ISSN: 0976-3376

Available Online at http://www.journalajst.com

ASIAN JOURNAL OF SCIENCE AND TECHNOLOGY

Asian Journal of Science and Technology
Vol. 08, Issue, 08, pp.5256-5261, August, 2017

RESEARCH ARTICLE

SCREENING OF MOLLUSCICIDAL POTENTIAL OF INDIGENOUS MEDICINAL PLANTS *TERMINALIA ARJUNA* AND *TAMARINDUS INDICA* AGAINST FASCIOLOSIS VECTOR: *LYMNAEA ACUMINATE*

Neelam Soni and *Vinay Kumar Singh

Malacology laboratory, Department of Zoology, DDU Gorakhpur University Gorakhpur 273009 (U.P.), India

ARTICLE INFO

Article History:
Received 12th May, 2017
Received in revised form
13th June, 2017
Accepted 20th July 2017
Published online 31st August, 2017

Key words:

Fasciolosis,
Plant molluscicide,
Terminalia arjuna,
Tamarindus indica,
Toxicity.

ABSTRACT

Snail control is one of the important tools to reduce the incident of fasciolosis. To attain this objective the present study undertaken to evaluate the molluscicidal potential of *Terminalia arjuna* bark and *Tamarindus indica* seed. The toxicity of both of plants were time and concentration-dependent. The toxicity of *T. indica* seed (12.00 mg/l) was more pronounced than that of *T. arjuna* bark (57.47 mg/l). Ethanol extract of both plants were more effective than other organic solvent extract. The 96h LC_{50} of column purified fraction of *T. arjuna* bark (3.12 mg/l) and *T. indica* seed (0.71 mg/l) was found to be highly effective against snail. Arjunolic acid and procynadine were isolated by column chromatography and characterized as active molluscicidal components in *T. arjuna* bark and *T. indica* seed respectively. Co-migration of pure arjunolic acid (R*f* 0.80) and procynadine (R*f* 0.77) with column purified extract of *T. arjuna* bark (R*f* 0.80) and *T. indica* seed (*Rf* 0.77) demonstrate the same R*f* value, confirm the presence of arjunolic acid and procynadine in their respective column purified fractions. The result of the present study clearly indicate that the *T. arjuna* and *T. indica* are the potential source of plant molluscicides.

INTRODUCTION

Endemic fasciolosis is a serious parasitic disease affecting domestic ruminants as well as human population (Haridy *et al.*, 2002, Ashrafi *et al.*, 2016). The disease is closely linked to summer rainfall which favors fluke development and provides an optimum habitat for intermediate host, the snail. The two species most commonly associated as the causative agent of fasciolosis are *Fasciola hepatica* and *Fasciola gigantica* (Gebrie *et al.*, 2015, Singh *et al.*, 2015).). Certain aquatic snail (Lymnaeidae and Planorbidae) are of great economic importance because they act as intermediate hosts of trematode (Kumar *et al.*, 2014, Soni and Singh 2015). The economic losses due to the fasciolosis throughout the world are more than $ 3.0 billion and these losses are associated with mortality, morbidity and reduced growth rate, contamination of bulky liver, and increased susceptibility to secondary infection and expense of control measure and treatment (Ayaz *et al.*, 2014, Gebrie *et al.*, 2015). Treatment of *Fasciola* in mammalian host required multiple doses of antihelminthic drugs, which pose frequent side effect (Abdul-Samie *et al.*, 2010). Therefore the best method to control trematode infection is to control the population of vector snail by the use of molluscicide either synthetic or plant origin (Agarwal and Singh, 1988, Upadhyay *et al.*, 2013, Quijano-Aviles *et al.*, 2016).). The high cost of synthetic molluscicides and their negative impact on environment as well as snail resistance to these compounds have given a line to study the plant molluscicides (Singh *et al.*, 1996, Singh *et al.*, 2014, Osman *et al.*, 2014). Thus the use of bio-molluscicides has received increased interest, primarily because it could be an appropriate and inexpensive technology for snail control (Adadesanmi *et al.*, 2007). The present study describes the molluscicidal activity of the plant of *Terminalia arjuna* (Family- Cobmretaceae) and *Tamaridus indica* (Family-Leguminaceae) against *Lymnaea acuminata*. Though both plants have great pharmacological significance in Indian Traditional medicine system (Amalraj and Gopi., 2017, Javed *et al.*, 2016, Shaikh *et al.*, 2017, Ribeiro *et al.*, 2015).

