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MOLLUSCICIDAL PROPERTIES OF *CESTRUM* Sp. AGAINST FRESHWATER SNAIL : *LYMNEA ACCUMINATA* LINN.

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A B S T R A C T

Synthetic molluscicides have proved too expensive for most countries wanting to include snail control in their anti-Schistosomiasis programme. An alternative that is not only cheaper but also safe, non-hazardous and degradable bio-molluscicides. It promotes self-reliance and empowerment of the affected communities, is the use of molluscicidal plants. Molluscicidal properties of two Cestrum spp were confirmed and active principle was isolated, purified and identified as steroidal saponin. The Lc_{50} value of isolated saponin along with aqueous and alcoholic extracts of leaves of two Solanaceous plants viz. Cestrum nocturnum Linn and Cestrum diurnum Linn were determined. The Lc_{50} values were obtained for 24 h, 48 h and 72 h by bioassay method against freshwater snail, Lymnea. During experimentation, the pulmonate exhibit behavior similar to contact toxicants.

Key words: Bioassay, Bio-molluscicides, *Cestrum, Lymnea*, Schistosomiasis, Saponin,

INTRODUCTION

Plant secondary metabolites, have no apparent function in plant physiology but where major role in it is reportedly defensive and hence uses commercially as pesticides (Sharma, 1982; Brackenbury, 1999). These phytochemical are widely used as important constituents in medicines and agriculture products as antibacterial, fungicidal, insecticidal, larvicidal, piscicidal, molluscicidal, cytotoxic, anti-tumor, anti-edema, anti-fertility agents (Rao and Gurfinkel, 2000; Bilia et al., 2000; Patole and Mahajan, 2007a&b). Recent investigations on toxicological action of these compounds provide a clue for new biodegradable source and many fundamental properties of them. Thus efforts should be exercised together for increasing the knowledge of underlying mechanism of toxicity in different animals like snail, insects, fishes etc for the development of effective biocides. The fresh water pulmonate, *L. accuminata* is commonly found in river, lake, pond or streams. It is hermaphrodite, breed almost throughout the year and serves as an intermediate host as well as vector for larval forms of helminthes parasites, *Schistosoma.*

This trematode causes an endemic disease Schistosomiasis to human and domestic animals. According to World Health Organization (WHO), this disease affects more than 200 million peoples and places other 600 million at risk of infection in more than 70 countries in tropics (Nelymar et al., 1999). On other hand *Lymnea* is also serious pest in horticulture and agriculture for their control various molluscicidal chemicals viz Copper sulphate, Aldrin, Lindane and other synthetic chemicals were used (Ebele et al., 1990). However, growing awareness towards adverse effect of these chemicals and high cost of synthetic molluscicides, has initiated the search for safe, non-haz-

ardous, readily availability and economical for users. Ideally, aqueous and alcoholic extracts, which does not involve much complicated technologies should be satisfactory for application of phytoconstituents (Singh and Singh, 1997). In present investigation, aqueous, alcoholic and isolated saponin extracts of two species of Solanaceous plants, namely *C. nocturnum* and *C. diurnum* were used as molluscicides against fresh water snail, *L. accuminata*. Both are ornamental plants, their phytochemical constituents like saponin, alkaloids, hydrocarbons, fatty acids and essential oils were reported the significant piscicidal and haemolytic plants (Patole and Mahajan, 2004; 2007a).

