JETIR.ORG ISSN: 2349-5162 | ESTD Year : 2014 | Monthly Issue
JOURNAL OF EMERGING TECHNOLOGIES AND INNOVATIVE RESEARCH (JETIR)
An International Scholarly Open Access, Peer-reviewed, Refereed Journal

Anti leech effect of selected plant extracts on Indian leech, *Haemadipsa sylvestris* (Blanchard:1894)

P DHOTTAMMA,[1] J EBANASAR*[2] KAYALVIZHI. R[3]

[1]Ph.D Research scholar, Department of Zoology, Government Arts College, Udhagamandalam-643002, Tamilnadu.

[2]Associate Professor and Head, Department of Zoology, Government Arts College,Udhagamandalam-643002 Tamilnadu.

[3]Ph.D Research Scholar, Department of Zoology, Government Arts College, Udhagamandalam- 643002 TamilNadu, India.

*Corresponding Author- ebanasar@gmail.com

ABSTRACT:

Terrestrial leeches are common in the rural areas at higher elevations of moist deciduous forests and ajoining plantations and it causes discomfort to people and livestock. The present study was carried out to determine the leech repellent activities of some locally available plant species on the land leech *Haemadipsa sylvestris.* A total of five medicinal plants were used as leech repellents. Aqueous extracts of *Artemisia vulgaris*(Burm f), *Crassocephalum crepidioides* (Benth S. Moore), *Acalypha indicia* L., *Sida cartifolia* L., and *Stachytarpheta cayennensis*(Rich Vahl) were prepared and tested on the terrestrial leech. The experiment was designed to assess the effect of three concentrations of aqueous extract *viz.*, 1%, 3% and 5% for each concentration ten leeches were used. The following indices of repellency activity *viz.*, repellency (%), effective period of plant extracts and Effectiveness repellency concentration (ECR) ECR-50, ECR-75 and ECR-100 in 3hours and 6hours interval. The aqueous extract of *A. vulgaris* showed high repellency of (81.43%) at 5% concentration after 3hrs of exposure and *S. cartifolia* showed least repellency of (27.43%) at 1% concentration after 6hours exposure. *A. vulgaris* has effective concentration of 50% repellency (ECR-50) 2.304% at 3 hours of interval and 100% repellency (ECR-100) is 5.813%. Repellency by *A. vulgaris* afforded for better protection for 3hours against the bites of *H. sylvestris* The study reveals that *A. vulgaris* is highly repellent as well as toxic to land leeches, and can be effectively used to prevent leech bites in the field areas.

Key Word: Leech, Aqueous extract, Repellent, Medicinal plants, *Haemadipsa sylvestris.*

INTRODUCTION:

Leeches are hematophagous, predatory or parasitic annelids. Leeches make up a third large group of annelids. Most species of leech live in fresh water, terrestrial and some in marine environment. The majority of the land species are distributed in tropical and subtropical areas (Sawyer, 1986). Land leeches are found on the surface of the plants, grasses and under stones in damp places (Henderix, 1998). In some animals, leeches enter through the mouth and attach to the upper respiratory system or the mucosa of digestive system (Pandey *et al.*, 2000). *H. sylvestris* is commonly known as the Indian land leech. This leech may have a potential role to attack cattles and human beings as well as their bites result in prolonged bleeding (Blanchard, 1894). Its bite is very painful. They also have impact on ecotourism as it often bites the tourists. Land leeches are inactive during heavy rain and hot weather but became active during slight drizzle (Saxena *et al.*,1974). Leech bites can also lead to secondary bacterial infections (Heukelbach *et al.*, 2009). Plants and plant-derived substances have been used traditionally to repel or kill leeches. Infestation of leeches is common in various ecotourism locations of Nilgiri biosphere reserve.The commercial repellents are prepared by using chemicals like, N-N- diehyl-m-toluamide (DEET), N, N-diethyl mendelic acid amide (DEM), dim-ethyl

phthalate (DMP) and allethrin. These chemical repellents are not safe for public use (Das *et al.,* 2000; Zandikoffi *et al.,* 1979). Leeches are naturally aggressive and causes much infection on different animals including donkey, hen, cow, dog, goat, sheep and lamb (Bahmani *et al.,* 2013). Natural products are always safe for human when compared to that synthetic compounds (Sharma *et at.,* 1993). Numerous studies are reported on the effect of repellents on mosquito's repellents but only few are reported in leech repellents. Hence in the present study, attempts to evaluate five traditionally plant extracts used against *H. sylvestris.*

