19
48h	CCl_4 Extract	130.90	105.36-237.99	2.31±.63	3.66	0.29	0.13
	Acetone Extract	115.73	96.74-182.30	2.62±.69	3.79	0.27	0.14
	Chloroform Extract	99.72	84.08-140.94	2.23±.60	3.69	0.28	0.11
	Column Purified	29.51	23.79-42.92	1.91±.40	4.81	0.17	0.15
	Apigenin	9.31	5.92-30.68	1.07±.29	3.73	0.28	0.16
	Bark Powder	224.37	200.86-253.19	3.59±.76	4.74	0.17	0.21
	Ethanol Extract	54.86	43.76-68.15	1.92±.47	4.09	0.23	0.09
	Ether Extract	80.61	62.89-152.16	1.56±.47	3.29	0.36	0.13
72h	CCl_4 Extract	80.13	68.02-95.32	2.51±.60	4.17	0.22	0.12
	Acetone Extract	78.28	67.72-91.15	2.83±.66	4.29	0.21	0.11
	Chloroform Extract	75.76	60.82-91.81	2.16±.60	3.63	0.29	0.11
	Column Purified	16.59	12.25-21.20	1.58±.38	4.22	0.22	0.13
	Apigenin	4.60	3.06-9.41	0.92±.26	3.47	0.32	0.11
	Bark Powder	173.17	141.38-194.12	3.57±.77	4.65	0.18	0.20
	Ethanol Extract	36.50	19.86-46.66	1.64±.47	3.50	0.31	0.11
	Ether Extract	59.26	45.17-82.89	1.54±.47	3.31	0.35	0.11
96h	CCl_4 Extract	64.64	52.32-73.99	2.83±.61	4.62	0.18	0.12
	Acetone Extract	62.91	48.92-72.47	2.69±.66	4.10	0.23	0.11
	Chloroform Extract	56.18	35.31-67.87	2.14±.60	3.54	0.31	0.11
	Column Purified	8.69	6.01-10.73	2.59±.46	5.68	0.12	0.38
	Apigenin	1.92	1.20-2.58	1.33±.27	4.98	0.16	0.28

Mortality was determined at every 24h up to 96h. Each set of experiment was replicated six times.

Abbreviation: LCL= lower confidence limit; UCL= upper confidence limit. Significant negative regression ($p < 0.05$) was observed between exposure time and LC_{50} of treatments. T_s= testing significance of the regression coefficient of Morus nigra bark powder (13.44^{+}), ethanol extract (10.22^{+}), ether extract (5.69^{+}), carbon tetrachloride extract (9.33^{+}), acetone extract (10.86^{++}), chloroform extract (15.86^{+}), column-purified (9.37^{+}), apigenin (7.20^{+}). (+) Linear regression between x and y. (++) Non-linear regression between log x and log y.

DISCUSSION

The results of the present study clearly indicate that the fruit, bark and leaf powder of *M. nigra* are the potent molluscicides. Their toxic effects are time and concentration dependent as evident from negative regression between exposure period and LC_{50} of different treatments. The time dependent toxic effect of *M. nigra* 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 duration or it might be possible that the active compound(s) could change into more toxic forms in the aquarium water or in the snail's body due to the action of various enzymes. Among the organic solvent extracts, the higher toxicity of ethanolic extracts of *M. nigra* fruit, bark and leaf powder indicates that the molluscicidal component present in fruit, bark and leaf are more soluble in ethanol than other organic solvents. It is evident from co-migration on thin layer chromatographic plates that the molluscicidal activity of *M. nigra* fruit, bark and leaf may be due to the presence of quercetin, apigenin and morusin.

Morus nigra L. (*Moraceae*) belongs to the genus

Table 4. Toxicity of *Morus nigra* leaf powder, its different organic solvent extracts, column-purified fractions and active component morusin against the snail *Lymnaea acuminata* at different exposure periods.

