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MOLLUSCICIDAL EFFECT OF BINARY COMBINATIONS [1:1] OF AQUEOUS STEM BARK EXTRACT WITH ACTIVE CONSTITUENTS OF *EUPHORBIA TIRUCALLI* ON FRESHWATER SNAIL *INDOPLANORBIS EXUSTUS*

Reshmina Firoz Khan* and Ram P. Yadav

Natural Product Laboratory, Department of Zoology, D.D.U Gorakhpur University Gorakhpur, Gorakhpur (U.P) India.

***Corresponding Author: Reshmina Firoz Khan**
Natural Product Laboratory, Department of Zoology, D.D.U Gorakhpur University Gorakhpur, Gorakhpur (U.P) India.

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ABSTRACT
The binary combinations of the aqueous extract of stem bark and the active moieties of the plant *Euphorbia tirucalli* were tested against freshwater vector snail *Indoplanorbis exustus* for their molluscicidal activity. Accordingly, binary (1:1) combinations of the gallic acid and quercetin with aqueous stem bark extract of *Euphorbia tirucalli*, were studied for their toxic activity on the vector snail. The result was observed that molluscicidal activity of *Euphorbia tirucalli* aqueous stem bark with the other two active constituents against freshwater vector snail *Indoplanorbis exustus* was both time as well as dose dependent. A significant negative correlation was observed between the LC_{50} values and exposure periods, thus the LC_{50} values in binary (1:1) combinations of the gallic acid and quercetin with aqueous stem bark extract of *Euphorbia tirucalli* decreased from 6.28 mg/L (24h) to 3.79 mg/L (96h) and from 4.90 mg/L (24h) to 1.71 mg/L (96h) respectively, against the vector snail *Indoplanorbis exustus.* The toxicity test of binary combinations showed that the combination of active compound quercetin with stem bark of *Euphorbia tirucalli* was more toxic than the binary combination of active compound gallic acid with the stem bark of *Euphorbia tirucalli.* Thus, it can be concluded that the above binary combinations can be used as more effective and potent molluscicides.

KEYWORDS: Molluscicides, *Euphorbia tirucalli*, quercetin, gallic acid, *Indoplankrbis exustus.*

INTRODUCTION
As per their agreement, an emerging zoonosis is defined as one that has not been previously identified, has recently evolved, or has occurred previously but exhibits an increase in incidence or expansion in geographical, host, or vector range. This definition was reached by the Food and Agriculture Organisation (FAO), the World Organisation for Animal Health (OIE), and the World Health Organisation (WHO). The World Health Organisation defines zoonotic diseases as infectious illnesses that can naturally spread from vertebrate animals to humans. Animals are therefore crucial to the persistence of zoonotic illnesses in the natural world. Nearly everyone has some kind of interaction with animals. Zoonotic illnesses account for more than 60% of human illnesses. It includes a wide variety of bacteria, viruses, fungi, protozoa, parasites, and other pathogens.

Large liver flukes, called *Fasciola hepatica* or the sheep liver fluke, are trematodes that are mainly found in domestic and wild ruminants, which are their primary definitive hosts. However, they can also cause fascioliasis in pastoral animals. *Lymnaea acuminata* and *Indoplanorbis exustus* utilise *Fasciola hepatica* and *Fasciola gigantica* as intermediate hosts; these parasites are especially harmful to India's domestic animals. Fasciola hepatica is the common cause of fascioliasis in sheep, cattle, goats, and other herbivorous animals worldwide.[1,2] Little ponds, pools, rivers, and streams are home to the snail species *Indoplanorbis exustus*. In addition, semi-permanent ponds formed in flooded areas of fields are home to snails, which burrow in the mud to survive the dry season. While immature snails have a relatively low tolerance to desiccation, adult snails have a great tolerance.