Corresponding author: **Vinay Kumar Singh**
Malacology laboratory, Department of Zoology, DDU Gorakhpur University Gorakhpur 273009 (U.P.), India.

MATERIALS AND METHODS

Animal collection

Adult *L. acuminata* lengths (2.6±0.30 cm) were collected locally from Ramghar Lake, located almost adjacent to D.D.U. Gorakhpur, University Campus India. Snails were acclimatized for 72 h in laboratory condition in dechlorinated tap water. Ten experimental animals were kept in glass aquaria containing 3 liter of dechlorinated tap water at 24±1^{0}C. The pH of the water was 7.1-7.3 and dissolved oxygen, free carbon

dioxide and bicarbonate alkalinity were 6.5-7.3 mg/l, 6.2-6.5 mg/l and 102-106 mg/l, respectively.

Plants

The bark of *T. arjuna* commonly called as arjuna and seed of *T. indica* locally known as imli were collected from the Botanical garden of University campus and identified by Dr. S.K. Singh, Retired professor (plant taxonomist), Department of Botany D.D.U. Gorakhpur University, Gorakhpur, India.

Preparation of crude extract

The freshly collected stem bark of *T. arjuna* and seed of *T. indica* were kept in incubator at 45^0 for 24h. The dried parts of both plants were pulverized separately in electric grinder to obtained crude powder. The crude powder then sieved with the help of fine meshed cotton cloth to obtained fine crude powder, thus obtained were used for the toxicity experiment.

Organic solvent extract

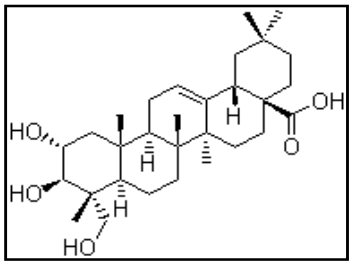
Five gram of crude powder of both of plants parts were extracted separately with 100 ml each chloroform (99%), ether (98%), Acetone (99%), carbon tetra chloride (95.5%) and alcohol (95%) at the room temperature for 24h. Each extracts were subsequently evaporated under vacuum at room temp. The residues thus obtained were used for determination of molluscicidal activity. The bark powder of *T. arjuna* yielded 43 mg of chloroform extract, 90 mg of ether extract, 730 mg of acetone extract, 70 mg of carbon tetra chloride extract and 1008 mg of alcoholic extract. *T. indica* seed yielded 78 mg of chloroform extract, 93 mg of ether extract, 220 mg of acetone extract, 65 mg of carbon tetra chloride extract and 1110 mg of alcoholic extract.

Column Chromatography

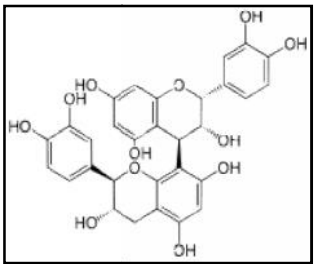
50 ml ethanol extract of seed of *T. indica* extract subjected separately to silica gel (60-120) mesh Qualigens glass, Precious Electro Chemindus Industry Privet Limited, Mumbai, India) Chromatography through 95×45 cm column. Five milliliters fractions of elutents were eluted with 95% ethanol for each column preparation. Ethanol was evaporated under vacuum and the remaining solids obtained from all 5 ml elutents were used for the determination of molluscicidal activity.

Pure Compounds

Arjunolic acid (2,3,23-Trihydroxyolean-12-en-28-oic acid) and Procynadine (cis,trans″-4,8″-Bi-(3,3′,4′,5,7-Pentahydroxyflavane) were purchased from sigma chemical Co. U.S.A.



Arjunolic acid



Procyanadine

Thin layer Chromatography

Thin layer chromatography (TLC) was performed by the method of Jaiswal and Singh (2008) to identify active molluscicidal component present in the *T. arjuna* bark and *T. indica* seed. TLC was done on 20×20 cm precoated silica gel (Precious Electro Chemindus Industry Private Limited, Mumbai, India) using benzene/ethyl acetate (9:1,V:V) as the mobile phase. Co-migration of column purified fraction of plant along with their respective active component arjunolic acid and procynadine was done for identification of molluscicidal component. TLC plate was developed by iodine.