Exposure Period	Tested Materials	LC_{50} mg/l	Limits LCL-UCL	Slope Value	t-ratio	g-value	Heterogeneity
24h	Leaf Powder	377.90	310.86-650.11	3.39±.88	3.84	0.26	0.13
	Ethanol Extract	139.80	103.22-320.08	2.47±.63	3.93	0.25	0.25
	Ether Extract	140.29	101.55-357.31	2.21±.59	3.76	0.27	0.20
	CCl_4 Extract	143.49	102.50-393.90	2.15±.59	3.67	0.29	0.19
	Acetone Extract	154.74	118.18-383.16	2.57±.59	3.41	0.33	0.15
	Chloroform Extract	146.88	101.87-487.38	1.93±.56	3.47	0.32	0.22
	Column Purified	65.61	41.58-178.51	1.18±.26	4.46	0.19	0.21
	Morusin	13.40	8.33-46.16	1.39±.35	4.02	0.24	0.22
48h	Leaf Powder	310.42	266.35-452.61	3.05±.79	3.86	0.26	0.09
	Ethanol Extract	78.82	64.71-115.44	2.00±.49	4.12	0.23	0.11
	Ether Extract	106.56	80.80-232.17	1.82±.50	3.61	0.30	0.14
	CCl_4 Extract	103.89	79.58-214.13	1.85±.50	3.67	0.28	0.13
	Acetone Extract	134.08	106.04-288.89	2.37±.70	3.37	0.34	0.14
	Chloroform Extract	111.63	85.62-220.98	2.06±.53	3.92	0.25	0.15
	Column Purified	21.75	15.28-33.54	1.09±.24	4.50	0.19	0.22
	Morusin	9.31	5.92-30.68	1.07±.29	3.73	0.28	0.16
72h	Leaf Powder	231.87	202.44-276.11	2.88±.75	3.86	0.26	0.15
	Ethanol Extract	57.10	45.70-72.36	1.87±.47	3.98	0.24	0.11
	Ether Extract	71.85	58.92-101.76	1.90±.48	3.97	0.24	0.11
	CCl_4 Extract	67.54	54.98-94.06	1.82±.47	3.85	0.26	0.13
	Acetone Extract	78.59	64.45-97.81	2.19±.65	3.38	0.34	0.10
	Chloroform Extract	77.97	62.94-121.49	1.81±.48	3.78	0.27	0.14
	Column Purified	7.88	3.14-12.04	0.97±.25	3.94	0.25	0.15
	Morusin	3.79	2.67-5.81	1.11±.27	4.18	0.22	0.17
96h	Leaf Powder	173.69	153.50-188.95	5.10±.82	6.20	0.10	0.29
	Ethanol Extract	30.79	22.24-36.92	2.87±.53	5.44	0.13	0.43
	Ether Extract	35.73	19.63-45.59	1.68±.47	3.58	0.30	0.12
	CCl_4 Extract	45.73	35.19-54.62	2.12±.47	4.49	0.19	0.15
	Acetone Extract	55.60	35.10-66.51	2.29±.66	3.49	0.32	0.11
	Chloroform Extract	50.57	36.21-64.81	1.60±.46	3.45	0.32	0.09
	Column Purified	4.97	2.82-6.76	2.01±.37	5.46	0.13	0.40
	Morusin	2.12	1.38-2.84	1.32±.27	4.95	0.16	0.32

Mortality was determined at every 24h up to 96h. Each set of experiment was replicated six times.

Abbreviation: LCL= lower confidence limit; UCL= upper confidence limit. Significant negative regression ($p < 0.05$) was observed between exposure time and LC_{50} of treatments. T_s= testing significance of the regression coefficient of Morus nigra leaf powder (14.36^{+}), ethanol extract (8.31^{+}), ether extract (29.78^{+}), carbon tetrachloride extract (29.11^{+}), acetone extract (8.50^{+}), chloroform extract (14.30^{++}), column-purified (10.61^{+}), morusin (6.04^{+}). (+) Linear regression between x and y. (++) Non- linear regression between log x and log y.