It is undeniable that managing veterinary infections in addition to human illnesses is now imperative. Destruction of the carrier snails and the removal of a crucial link in the fluke's life cycle represent more effective approaches to combat fascioliasis. The most significant way to eradicate aquatic snails is to apply molluscicide.[3] The study of plant molluscicides has received a new lease on life due to the exorbitant expense of imported synthetic compounds and growing concerns about the compounds' potential to develop

resistance in snails and their toxicity in non-target organisms.[4,5]

As already it has been reported that the molluscicidal activity of aqueous extracts of *Phyllanthus niruri* and *Euphorbia tirucalli* is found on fresh water snails *Lymnaea* (Radix) *acuminata* and *Indoplanorbis exustus* (as target organisms) and on *Channa punctatus* fish (as non target organism), sharing the same habitat,[16] the intention of this work is to evaluate the full potential of molluscicidal activity of active compound quercetin and galic acid as in binary combinations with aqueous stem bark extract of *Euphorbia tirucalli* plant, in ratio (1:1).

MATERIALS AND METHODS

2.1 Test Plants

The plants under investigation *Euphorbia tirucalli* was collected easily, during the rainy season, from the Botanical Garden of Deen Dayal Upadhyay Gorakhpur University, Gorakhpur *Euphorbia tirucalli* (commonly called milk bush or pencil tree) belongs to the family Euphorbiaceae, the milky latex of which is extremely irritating to the skin and is toxic and therefore should be carefully handled.

Fig. 1: *Euphorbia tirucalli* plant.

2.2 Preparation of Aqueous Extracts of Stem Bark

Fresh stem bark from *Euphorbia tirucalli* were minced with distilled water, homogenized for 5 minutes, and then centrifuged at 1000g for around 10 minutes. The molluscicidal activity of the obtained supernatant was tested.

Table 1: Doses used for determining the LC values of stem bark extract with combinations of binary (1:1) with gallic acid and quercetin.

Stem Bark+ Gallic acid	3.0	4.0	5.0	6.0
Stem Bark+ Quercetin	1.0	2.5	3.5	4.5

Gallic acid $(HO)_3C_6H_2CO_2H$) (3,4,5-Trihydroxybenzoic acid) (EC NO-205-749-9), **Quercetin** ($C_{15}H_{10}O_7$)(2-(3,4-Dihydroxyphenyl)-3,5,7-trihydroxy-4H-1-benzopyran-4-one,3,3′,4′,5,6-Pentahydroxyflavone) (EC NO-204-187-1) supplied by Sigma Chemical Co. P.O. Box 14508 St. Louis.Mo.63178 USA 314-771-5750, were studied in toxicity analysis in **binary combination** with stem bark of *Euphorbia tirucalli*, against *Indoplanorbis exustus*, for four different concenterations of doses as in Table 1. Gallic acid and Quercetin is found in stem bark of *Euphorbia tirucalli*.[6,7]

2.3 Test Animals

The target organisms for this research study, adult freshwater snails, *Indoplanorbis exustus*, (1.5 ± 0.2cm in shell height) were collected from pool alongside the campus of Veer Abdul Hameed P.G. College, Medical Road, Gorakhpur district. The collected animals were kept in glass aquaria containing de-chlorinated tap water to acclimatize to laboratory conditions. The water in the aquaria was changed daily and in order to prevent the water fouling any dead animal were removed periodically.

Fig. 2: *Indoplanorbis exustus* snail.

2.4 Toxicity Experiments

The Singh and Agarwal.[8] method was used to conduct the toxicity test for both snails, ten snails were housed in glass aquaria with 3L dechlorinated tap water. The experimental snails were exposed for 96 h to four different concentrations of stem bark of *Euphorbia tirucalli* in binary combinations with gallic acid and quercetin. For each concentration, six similar aquaria were set up. The snails were grown in the same way as the control group, but without any toxicity treatment. Snail behavioral reactions were detected for up to 2 hours after the treatment. Every 24h, 48h, 72h, and 96h, mortality was recorded LC_{10}, LC_{50}, and LC_{90} values, upper and lower confidence limits (UCL LCL), slope value 't' ratio, 'g' factor and heterogeneity were determined using the POLO computer program[9] utilizing the probit log analysis approach regression coefficient was also determined between exposure time and different values of LC_{50}.[10]

RESULTS

Experimental conditions of water were calculated using APHA/WPCF method (1998).[11] Accordingly, the parameters and their values determined were as follows

Atmospheric temperature 35.0 - 36^{0}C
Water temperature 28.0- 29.0 ^{0}C
pH of water 7.2-7.4
Dissolved Oxygen 6.9-7.4
Free carbon dioxide 4.6-6.7
Bicarbonate alkalinity 110.0- 111.0.