Materials and Methods

Test Leeches

Uniform sized *H. sylvestris* were collected from O'valley area ($11^0$27'19''N& $76^0$28'79''E) of Gudalur, Tamilnadu (Figure-1). It is collected from their natural micro habitat, *viz.*, under rocks, soil, sticks, grazing lands and decaying leaves. Multiple field visits were conducted. Leeches were collected by hand picking and transferred to plastic container provided with little amount of soil mixed with water to keep in moist condition. Test leeches were identified using hand book: Leeches of India by (Mahesh Chandra 1991). The collected specimens were brought acclimatized to laboratory condition and reared.

Preparation of repellents:

The following plants are selected for preparing anti leech aqueous extracts. They are,

Artemisia vulgaris (Burm f) (Fig-1), *Crassocephalum crepidioide* (Benth, S. Moore) (Fig-2), *Acalypha indica* L.(Fig-3) *Stachytarpheta cayennensis* (Rich vahl) (Fig-4), *Sida cartifolia* L (Fig-5). The plant species were identified by (Gamble, 1925). The plant leaves of selected healthy plants were collected and crushed and pure filtrate sample were prepared. The fresh aqueous extract samples were weighed for the experiments *viz.*, 1%, 3% and 5% concentrations and they are incorporated in cloth strips for 2 minutes and gms/cm^2 were calculated (Table-1). The five medicinal plants were studied for repellency against *H. sylvestris* at three different concentrations (1%, 3%, 5%) were prepared using distilled water and two different time intervals (3 hours and 6 hours).

Repellency testing methods: The repellency was tested as per method of (Ribbands, 1946) and it modified by (Ramachandran *et al*., 1971). In this method poplin cloth were used. The cloth cut into four strips with proportion of 5cm width and 96cm of length. Before using the cloth strips are washed clean, rinsed and well dried. A set of for cloth strips were soaked in required concentration of plant extracts for 2 minutes. The glass panel was kept dry during the test. The cloth strips are assembled in a surface around the glass panel. Test leeches are released into the glass panel. The repellency was calculated by using the following formula is,

$$\text{Repellency} = \frac{\text{No. Probes attempted} - \text{No. Cross over}}{\text{No. Probes attempted}} \times 100$$

Untreated cloth strips soaked in distilled water was used as control.

Statistical Analysis:

The test difference between control and treated to groups analyzed by Duncan Multiple Range Test (DMRT) with SPSS 2.0 software. The repellency effect of different concentration of plant extracts was calculated using Curve Expert 1.4 software and also include with effective of concentration for 50% (ECR-50) repellency, 75% (ECR-75) repellency, 95% (ECR-95) repellency and 100% (ECR-100) repellency were calculated.

Result and Discussion:

The repellency of five aqueous extracts after different periods of exposure is presented in Table-2. All the extracts revealed concentration dependent action with high repellency at higher concentration and declining with the decrease in concentration. After 3 hrs of exposure repellency activity of five extracts differed significantly ($p< 0.01$). The repellency of *A. vulgaris* exhibited highest activity of 81.43% and 48.72 % in 3 hrs and 6hrs respectively while *C. crepidioides* 74.05 % in 3 hrs and 48.97 % in 6 hrs and *S. cartifolia* 60.95% in 3hrs and 41.26% in 6hrs were comparatively less repellent than *A. vulgaris*.