Morus which is widely distributed in Asia, Europe, North and South America and Africa. Mulberry (genus *Morus*) is an economically important plant used for sericulture, as a feed for the domesticated silkworm, *Bombyx mori* [22] and has a long history of medicinal use in Chinese medicine as a herbal medicine called "Sang Bai-Pi" [23]. The fruits are one of the constituent of Unani medicine named "Tut-i-aswad" which is said to be against cancer [24]. The ripe fruit contains about 9% sugar, with malic and citric acid. The juice forms a grateful drink during convalescence after febrile diseases; it checks thirst and cools the blood. The extracts of *M. nigra* fruit were reported to have a protective action against peroxidative damage to biomembranes and biomolecules [25] while the roots methanolic extract showed mushroom tyrosinase inhibitory activity [26]. The bark is purgative and vermifuge. Root bark contains tannins, phlobaphenes, a sugar, a phytosterol (m.p.132°), ceryl alcohol, fatty acids and phosphoric acid. The bark of *M. nigra* was reputed to be used to expel tape worm. The decoction of the leaves possesses blood purifying properties, reduces fever and is diuretic [27]. Black mulberry genotypes have a higher bioactive content. A study was conducted to investigate the chemical constituents in the barks of *M. nigra*. In this study, nine compounds were isolated and identified as olcancolic acid, apigenin, cyclocommunol, morusin, cyclomorusin, kuwanon C, daucosterol, ursolic acid and 63-sitosterol [28].

Quercetin has been isolated from fruit of *M. nigra*. Quercetin targets cysteine string proteins (CSPα) and impairs synaptic transmission [29]. Apigenin has been isolated from bark of *M. nigra*. Apigenin is a natural product belonging to the flavones class that is the aglycone of the several naturally-occurring glycosides. It is a yellow crystalline solid. Apigenin may contribute to the chemopreventive action of vegetables and fruits [30]. It was recently shown that apigenin induces a process called autophagia (a kind of cellular dormancy) that may well explain its chemopreventive properties, but at the same time it induces resistance against chemotherapy [31]. Apigenin is a potent inhibitor of CYP2C9, [32] an enzyme responsible for the metabolism of many pharmaceutical drugs in the body. Apigenin has been shown to reverse the adverse effects of cyclosporine. Research has been conducted to study the effects of apigenin on reversal of cyclosporine-induced damage, and this was assessed by immunohistochemical estimation of expression of *bcl-2*, and estimation of apoptosis in histopathological sections [33]. Morusin has been isolated from leaf of *M. nigra*. Morusin, a flavonoid has high cytotoxicity against murine leukemia cell P-388, IC_{50} 3.1µg/mL [17].

A comparison of the molluscicidal activity of the active components present in *M. nigra* fruit, bark and leaf powder with synthetic molluscicides clearly demonstrates that these components are more potent against *L. acuminata*. The LC_{50} at 96h of quercetin (1.11), apigenin (1.92) and morusin (2.12) 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) [18 ,19]. LC_{50} at 96h of crude powder of *M. nigra* fruit (166.92), bark (173.17) and leaf (173.69) against *L. acuminata* are lower than the crude powder of *Canna indica* root (359.02 mg/L) [34], *Thuja orientalis* leaf powder (250.55 mg/L), *Thuja orientalis* fruit powder (255.12 mg/L) [35], *Zingiber officinale* rhizome (273.80 mg/L), *Allium cepa* bulb (253.27 mg/L) [36].

It is evident from the steep slope values that a small increase in the concentration of different treatments causes a marked mortality in snails. A t-ratio value greater than 1.96 indicates that the regression is significant. Values of heterogeneity factor less than 1.0 denote that in the replicate tests of random samples the concentration response 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 values of the mean are within the limits at all probability levels (90, 95, 99) as it is less than 0.5.

CONCLUSION

In conclusion it can be stated that molluscicidal activity of *M. nigra* is due to quercetin, apigenin and morusin. *M. nigra* can be used as potent molluscicide as it is easily available and ecologically and culturally more acceptable by livestock keepers. Toxicity of active components and crude extracts is more than the other plant extracts and synthetic molluscicides, respectively. Further studies will assess the mode of action of purified molluscicidal components of the plant in snail *L. acuminata*.

TRANSPARENCY DECLARATION

The authors declare no conflicts of interest.

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