Effects on Behavioural Changes and Poisoning Symptoms

After five to ten minutes of exposure to the toxicity test, the freshwater snails *Indoplanorbis exustus* showed significant behavioural changes due to exposure to the aqueous extracts of *Euphorbia tirucalli* stem in binary combinations with Gallic acid and Quercetin. Initially, the aquaria showed hyperactivity of the sluggish snails, but over time, they became intoxicated and displayed spiral twisting and twitching of their muscles, which ultimately led to paralysis and the snails' death.

Dose-mortality Response

LC values ($LC_{10,50,90}$) of aqueous extracts of stem bark of *Euphorbia tirucalli* in combination with gallic acid and quercetin, and for period ranging from 24h to 96h for the snails, *Indoplanorbis exustus* have been given in (Tables 2-3). The snail toxicity was time as well as dose dependent. There was a significant negative correlation between LC_{50} values and exposure time (Tables 2-3). Thus increase in exposure time the LC_{50} of aqueous stem of *Euphorbia tirucalli* in combination with gallic acid, decreased from 6.28mg DW/L (24h);> 4.84mg DW/L (48h);> 4.11mg DW/L (72h);> to 3.79mg DW/L (96h) and in case aqueous stem of *Euphorbia tirucalli* in combination with quercetin, decreased from 4.90mg DW/L (24h); > 4.09mg DW/L (48h);> 2.28mg DW/L(72h); > to 1.71mg DW/L(96h) against *Indoplanorbis exustus* respectively.

The results were found to be within the 95% confidence limits of LC values; the potency estimation test ('g' value) was less than 0.5 at all probability levels, the regression test ('t' ratio) was greater than 1.96, and the slope values given in toxicity (Tables 2-3) were steep.

Table 2: Toxicity (LC_{10}, LC_{50} and LC_{90}) of stem bark extract of *Euphorbia tirucalli* + gallic acid against *Indoplanorbis exustus* at different time intervals.

Exposure periods	Effective dose(W/V) (mg DW/L)	Limit (mg DW/L		Slope value	't' ratio	'g' value	Heterogeneity
		LCL	UCL				
24h	LC_{10}= 1.65 LC_{50}= 6.28 LC_{90}= 13.91	0.45 5.28 11.88	2.40 10.36 18.93	0.57±0.37	2.91	0.23	0.11
48h	LC_{10}= 1.38 LC_{50}= 4.84 LC_{90}= 11.95	0.12 3.24 10.56	2.08 5.95 32.49	0.54±0.35	3.18	0.54	0.12
72h	LC_{10}= 1.11 LC_{50}= 4.11 LC_{90}= 10.51	0.84 3.64 8.08	2.15 4.55 18.81	0.55±0.35	4.21	0.33	0.21
96h	LC_{10}= 1.00 LC_{50}= 3.79 LC_{90}= 7.54	1.34 2.43 6.51	2.32 4.09 9.76	0.382±0.60	5.51	0.33	0.39

- Batches of ten snails were exposed to four different concentrations of combination of aqueous extracts of stem bark of *Euphorbia tirucalli* + Gallic acid.
- Concentrations (Dry weight of stem and leaves) given are the final concentrations W/V in aquarium water.
- Regression coefficient showed that there was significant negative regression between exposure time and different LC values, LCL: lower confidence limit UCL; upper confidence limit.
- There was no mortality in control groups.

Table 3: Toxicity (LC_{10}, LC_{50} and LC_{90}) of stem bark extract of *Euphorbia tirucalli* + quercetin against *Indoplanorbis exustus* at different time intervals.