All the extracts exhibit significantly higher repellent action at higher concentration, while substantial action could also be prominent in the lowest concentrations as well. The effect of different treatment on 3 hrs of exposure is presented in (Table-3). It reveals that least ECR50 rate of 2.304% is found in *A. vulgaris* and highest ECR 50 rate of 5.583% it found in *S.*

cartifolia. Similar trend was found ECR 75 and ECR 100. The least value of ECR 100 is found in *A. vulgaris* which shows that the *S. cartifolia* has high activity even at low concentration 5.813%.

The effect of different treatment on 6 hrs of exposure is presented in (Table-4). It also reveals that least ECR50 rate of 3.348 is found in *A. vulgaris* and highest ECR 50 rate of 6.433 found in *S. cartifolia*. Similar trend was found ECR 75 and ECR 100. The least value of ECR100 is found in *A. vulgaris* 8.817 which shows that the *S. cartifolia* has high activity even at low concentration 14.402%.

The concentration of the extracts is increased at the same time repellency also increased. According to (Shah *et al.*,2020) the methanol extract of *Dillenia suffruticosa (*Dilleniaceae) was applied against *Zeylanicobdella arugamensis* (marine leech) concentration of 100mg/mL, and it took 14.39 and 4.88 min to kill all the leeches. However the result of present study reveals that the aqueous extract of *A. vulgaris* took less concentration (5%) than *D. suffruticosa* to kill the leeches. (Eftekhari *et al.*, 2012) the methanol extracts of *Allium sativum* (600µg/mL) were tested against the aquatic leech *Limntis nilotica* and took 144.55min for total mortality. Previous report revealed that *Artemisia kermanensis (Artemisia spp)* has the strong effect on the leech death (Bahmani *et al.*, 2012). However our present study reveals that the aqueous extract of *A. vulgaris* (5%) at (3hours) has a strong effect on the bites of leech. According to Venmathi maran *et al.*, (2021) the aqueous extracts of *Azadirachta indica* leaves exposure (100 mg/mL) 11.9±1.11 min killed marine parasitic leech. Similar study by (Yit *et al.*, 1985) benzone and methonal extracts of *Artemisia vulgaris* had repellent activity against *Aedes aegyti*. Therefore the present study indicates the aqueous extract of *A. vulgaris* (without using any solvent) has strong effect on land leeches. Hence, *A. vulgaris* can also be used as an effective repellent on mosquitos.

Conclusion

The present study, it was proven that aqueous extract of *A. vulgaris* exhibit high degree of protection against terrestrial leeches. The five medicinal plant species can be used as natural repellents. It is not more expensive and easily available to field areas. There is no previous tested report on use of *A. vulgaris, C. crepidioides, A. indica, S. cartifolia,* and *S. cayennensis* as leech repellents. These plants are commonly available the ecotourism, rural areas and other visitors can effectively use the plants to protect themselves from leech bite. Therefore, use of these different types of plant extracts could reduce the cost and environment effects and also easily biodegradable. The plant products used as natural repellents to repel or kill biting insects.

Acknowledgement

Authors are thankful to Dr. M. Eswaramoorthy Principal of Government Arts College, Uhagamandalam for providing facilities to carry out this work. Special thanks to Dr. B.D Sheeja, Assistant Professor Department of Botany for plants identification and valuable suggestions carrying out this investigation.

Tables

Table1: Relative repellency of aromatic oils against *H. sylvestris*

Plant extracts	Concentration (%)	Quantity of plant extract used per g/cm^2
Artemisia vulgaris(Burm f),	1%	0.00339
	3%	0.01017
	5%	0.01695
Crassocephalum crepidioides (Benth) S. Moore	1%	0.00382
	3%	0.01148
	5%	0.05743
Acalypha indica L.	1%	0.00359
	3%	0.01078
	5%	0.05394

Sida cartifolia L.	1% 3% 5%	0.00309 0.00929 0.01548
Stachytarpheta cayennensis (Rich Vahl)	1% 3% 5%	0.00342 0.01028 0.01714

Table:2 The efficiency of different medicinal plants against land leech *H. sylvestris* during different periods after application.