Toxicity experiment

Treatment protocol for concentration response relationship

Toxicity experiments were performed by the method of Singh and Agarwal (1984). Ten experimental animals were kept in glass aquarium containing 3l of dechlorinated tap water. Snails were exposed continuously for 96h to different concentration of plant products separately. Six aquaria were set up for each concentration. The control animals were kept in the equal volume of water under similar condition without treatment. Mortality of snails was recorded at time interval of 24h up to 96h. The dead animals were removed immediately to avoid any contamination of aquarium water. The mortality of snail was established by concentration within the shell, no response to needle probe was taken as evidence of death. The LC values lower and upper confidence limits (LCL-UCL) slope value, t-ratio, g-value and heterogeneity factor were calculated by using Polo-Computer program software of Robertson et al (2007). The regression coefficient between exposure time and different value of LC_{50} was determined by the method of Sokal and Rohlf (1995).

RESULTS

The toxicity of crude bark powder of *T. arjuna* and seed powder of *T. indica* and their organic solvent extracts against *L. acuminata* were time and concentration dependent. There was a significant negative regression in between the exposure period and LC_{50} all treatments ($P<0.05$).

Table 1. Concentration used for toxicity determination of different of preparation of *T. arjuna* bark and *T. indica* seed and their active component against *L. acuminata*.

Material used	Test concentration (mg/l)
T. arjuna bark powder	50, 70, 90, 110,
Ethanol extract	7, 9, 11, 13
Ether extract	9, 11, 13, 15
Aceton extract	9, 11, 13, 15
Chloroform extract	9, 11, 13, 15
Carbon tetra chloride extract	9, 11, 13, 15
Column purified	3, 5, 7, 9,
Arjunolic acid	1, 3, 5, 7
T. indica seed powder	10, 15, 20, 25
Ethanol extract	0.9, 1.5, 3.0, 5.0
Ether extract	1, 3, 5, 7
Aceton extract	1, 3, 5, 7
Chloroform extract	1, 3, 5, 7
Carbon tetra chloride extract	1, 3, 5, 7
Column purified	0.7, 0.9, 1.1, 1.3
Procynadine	0.3, 0.5, 0.7, 0.9

Table 2. Toxicity of *Terminalia arjuna* bark powder, different organic extract, column purified fraction and its active component against *Lymnaea acuminata* at different exposure period

Exposure Period	Treatment	LC_{50} (mg/l)	LCL	UCL	Slope value	t-ratio	g- value	Heterogeneity
24h	*T. arjuna* bark powder	116.25	101.48	151.76	3.79±0.77	4.89	0.16	0.20
	Ethanol extract	13.18	11.92	15.92	4.91±0.97	5.04	0.15	0.17
	Acetone extract	14.95	13.77	17.42	5.80±1.15	5.04	0.15	0.24
	Ether extract	15.35	14.09	18.07	5.98±1.18	5.04	0.15	0.74
	Chloroform extract	15.45	14.23	18.00	6.44±1.23	5.22	0.14	0.27
	Carbon tetra chloride extract	14.64	13.66	16.44	6.75±1.19	5.65	0.12	0.66
	Column purified	8.15	6.99	9.85	3.15±0.54	5.82	0.13	0.16
	Arjunolic acid	8.00	6.05	14.40	1.66±0.33	4.94	0.19	0.15
48h	*T. arjuna* bark powder	89.57	80.28	104.53	3.49±0.68	5.08	0.14	0.22
	Ethanol extract	11.05	10.14	12.47	4.50±0.85	5.14	0.14	0.27
	Acetone extract	12.90	12.06	14.13	5.68±1.06	5.35	0.13	0.22
	Ether extract	13.72	12.76	15.42	5.54±1.88	5.13	0.14	0.17
	Chloroform extract	13.58	12.67	15.00	5.79±1.08	5.33	0.13	0.27
	Carbon tetra chloride extract	13.07	12.18	14.44	5.46±1.05	5.15	0.14	0.27
	Column purified	7.03	6.08	8.63	2.67±0.50	5.30	0.13	0.17
	Arjunolic acid	6.17	4.13	15.06	1.98±0.27	3.62	0.29	0.20
72h	*T. arjuna* bark powder	70.88	61.31	79.70	3.25±0.66	4.88	0.16	0.21
	Ethanol extract	8.96	7.94	9.83	4.09±0.84	4.82	0.16	0.29
	Acetone extract	11.19	10.43	11.89	6.38±1.06	5.99	0.10	0.36
	Ether extract	11.50	10.63	13.36	5.46±1.03	5.25	0.13	0.26
	Chloroform extract	11.58	10.84	12.33	6.23±1.06	5.93	0.10	0.53
	Carbon tetra chloride extract	11.42	10.63	12.19	6.02±1.05	5.72	0.11	0.36
	Column purified	4.49	3.62	5.24	2.49±0.48	5.19	0.14	0.19
	Arjunolic acid	2.20	1.39	3.00	1.29±0.26	4.65	0.17	0.14
96h	*T. arjuna* bark powder	57.47	48.94	63.73	4.26±0.72	5.91	0.11	0.46
	Ethanol extract	7.34	6.20	8.08	4.92±0.92	5.31	0.13	0.65
	Acetone extract	10.08	9.29	10.68	7.39±1.45	6.64	0.09	0.58
	Ether extract	10.11	9.20	10.77	6.51±1.10	5.87	0.11	0.73
	Chloroform extract	10.30	9.48	10.93	6.83±1.11	6.13	0.10	0.75
	Carbon tetra chloride extract	10.05	9.23	10.66	7.19±1.39	6.31	0.09	0.59
	Column purified	3.12	2.39	3.18	2.92±0.52	5.61	0.12	0.35
	Arjunolic acid	1.30	0.79	1.75	1.16±0.28	5.74	0.11	0.40