Exposure periods	Effective dose(W/V) (mg DW/L)	Limit (mg DW/L		Slope value	't' ratio	'g' value	Heterogeneity
		LCL	UCL				
24h	LC_{10}= 1.60 LC_{50}= 4.90 LC_{90}= 9.61	0.19 3.75 7.30	1.98 8.40 12.76	0.58±0.135	3.842	0.16	0.10
48h	LC_{10}= 1.56 LC_{50}= 4.09 LC_{90}= 7.95	0.92 3.03 2.50	1.90 7.83 13.50	0.48±0.118	3.139	0.25	0.12
72h	LC_{10}= 1.35 LC_{50}= 2.28 LC_{90}= 4.631	0.11 1.81 1.95	1.71 2.80 19.72	0.47±0.11	4.632	0.28	0.32
96h	LC_{10}= 1.17 LC_{50}= 1.71 LC_{90}= 2.73	0.17 1.34 0.71	1.59 2.03 13.07	0.46±0.122	5.595	0.32	0.56

- Batches of ten snails were exposed to four different concentrations of combination of aqueous extracts of stem bark of *Euphorbia tirucalli* +Quercetin.
- Concentrations (Dry weight of stem and leaves) given are the final concentrations W/V in aquarium water.
- Regression coefficient showed that there was significant negative regression between exposure time and different LC values, LCL: lower confidence limit UCL; upper confidence limit.
- There was no mortality in control groups.

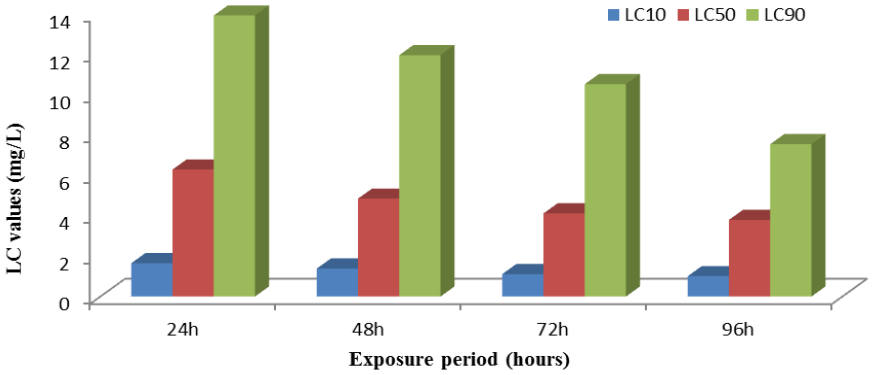

Fig. 3: Bar diagram showing toxicity (LC_{10}, LC_{50} and LC_{90}) of stem bark extract of *Euphorbia tirucalli* + gallic acid against *Indoplanorbis exustus* at different time intervals.

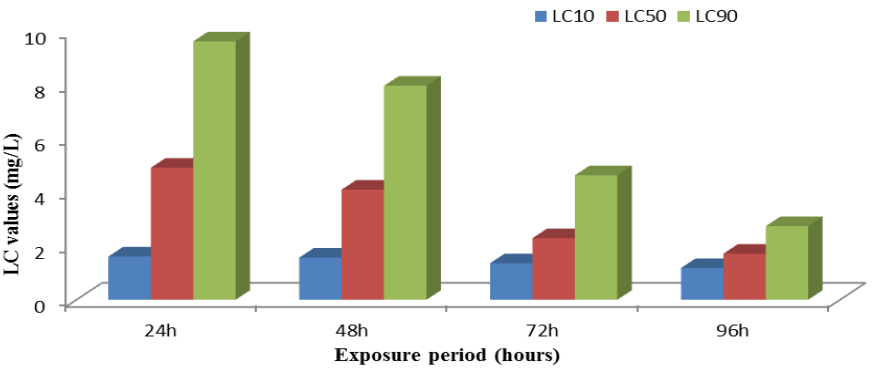

Fig 4. Bar diagram showing toxicity (LC_{10}, LC_{50} and LC_{90}) of stem bark extract of *Euphorbia tirucalli* + quercetin against *Indoplanorbis exustus* at different time intervals.