S. no	Time after exposure (hrs)	Concentration	Repellency in treatment %					
			Control	*Artemisia vulgaris*	*Crassocephalum crepidioides*	*Acalypha indica*	*Stachytarpheta cayennensis*	*Sida cartifolia*
1	3hrs	1%	0.00±0.00^{a*}	52.76±9.49^{d}	38.71±3.86^{b}	43.42±4.51^{c}	46.99±4.30^{c}	32.50±2.84^{b}
		3%	0.00±0.00^{a*}	64.43±5.97^{d}	43.63±4.24^{b}	47.23±4.35^{c}	51.59±5.20^{c}	35.27±3.04^{b}
		5%	0.00±0.00^{a*}	81.43±3.15^{d}	47.86±4.08^{b}	53.07±4.66^{c}	60.38±4.98^{c}	41.26±4.98^{b}
2	6hrs	1%	0.00±0.00^{a*}	44.41±6.03^{c}	32.22±2.74^{b}	35.11±4.26^{b}	38.98±3.61^{b}	27.43±3.12^{b}
		3%	0.00±0.00^{a*}	51.42±5.03^{d}	37.07±4.32^{b}	39.35±2.85^{b}	41.40±5.37^{c}	31.90±3.50^{b}
		5%	0.00±0.00^{a*}	60.32±7.18^{d}	41.18±3.82^{b}	47.86±3.74^{c}	50.09±4.00^{c}	36.63±5.62^{b}

Table 3: This table is showing repellency of selected plants during 3hours of exposure

Plants	Linear fit equation: Y=a+bx	Standard Error	Correlation Coeff.	ECR-50%	ECR-75%	ECR-95%	ECR-100%
Artemisia vulgaris(Burm f),	R=1.213+1.623C	15.782	0.8724	2.304	4.058	5.462	5.813
Crassocephalum crepidioides (Benth S.Moore)	R=1.520+1.862C	12.4937	0.7875	4.453	7.543	10.026	10.645
Acalypha indica	R=1.287+1.690C	13.9125	0.7829	3.881	6.704	8.962	9.526

L.							
Stachytarpheta cayennensis (Rich vahl	R=1.629+2.024C	14.7218	0.8063	3.301	5.764	7.734	8.227
Sida cartifolia L.	R=1.656+2.099C	10.3048	0.7982	5.583	9.202	12.097	12.821

Table 4: This table showing repellency of selected plants during 6hours of exposure.

Plants	Linear fit equation: Y=a+bx	Standard Error	Correlation Coeff.	ECR-50%	ECR-75%	ECR-95%	ECR-100%
Artemisia vulgaris(Burm f),	R=1.355+1.862C	14.163	0.821	3.348	5.767	7.703	8.187
Crassocephalum crepidioides (Benth S.Moore)	R=1.620+1.836C	10.374	0.800	5.484	9.051	11.909	12.618
Acalypha indica L.	R=1.284+1.848C	10.753	0.831	4.674	7.748	10.207	10.822
Sida cartifolia L.	R=1.733+2.015C	12.203	0.804	4.372	7.366	9.762	10.361
Stachytarpheta cayennensis (Rich vahl	R=1.823+2.326C	8.978	0.810	6.433	10.418	13.605	14.402

FIGURES: The photograph of plants used as leech repellency:

Fig-1 ***Ageratum conizoides***

Fig-2 ***Crassocephalum crepidioides***

Fig-3 ***Acalypha indica***

Fig-4 *Stachytarpheta cayennensis* **Fig-5 *Sida cartifolia***

REFERENCE:

Sawyer, R.T. 1986 Leech Biology and behavior: Feeding biology, Ecology and Systamatics. (Oxford University Press) USA. Volume 11.

Henderix, M.C. 1998. Diagnostic Vertinary Parasitology. 2ND edision. Mosby, *Elsevier* (USA) 234-238.

Pandey, C. K. Sharma, R. Baronia, A. Agarwal, A. Singh. 2000. An unusual case of respiratory distress: live leech in the larynx *Anesthesia Analogue*

Blanchard, R. Viaggio di Leonardo Fea in Birmanica e regioni vicine- L 1894. VII. Hirudinees. *Annali Museo civico di Storia Naturelle, Genova,* 14(2) :113-118.