Mortality was determined at every 24h up to 96h. Each set of experiment was replicated six times. Concentrations given are the final concentration (w/v) in aquarium water. UCL= upper confidence limits. Significant negative regression ($P<0.05$) was observed between exposure time and LC_{50} of treatments.
Ts, testing significance of the regression coefficient - *T. arjuna* bark powder , 9.25^{+}; Ethanol extract ,22.75^{+}; acetone , 10.89^{+}; ether extract , 16.71^{+}; chloroform , 15.72^{+}; Carbon tetra chloride , 37.98^{+}; column purified , 9.08^{+}; arjunolic acid , 6.06^{+}.
+, linear regression between x and y.
++, non-linear regression between log x and log y.
Abbreviation: *T. arjuna* bark powder = *Terminalia arjuna* bark powder LCL= lower confidence limit;

The LC_{50} of crude bark powder of *T. arjuna* and crude seed powder of *T. indica* at 24h was 116.25mg/l, 26.17 mg/l and at 96h was 57.47, mg/l 12.00 mg/l respectively (Table 2, 3). Among the organic extract the ethanol extract of all these two plants were more toxic (Table 2, 3). The ethanol extract of *T. indica* (1.18 mg/l) found to be more toxic the ethanol extract of *T. arjuna*. The maximum molluscicidal activities of both plants were observed in between15^{th} to 25^{th} of 5 ml fraction eluted from silica gel column. The column purified fractions of these plants were highly toxic. The 24h LC_{50} of column purified fraction of *T. arjuna* bark and *T. indica* seed were 8.15mg/l and 1.74 mg/l respectively. The 96h LC_{50} of column purified fraction of *T. indica* seed (0.71 mg/l) was higher than *T. arjuna* bark (3.12 mg/l). Thin layer chromatography (TLC) analysis demonstrate that *Rf* value of arjunolic acid (0.80) and procynadine (0.77) was equivalent to the *Rf* value of column purified fraction of *T. arjuna* bark (0.80) and *T. indica* seed (0.77), indicate the presence of arjunolic acid procynadine in *T. arjuna* bark and *T. indica* seed respectively and 96h LC_{50} value of arjunolic acid and procynadine was 1.30 mg/l and 0.31 mg/l respectively (Table 2, 3). In the control group of snail no mortality within the 96h after the exposure period was recorded. The slope values were steep and separated estimation of LC based on each replicates, were 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 0.05 at all the probability levels i.e. 90, 95, 99. There was a significant negative regression ($p<0.05$) between exposure time and LC_{50} values.