DISCUSSION

To comprehend the worldwide proliferation of fasciolosis, one must possess a worldwide outlook about the snail species that function as either hosts or vectors for Fasciola spp. It is necessary to compile specific studies on the disease's geographic spread and susceptibility as a valuable tool that could help with its control and prevention. Although they've fallen short of

expectations, these insecticides are effective. The potential for environmental contamination of these compounds has prompted an increasing number of efforts to identify plant-derived molluscicidal products. Given that they are products of biosynthesis, these might be biodegradable. Multiple kinds of compounds found in various plants have been proven to be harmful to snails. Numerous plant species that have certain active chemical ingredients have been recognised as inherently occurring molluscicides and are currently undergoing further investigation. Recent research has revealed the molluscicidal efficacy of some plant extracts, and saponins extracted from *Camellia oleifera* seeds have been used to create the novel chemical Luo-Wei, also referred to as tea-seed distilled saponin (TDS), which has been successfully tested in China and Egypt against intermediate host snails *Oncomelania hupensis*, *Biomphalaria alexandrina*, and *Bulinus truncates*.[12] Molluscicidal activity is attributed to terpene compounds, which are the most common type of bioactive plant chemical. It has been determined that thymol and α-pinene are both effective against *B. glabrata*, exhibiting concentration-dependent patterns of mortality and a lethal effect at doses in line with WHO recommendations (LC_{90} of 7.11 and 10.34 g mL1, respectively). Additionally, it has been reported that both compounds inhibit the acetyl cholinesterase of *B. glabrata* snails.[13]

Thus, it has been found that different alkaloids, flavonoids, sesquiterpenes, lactones, terpenoid, phorbol esters, saponins, tannins, alkenyl phenols, glycoalkaloids, and flavonoids are harmful to snails at acceptable doses of less than 1 to 100 parts per million.

The results show that *Euphorbia tirucalli* aqueous stem extract in binary combinations to above pure compounds Gallic acid and Quercetin, are poisonous to the freshwater snails *Indoplanorbis exustus*.

In the first thirty to forty minutes, the sluggish snails in the aquaria showed signs of hyperactivity. Over time, the snails became inebriated and exhibited spiral-twisted muscle twitches that ultimately led to paralysis and the snails' demise.

When conducting a laboratory study on the acute toxicity of *Euphorbia royleana*, *Euphorbia antisyphiliatica*, and *Euphorbia tirucalli* lattices on the snail *Lymnaea acuminata*, Singh and Agarwal (1988) observed comparable behavioural effects.[8]

The absence of any observable behavioural symptoms or mortality was observed in the control animals that did not receive treated water, indicating that factors other than plant moieties were responsible for the altered behaviour and mortality. Penetration rate, slope, unpredictability, and maximum effect are some of the factors that could be causing the animal's concentration-dependent response. The LC_{50} values in binary (1:1) combinations of the gallic acid and quercetin with aqueous stem bark extract of *Euphorbia tirucalli* decreased from 6.28 mg/L (24h) to 3.79 mg/L (96h) and from 4.90 mg/L (24h) to 1.71 mg/L (96h) respectively, against the vector snail *Indoplanorbis exustus.*

One possible explanation for reduced toxicity is either the adsorption of soil particles or the temperature-induced acceleration of the toxicant breakdown process.

Fish could be added to ponds four days after plant pesticides were sprayed, according to Perschbacher and Sarkar's[14] findings that "the toxicity of *Masea ramentacea* and tea seed cake was short-lived." Numerous factors, acting alone or in combination, may contribute to increased mortality with prolonged exposure times. For instance, a progressive increase in the drug's entrance and effects in the snail body is caused by the time-dependent updating of the active moiety. Death and exposure times are influenced by the rate of detoxification in the animal body as well as the stability (life span) of the pesticide's active moiety in the environment.

Numerous important facts are revealed after a statistical examination of the toxicity data. The mortality counts were not statistically heterogeneous, according to the 2 test for goodness of fit (heterogeneity), and resistance and other variables did not significantly affect the LC_{50} values, which were found to be within the 95% confidence intervals. Thus, the slope indicates how susceptible the target animal is to plant molluscicides. A steep slope also suggests rapid absorption and the beginning of effects. Although the slope by itself is not a very good indicator of the toxicological process, it is nevertheless a useful statistic in this kind of study. It is obvious that the concentration response lines in a replication test using random samples would fall within the same range given that the LC_{50} of the aqueous extracts was within the 95% confidence levels.[15]

CONCLUSION

In conclusion it may be stated that the binary (1:1) combinations of the gallic acid and quercetin with aqueous stem bark extract of *Euphorbia tirucalli* can be used as alternative molluscicides of plant origin to control the vector freshwater snail *Indoplanorbis exustus*, without harming the environment.

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