Saxena, B. N. Dubey, D. N. & Nair, L. N. 1974. Studies on the insecticidal and repellent properties of the seed extract of *Tephrosia purpuria* (Linn.) Pers. *Defence Science Journal*. 24(2), 43-48.

Heukelbach, J. Hengge, U. R. 2009. Bed bugs, leeches and hookworm larvae in the skin. *Clinics in Dermatology.* (27) 285-290.

Das, N. G. Nath, D. R. Baruah, I. Talukadar, P. K. Das, S. C. 2000. Field evaluation of herbal mosquito repellents. *Journal of Communication Disorders*. 31(4): 241-5.

Zandikoff, C. M. 1979. Toxic encephalopathy associated with use of insect repellent. *Journal of Paediatrics*. 95 (I) L: 140-2.

Bahmani, M. Golshahi H, Ghotbian F, Bahmani F. 2013. Internal hirudiniasis in a hen (*Gallus gallusdomesticus)*-the first report in literature. *Asian Pacific Journal Tropical Diesase.* 3: 232-234.

Sharma, V. P. Nagpal B. N. 1993. Srnivastava Aruna. Effectiveness of neem oil mats in repelling mosquitos. *Transactions of the Royal Society Tropical Medicine and Hugiene*.87: 627-8.

Chandra, M. 1991. The leeches of india –A hand book. Zoological survey of india. Calcutta. p.130.

Bishen Singh Mahendra Pal, Singh, Gamble, J. S. 1925. Flora Presidence of Madras. Volumes II, III. 2017.

Ribbands, C R. 1946. Experiments with leech repellents. *Annals.Tropical.Medicine and Parasitology*.(40) 314-319.

Ramachandran, P. K. Koshy, T. Shastry, K. G. K. Singh, S. P. Srinivasan, M. N. and Ganguly, S. K. 1971. Studies on leech repellents. *Journal of.Economic,Entomology.* (64) 1293-1294.

Manishaben Jaiswal, "CRYPTOCURRENCY AN ERA OF DIGITAL CURRENCY", International Journal of Creative Research Thoughts (IJCRT), ISSN:2320-2882, Volume.8, Issue 1, pp.60-70, January 2020, Available at :http://www.ijcrt.org/papers/IJCRT2001010.pdf

Shah, M. D. Venmathi Maran, B. A. Iqbal, M. Ching, F. F. Mohamad Lal, M. T. Binti Othman, R. Shapawi, R. 2020. Antiparasitic activity of the medicinal plant *Dillenia suffruticosa* against the marine leech *Zeylanicobdella arugamensis* (Hirudinea) and its phytochemical composition. *Aquaculture.Research.*.(51) 215-221.

Eftekhari, Z. Bahmani, M. Mohsenzadeghan, A. Gholomi Ahangaran, M. Abbasi, J. Alighazi, N. 2012. Evaluating the anti-leech (*Limnatis nilotica)* activity of methanolic extract of *Allium sativum* L.Compared withlevamisole and metronidazole. *Comparative.Clinical.Pathoogy*. 21.1219-1222.

Bahmani, M. Avijgan, M. Gholami-Ahangaran, M. Rafieian, M. 2012.The comparison on anti *Limnatis nilotica* effects of albendazole and some of the Iranian medicinal plants. *Iranian South Medical Journal*. 15(1). 25-34.

Venmathi Maran, B. A. Josmeh, D. Tan, J. K. Yong, Y. S. Shah, M. D. 2021. Efficacy of the extract of *Azadirachta indica* Against the marine leech Parasitic Leech and its Phytochemical Profiling. *Molecules* 26.1908.

Yit, H. S. Ku-Hua, W. V. Kumamoto, J. H. Axelrod, Mulla M. S. 1985. Isolation and identification of mosquito repellent in *Artemisia vulgaris. Journal Chemical Ecology*. 11: 1297-306.