DISCUSSION

The result of the present study clearly indicate that the plant of *T. arjuna* and *T. indica* are potent mollusccicides. It has been shown that toxicity of crude or purified plant product is potent molluscicidal if, the LC_{50} is less than 100 mg/l (Hostettmann and Lea, 1987). In the present Study 96h LC_{50} of both plants are less than 100 mg/l. The higher toxicity of ethanol extract of *T. arjuna* bark powder and *T. indica* seed powder as compared to other organic solvent extract indicate that the molluscicidal component in the bark and seed are more soluble in ethanol extract. The phytochemical investigation has reported that the bark of *T. arjuna* has active component arjunolic acid (Verma *et al.*, 2012). Experimental study of *T. arjuna* bark revealed that its bark shows significant antihelminthic activity (Yadav *et al.*, 2013), antibacterial activity (Shinde *et al.*, 2009, Jethinlalkhosh and Antony, 2013)) and analgesic activity (Biswas *et al.*, 2011). The seed of *T. indica* found to have great pharmacological significance as it shows anti-inflammatory and analgesic activity (Nakchat *et al.*, 2014) , (Suralkar *et al.*, 2012) antioxidant activity (Sandesh *et al.*, 2014). Kalra et al (2011) noted peptic ulcer protective effect due to presence of

Table 3. Toxicity of *Tamarindus indica* seed powder and its different organic extract, column purified fraction and its active compound (Procynadine) against the snail *Lymnaea acuminata*

Exposure period	Treatment	LC_{50} (mg/l)	LCL	UCL	Slope value	t-ratio	g- value	Heterogeneity
24h	*T. indica* seed powder	26.17	22.50	34.94	3.31±0.6	4.96	0.15	0.21
	Ethanol extract	5.22	3.96	8.58	1.85±0.34	5.38	0.13	0.20
	Acetone extract	10.43	8.60	15.38	2.90±0.54	4.88	0.16	0.22
	Ether extract	10.64	7.10	27.26	1.40±o.32	4.29	0.20	0.23
	Chloroform extract	10.03	8.27	14.80	2.71±0.56	4.80	0.16	0.20
	Carbon tetra chloride extract	10.52	8.55	16.30	2.65±0.56	4.65	0.17	0.16
	Column purified	1.74	1.45	2.70	2.99±0.71	4.18	0.22	0.15
	Procynadine	0.95	0.78	1.40	2.52±0.53	4.76	0.17	0.15
48h	*T. indica* seed powder	19.67	17.30	23.54	2.76±0.59	5.02	0.15	0.23
	Ethanol extract	3.50	2.71	5.29	1.55±0.30	5.03	0.17	0.15
	Acetone extract	7.23	6.29	8.83	1.83±0.51	5.54	0.12	0.22
	Ether extract	6.21	4.38	12.29	1.15±0.27	4.15	0.22	0.26
	Chloroform extract	7.65	6.45	10.22	2.32±0.49	4.67	0.17	0.18
	Carbon tetra chloride extract	8.13	6.72	11.72	2.14±0.49	4.13	0.20	0.18
	Column purified	1.32	1.84	1.57	3.59±0.68	5.27	0.13	0.19
	Procynadine	0.63	0.55	0.75	2.74±0.44	5.53	0.12	0.14
72h	*T. indica* seed powder	15.09	12.29	17.30	2.78±0.5	4.87	0.16	0.22
	Ethanol extract	1.96	1.44	2.61	1.39±0.29	4.69	0.17	0.21
	Acetone extract	5.25	4.51	6.03	2.83±0.48	5.97	0.11	0.36
	Ether extract	3.22	2.24	4.60	1.16±0.26	4.39	0.19	0.37
	Chloroform extract	5.68	4.77	6.18	2.31±0.47	4.83	0.16	0.25
	Carbon tetra chloride extract	5.62	4.69	6.76	2.25±0.47	4.72	0.17	0.21
	Column purified	0.83	0.68	0.95	3.12±0.65	4.76	0.16	0.18
	Procynadine	0.44	0.34	0.52	2.30±0.47	0.82	0.16	0.22
96h	*T. indica* seed powder	12.00	0.82	13.43	3.89±0.62	6.22	0.09	0.57
	Ethanol extract	1.18	2.95	1.50	1.79±0.32	5.54	0.12	0.48
	Acetone extract	3.73	4.33	4.33	2.91±0.50	5.77	0.11	0.49
	Ether extract	1.69	2.24	2.24	1.49±0.27	5.48	0.12	0.72
	Chloroform extract	4.15	4.77	4.77	2.85±0.49	5.74	0.11	0.63
	Carbon tetra chloride extract	4.03	4.67	4.67	2.75±0.49	5.57	0.12	0.56
	Column purified	0.71	0.57	0.18	3.98±0.72	5.53	0.12	0.41
	Procynadine	0.31	0.23	0.38	2.85±0.52	5.49	0.12	0.42

Mortality was determined at every 24h up to 96h. Each set of experiment was replicated six times. Abbreviation: *T. indica* bark powder = *Tamarindus indica* bark powder; LCL= lower confidence limit; UCL = upper confidence limits. Significant negative ($P<0.05$) was observed between exposure time and LC_{50} of treatments.