MATERIALS AND METHODS

The snail, L. accuminata were collected from local ponds of Panzara river (Sakri). Healthy and active snails of moderate size (2-3 cm) weighed about 1.5 to 2 g were selected irrespective of sex. They were maintained in glass aquaria at laboratory condition. Water was replaced at 2 days interval. The plant material, *C. nocturnum* (Rat-rani) and *C. diurnum* (Din-ka-raja) were collected from local garden. Plant leaves was dried in shade, ground in a knife mile. Aqueous and alcoholic extracts was prepared by mixing of 10 g plant materials with 200 ml of usual solvent. Kept the mixture in 250 ml conical flask at room temperature. The flask was plugged with cotton. After 48 h, the mixture was filtered by double muslin cloth and refiltered through Whatmann filter paper no. 1. The excess solvent was removed by rotary evaporation under reduced pressure and temperature below 45 ^{0}C. The resulting crude extracts were kept in the dessicator until assayed. The saponins of both plants were isolated by the method (Chakravarti et al., 1962). The leaves were extracted with alcohol, the extract were hydrolyses with diluted solution of NaCl and HCl mixed with 50 % butanol and repeatedly crystallized with diluted alcohol. The residue was evaporated to yield crystalline saponin having melting point 242 ^{0}C and 269 ^{0}C from *C. diurnum* and *C. nocturnum* respectively. The isolated saponin were dried and preserved in dessicator.

A preliminary screen to detect molluscicidal property was evaluated for aqueous, alcoholic and isolated saponin extracts. It was run using the WHO guidelines (WHO, 1983) employing 10 adult snails. They were kept submerged in 500 ml beaker containing desired dose concentrations of each extracts in dechlorinated water. The data of percent mortality were noted at 24 h duration.

RESULTS AND DISCUSSION

Molluscicidal activity of different dose concentrations of aqueous, alcoholic and isolated saponin extracts of both species experimental plant against snail is presented in table 1. It shows, among the aqueous and alcoholic extract, the alcoholic extract was the most toxic with 100 % mortality occurs at concentration of 4.0, 3.0 and 2.5 mg/l within 24, 48 and 72 h respectively. Extract from *C. diurnum* killed the snail at concentration one order of magnitude lower than the *C. nocturnum*. Aqueous extract of *C. nocturnum* and *C. diurnum* caused 100 % mortality at 4.0 and 5.0 mg/l concentration in 48 h respectively. Alcoholic extract of *C. nocturnum* was quite toxic causing 100 % mortality at 4.0 mg/l within 24 h, while same extract of *C. nocturnum* and *C. diurnum* cause 100 % mortality in 48 h at one order of magnitude i.e. 3.0 & 4.0 mg/l lower respectively. Isolated saponin from *C. nocturnum* and *C. diurnum* caused 100 % mortality within 48 h at 2.5 and 3.0 mg/l doses respectively. Table 2, represents Lc_{50} values of plant extracts at different period i.e. 24 h, 48 h and 72 h. Table clearly shows, based on toxicological property against *L accuminata*, the alcoholic extract was most toxic than aqueous extracts of both plants. Isolated saponins have the same range of Lc_{50} values i.e. 1.2 to 1.3 mg/l at 72 h. During experimentation, snail showed typical behaviors, as they close their operculum tightly, there was excessive secretion of mucus and attempts were made to surface off water. However in due course of time, the toxic effects was revealed in terms of sluggish movements, settlement of snails at the bottom with slightly unwrap operculum and finally leads to death.

Many Snails acts as intermediate hosts for helminthes parasites, which causes severe disease to domestic animals and man. Synthetic molluscicidal chemicals can check life cycle of diseases causing parasite but the hazardous chemical harm the livestock, when they are grazing on polluted food and water. Hence, there is an urgent need for the plant molluscicidal agents (Sturrock, 1995). In present study, the