Ts, testing significance of regression coefficient - *T. indica* seed powder , 8.26^{++}; Ethanol extract , 4.89^{++}; acetone extract , 7.42^{++}; ether extract , 3.78^{++}; chloroform , 46.76^{++}; Carbon tetra chloride , 5.88^{++}; column purified , 16.25^{++}; Procynadine , 7.37^{++}.

+, linear regression between x and y;

++, non-linear regression between log x and log y.

its polyphenolic compounds mainly procynadine epicatechine and polymeric tannin. Their toxicities are time and concentration dependent, as evident from the negative regression between exposure period and LC_{50} value of the different treatment. The time dependent toxic effect of tested plants product may be due to uptake of active component by snail, which progressively increased in the body with an increased in exposure duration. It is also possible that the active compounds could change in to more toxic form in the aquarium water or in the snail body due to the action of various enzymes. In the toxicity study it was found that 96h LC_{50} of *T. arjuna* bark (57.47 mg/l) was higher than the *T. indica* seed (12.00 mg/l), indicate that the seed of *T. indica* are potentially more toxic molluscicides than *T. arjuna* bark. Co-migration of column extract of both plants and active component arjunolic acid and procynadine on TLC plate was demonstrated, it indicate the same *Rf* value. It clears the presence of active component arjunolic acid procynadine in bark of *T. arjuna* and seed of *T. indica* respectively. A compression of molluscicidal activity of column purified fraction of *T. arjuna* bark and *T. indica* seed with synthetic molluscicides clearly demonstrated that the former are more potent. The 96h LC_{50} of column purified extract of *T. arjuna* bark (3.12 mg/l) and *T. indica* seed (0.71 mg/l) lower than those of synthetic molluscicides *Carbaryl* (14.4 mg/l), *phorate* (15.5 mg/l), *formothion* (8.5 mg/l)P (Singh and Agarwal., 1983).

The 96h LC_{50} of crude powder of *T. arjuna* bark (57.47 mg/l) and *T. indica* seed (12.00 mg/l) against *L. acuminata* was lower than the plant products has been reported earlier in our laboratory. The crude powder of *Cinnamonum tamala* leaf powder (830.90 mg/l) (Srivastava and Singh, 2005) *Sapindus mukorossi* fruit (119.57 mg/l), *Terminalia chebula* fruit (93.59 mg/l) (Upadhyay and Singh, 2011), *Mimusops elengi* bark (108.15mg/l), *Bauhinia variegata* leaf (238.17 mg/l) (Singh *et al.,* 2015), *Moringa oleifera* leaf (22.52 mg/l) and *Momordica charantia* fruit (318.29 mg/l) (Upadhyay *et al.,* 2013). It is evident from steep slope value that small increase in concentration of different treatment caused marked mortality in snails. A t-ratio value is greater than 1.96 indicate that regression is significant. The value of heterogeneity factor is less than 1.0 denotes that in the replicate tests of random sample the concentration response line would fall within 95% confidence limits and thus model fits the data adequately. The index of significance of potency estimation value indicate that the value of mean are within the limits of all probability levels (90,95,99) as it is less than 0.5.(Robertson *et al.*, 2007).

Conclusion

In the present study it can be concluded that the plants of *T. arjuna* and *T. indica* are the potent molluscicides. Both plants are native therefore easily available, economical, ecologically sound, and culturally more acceptable. For the proper

utilization of these plants products as molluscicides further extensive study are required to explore the mode of action of these components inside the snail body.

REFERENCES

Abdul-Samie, R. E., Soliman E. O., El-Nemr, H., and Masou, A. 2010. Study of I11b, I15 and Ige before and Mirazid therapy in children with intestinal schistosomiasis and fasciolosis, *New York Science Journal*, 3, 116.

Adadesanmi, A.J., 2007. Tetrapleura Tetraptera: Molluscicidal activity and Chemical constituent, African *Journal of Traditional Complementry and Alternative Medicine*, 4, 23.

Agarwal, R. A., and Singh, D. K. 1988. Harmful gastropods and their control. Acta Hydrochimica et Hydrobiologica 16, 113.

Amalraj, A. and Gopi, S. 2017. Medicinal properties of *Terminalia arjuna* (Roxb) Wight & Arn. *Journal of Traditional and Complimentary Medicine*, 7, 65.

Ashrafi, K., Massoud, J., Holakouei, K., Mahmoodi, M., Joafshani, M. A., Valero, M. A., Fuentes M. V., Khoubbane, M., Artigas, P., Bargues M. D. and Mas-Coma S. 2016. Evidence suggesting that *Fasciola gigantica* may be the most prevalent causal agent of Fasciolosis in northern Iran. *Iranian Journal of Public Health*, 33, 31.

Ayaz, S., Ullah, R., Naser, M., Abdel-Salam., Shams S., and Niaz S. 2014. *Fasciola hepatica* in some buffaloes and cattle by PCR and microscopy. *The Scientific World Journal*, 1-5. Article ID 462084.

Gebrie, Y., Gebreyohanes, M., and Tesfaye. 2015. Prevalence of bovine fasciolosis in and around Bohir Dar North West Ethiopia. *Journal of Parasitology and Vector Biology*, 7, 74.

Haridy, F. M., Morsy, T. A, Gawish, N. I., Antonios, T. N., and Abdel-Gawad, A. G. 2002. The potential reservoir role of donkey and horse in zoonotic fasciolosis in Gharbia Governortate, *Egyptian Journal of Egyptian Society Parasitology*, 32, 561.

Hostettmann, K., and Lea, P. J. 1987, Biologically active natural products. Oxford Sciences Publisher. 65.

Jaiswal, P., and Singh, D. K. 2008. Molluscicidal activity of *Carica papaya* and *Areca catechu* against the fresh water snail *Lymnaea acuminata*. *Veterinary Parasitology*, 152, 264.

Javed, T., Riaz, S., Uzair, M., Mustafa, G., Mohyuddin, A., and Ahamad B. C. 2016. Biological activity of *Terminalia arjuna* on human pathogenic microorganisms. *Pakistan Journal of Pharmaceutical Research*, 2(1), 23.

Jethinlalkhosh, J. P., and Antony A. (2013). Antibacterial and cytotoxic activity of aqueous and methanolic extract of *Terminalia arjuna*. *International Journal of Research in Pharmaceutical Sciences*, 4(1), 36.

Kalra, P., Sharma, S., Suman, and Kumar, S. 2011. Antiulcer effect of the methanolic extract of *Tamarindus indica* seed in different experiment models. *Pharmaceutical Bioallied Science*, 3, 236.

Kapoor, D., Vijayvergiya, R., and Dhawan, V., 2014 *Terminalia arjuna* in coronary artery disease. Ethnopharmacology, Pre-clinical and Safely Evolution, 155, 1029.

Kumar, P., and Singh, D. K. 2014. *In vitro* anthelmintic activity of *Allium sativum, Ferula asafetida, Syzygium aromaticum* and their active component against *Fasciola gigantica*. *Journal of Biology and Earth Science*, 4, B57.

Nakchat, O., Meksuriyen, D., and Ponqsamart, S. 2014. Antioxidant and antilipid peroxidation activities of *Tamarindus indica* seed coat in human fibroblast cell. *Indian Journal of Experimental Biology*, 52, 25.

Osman, G. Y., Mohamed, A. H., Sherin, S. K., and El-Nabi, S. E. H., 2014. Molluscicidal activity of Mirazid on *Biomphalaria alexandrina* snail; Biological and Molecular studies, *International Journal of Advance Research*, 2, 977.

Quijano-Aviles, M. F., Lara, G., Riera-Ruiz, C., Barragan-Lucas, A. D., Miranda M., and Manzano P. 2016. Evaluation of plant molluscicides against *Pomacea canaliculata*. *Emirates Journal of Food and Agriculture*, 28(3), 224.

Razali, N., Mat-junit, S., Abdul-Muthalib, A. F., Subramaniam, S., and Abdul-Aziz, A. 2012. Effect of various solvent on the extraction of antioxidant phenolics form the leaves, seeds and veins and skin of *Tamarindus indica* L. *Food Chemistry*, 131, 441.