natural compounds isolated from *C. nocturnum* and *C. diurnum* plant leaves was tested for their molluscicidal properties. The alcoholic extract of *C. nocturnum* leaves was potent and their Lc 50 value is 2.33 mg/l after 48 h of exposure. According to WHO, crude organic extracts should present Lc_{90} values bellow 20 ppm to be considered a good molluscicides candidates for direct application in infested water. Earlier workers evaluated similar experiments by using potent biomolluscicides. Some researchers used crude alcoholic and aqueous plant extracts for investigation of molluscicidal activity on different species of snails. They have reported that, the toxic effect was due to the plant secondary metabolites like triterpenoids, saponins, steroidal glycosides and alkaloids (Lopez and Garces, 1994; Sukumaran et al., 1995; Brackenbury, 1999). Various phytochemicals, viz., oils from Neem, Garlic, Ginger, Cedar etc individually or in combination, polyphenolic compounds, proanthrocyanins, flavonoid, azadirachtin, quinoline alkaloids, dinocophylline, epidinocophiline are isolated and successfully tested for potent molluscicidal activity on *Lymnea* and *Indoplanorbis* species (Singh and Singh, 1997; Muraleedharan et al., 1997; Hmumouchi et al., 1998; Nelymar et al., 1999; Bilia et al., 2000). The purification and analysis for chemical nature, confirmed the *C. nocturnum* compound as steroidal saponin. There was (Marston and Hostettmann, 199; Naqvi et al., 1996; Abdel et al., 1997) reported that, the saponin are molluscicidal compound. These reports support the present findings that the purified compound from *C. nocturnum* is saponin. In Nutshell, our screening revealed the molluscicidal property of different doses of both *Cestrum* plant species. Among them isolated saponin was most active molluscicides.

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Table 1 Per cent mortality of *Cestrum* spp against, L. *accuminata*

Plant species	Extracts	Concentration (mg/l)	* Per cent mortality within (h)		
		24 h	48 h	72 h	
Cestrum. nocturnum Linn	Aqueous	2.0	00.00	00.00	00.00
		2.5	00.00	33.33	70.00
		3.0	13.33	56.67	93.33
		3.5	30.00	90.00	100.00
		5.0	66.67	100.00	—
	Alcoholic	1.5	00.00	00.00	10.00
		2.0	00.00	33.33	73.33
		2.5	16.67	56.67	100.00
		3.0	30.00	100.00	—
		3.5	66.67	—	—
		4.0	100.00	—	—
	Isolated saponin	0.5	00.00	00.00	00.00
		1.0	00.00	10.00	16.67
		1.5	20.00	43.33	93.33
		2.0	43.33	90.00	100.00
		2.5	53.33	100.00	—
		3.0	100.00	—	—
Cestrum diurnum Linn	Aqueous	2.0	00.00	00.00	00.00
		2.5	10.00	40.00	56.67
		3.0	16.67	50.00	90.00
		3.5	26.67	66.67	100.00
		5.0	56.67	100.00	—
	Alcoholic	1.5	00.00	00.00	10.00
		2.0	10.00	20.00	63.33
		2.5	10.00	36.67	93.33

	3.0	20.00	46.67	100.00
	3.5	33.33	63.33	—
	4.0	40.00	100.00	—
Isolated saponin	0.5	00.00	00.00	00.00
	1.0	00.00	00.00	00.00
	1.5	13.33	16.67	60.00
	2.0	23.33	66.67	100.00
	2.5	40.00	90.00	—
	3.0	53.33	100.00	—

No mortality was observed in control. * Percent mortality data was obtained from three replicas.

Table 2

Lc $_{50}$ values of phytotoxicant of *Cestrum* spp against, *Lymnea accuminata*

Plant species	Nature of extract	*Lc $_{50}$ values (mg/l) within (h)		
		24 h	48 h	72 h
C. nocturnum	*Aqueous*	*4.62 ± 0.4185*	*2.71 ± 0.4731*	*2.36 ± 0.2152*
	Alcoholic	3.31 ± 0.3425	2.33 ± 0.3761	1.74 ± 0.2533
	Isolated saponin	2.26 ± 0.1436	1.56 ± 0.1621	1.23 ± 0.1211
C. diurnum	Aqueous	4.87 ± 0.2415	2.83 ± 0.1523	2.49 ± 0.1721
	Alcoholic	4.31 ± 0.3113	2.67 ± 0.2634	2.14 ± 0.1521
	Isolated saponin	2.85 ± 0.2164	1.65 ± 0.4165	1.32 ± 0.4125

* Lc 50 values are presented as average of four observations ± S.E.

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