Riberio, J. A., Serquiz, A. C., Silva, P. F., Barbosa, P. B., Sampaio, T. B., Araujo junior, R. F., Oliveira, A. S., Machado, R. J., Maciel, B. L., Uchoa, A. F., Santos, E.A., and Morais A.H. 2015. Trypsin inhibitor from *Tamarindus indica* seeds Linn. reduced weight gain and food consumption and increased plasmatic cholecystokinin levels. Clinics. (Sao Paulo). 70(2), 136.

Robertson, J. L., Russell, R. M., Preisler, H. K., Savin, N. E. 2007. Bioassay with arthropods: POLO computer Programme for analysis of Bioassay data, 2nd ed. Boca Raton: CRC Press;

Sandesh, P., Velu, V., and Singh, R. P. 2014. Antioxidant activities of tamarind (*Tamarindus indica*) seed coat extract using *in vitro* and *in vivo* models. *Journal of Food Science and Technology*, 51, 1965.

Shinde, S. L., Junne, S. B., Wadje, S. S., and Baig, M. M. V. 2009. The diversity of antibacterial compounds of *Terminalia* species (Combretace). *Pakistan Journal of Biological Science*, 12, 1483.

Singh, A., Singh, D. K., Mishra, T. N., and Agarwal, R. A. 1996. Molluscicides of plant origin. *Biological Agriculture and Horticulture*, 13, 205.

Singh, D. K., and Agarwal, R. A. 1983. *In vivo* and *in vitro* studies on synergism with anticholinesterase pesticides in the snail *Lymnaea acuminata*. *Archive of Environmental Contamination and Toxicology*, 12, 483.

Singh, D. K., and Agarwal, R. A. 1984. Correlation of the anticholinesterase and molluscicidal activity of the latex of *Euphorbia royleana* Bioss on *Lymnaea acuminata*. *Journal of Natural Product Research*, 47,702.

Singh, K. L., Singh D. K., and Singh, V, K. 2014, Binary combination of *Mimusops elengi* and *Bauhinia variegate* with other plant molluscicides against *Indoplanorbis exustus*. *International Journal of Traditional and Natural Medicine*, 4, 6.

Singh, K. L., Singh, D. K., and Singh, V. K. 2012. Characterization of molluscicidal activity of *Bauhinia variegata* and *Mimusops elengi* plant extracts against *Fasciola* vector *Lymnaea acuminata*. Revista de Instituto de Medicina Tropica de Sao Paulo. 54,135.

Sokal, R. R., and Rohlf, F. J. 1995. Introduction to Biostatistics, San Francisco: W.H. Freeman. 225.

Soni, N., and Singh, V. K. 2015. Molluscicidal activity of *Tamarindus indica* and *Terminalia arjuna* against *Indoplanorbis exustus*: A causative agent of Trematodiasis. Scientia Agriculturae. 2, 163.

Srivastava, P., and Singh, D. K. 2005. Control of Harmful snail: Tejpat (*Cinnamomum tamala*) a potential molluscicidal. *Journal of Applied Bioscience*, 31, 128.

Suralkar, A. A., Rodge, K. N., Kamble, R. D., and Maske, K. S. 2012. Evalution of anti-inflammatory and analgesic activities of *Tamarindus indica* seeds. *International Journal of Pharmaceutical Drug and Research*, 4(3), 213.

Upadhyay, A., and Singh, D. K. 2011. Molluscicidal activity of *Sapindus mukorossi* and *Terminalia chebula* against the freshwater snail *Lymnaea acuminata*. Chemosphere. 83, 468.

Upadhyay, A., Singh, V. K., and Singh D. K. 2013. Characterization of molluscicidal component of *Moringa oleifera* leaf and *Momardica charantia* fruits and their modes of action in snail *Lymnaea acuminata. Revista de Instituto de Medicina Tropica de Sao Paulo*, 55, 251.

Verma, N., and Vinayak, M. 2009. Effect of *Terminalia arjuna* on antioxidant defense system in cancer. *Molecular Biology Reports*, 36, 159.

Yadav, D. B., Shinde, A. M., Gupta, R. K. and Manjunatha, H. 2013. Antioxidant and anthelmintic activity of *Terminalia arjuna* Roxb. stem bark extract. *Asian Journal of Pharmaceutical and Clinical Research*, 6(4), 33.

****