Indian J Med Res 142 (Supplement), December 2015, pp 95-100
DOI:10.4103/0971-5916.176633

Insecticidal effect of plant extracts on *Phlebotomus argentipes* (Diptera: Psychodidae) in Bihar, India

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Received August 30, 2013

***Background & objectives*: *Phlebotomus argentipes* (Diptera: Psychodidae), the established vector for kala-azar is presently being controlled by indoor residual spray of DDT in kala-azar endemic areas in India. Search for non-hazardous and non-toxic biodegradable active molecules from botanicals may provide cost-effective and eco-friendly alternatives to synthetic insecticides. The present study was aimed at evaluating various plant extracts from endemic and non-endemic areas of Bihar for their insecticidal activity against sandfly to identify the most effective plant extract.**

***Methods*: Bio-assay test was conducted with larvae and adult of *P. argentipes* with different plant extracts collected in distilled water, hexane, ethyl acetate, acetone and methanol. Thin layer chromatography (TLC), column chromatography and high performance liquid chromatography (HPLC) were conducted for detection of active molecules.**

***Results*: Adults and larvae of sandflies exposed to the aqueous extract of *Nicotiana tabacum* resulted in 100 per cent mortality. The hexane extract of *Clerodendrum infortunatum* was found to kill 77 per cent adults but was ineffective against larvae. Bio-assay test of the ninth fraction (hexane extract-methanol phase) separated by column chromatography was found to be 63 per cent effective. The purple spot on the TLC of this fraction indicated the presence of a diterpenoid. HPLC of this fraction detected nine compounds with two peaks covering 20.44 and 56.52 per cent areas with retention time of 2.439 and 5.182 min, respectively supporting the TLC results.**

***Interpretation & conclusions*: The column separated 9th fraction of *C. infortunatum* extract was found to be effective in killing 63 per cent of adult *P. argentipes*. Compounds of this fraction need to be evaluated further for identification and characterization of the active molecule by conducting individual bio-assay tests followed by further fractionation and HPLC. Once the structure of the active molecule is identified and validated, it may be synthesized and formulated as a product.**

Key words *Clerodendrum infortunatum* - insecticides - leishmaniasis - plant extracts - sandflies

The State of Bihar has been known to be endemic for kala-azar since more than a century reporting around 80 per cent of the total global cases. DDT [1,1,1-trichloro-2,2-bis(4-chlorophenyl)ethane] is the insecticide of choice to control the disease by controlling the vector. However, resistance in vector has been developed against DDT in some parts of Bihar[1,2]. The synthetic pyrethroids (deltamethrin and permethrin) impregnated long-lasting insecticidal nets (LLIN) have not found to be very effective in controlling sandflies[3,4]. Prior to the discovery of chemical insecticides in the early 1940s, plant-based insecticides were important products used for pest management[5], since plants can defend themselves from predator insects[6]. After decades of indiscriminating use of synthetic insecticides, target organisms developed resistance and adverse effects were observed on non-target organisms and environment[7-9]. Organic and synthetic insecticides are hazardous and usually non-biodegradable, in addition to their deleterious influence on the environment. Unlike synthetic compounds that kill both pests and non-target organisms, natural insecticides are relatively inactive against the latter[5]. Over 98 per cent of sprayed insecticides reach a destination other than their target species[10]. However, as with any other pesticide, plant-based products must also be used properly[11-13]. These are also found to be safe for higher animals and environment[14]. Unlike conventional insecticides, which are based on a single active ingredient, plant derived insecticides comprise botanical blends of chemical compounds which impact on both behavioural and physiological processes. Thus, there is little chance of pests developing resistance to such products. Identification of bio-insecticides that are efficient as well as suitable and adaptive to ecological conditions is imperative for continued vector control management. Approximately 1,200 plant species have been reported to have insecticidal value[15]. Several groups of phytochemicals like alkaloids, steroids, terpenoids, essential oils and phenolics from different plants have been reported for their insecticidal activities[16]. It is essential to find out better alternatives to chemical insecticides that should not only be cost-effective, but also eco-friendly and can be used in the community. This study was undertaken to evaluate extracts of various plants collected from endemic and non-endemic areas of Bihar for their insecticidal activity against sandflies.

Material & Methods

The study was conducted at Rajendra Memorial Research Institute of Medical Sciences, Agamkuan, Patna, Bihar, India, during July 2012 to March 2013. Plants were collected from endemic (n=62) and non-endemic areas (n=35) of Bihar to determine their selective insecticidal effect. Specimens were preserved with respective voucher numbers. Crude extracts of plants were prepared in different solvents like water, hexane, ethyl acetate and methanol.

Bio-assay test: Laboratory bred three days old female *Phlebotomus argentipes* were exposed to plant extracts soaked in Whatman filter paper No. 1 along with deltamethrin at a concentration of 20 mg/m^2 as positive control and distilled water and the respective solvent as negative controls.

Adulticidal bio-assay: Crude extracts (50µl) of plant samples were blotted on a 10 cm^2 area of filter paper, dried at 40°C and placed in a 50 ml centrifuge tube. Sandflies (n=10) were exposed in the bio-assay tube for one hour and kept for 24 h in recovery tube with 10 per cent glucose solution soaked in a cotton ball along with control, positive control and negative control[17]. All bio-assay experiments were conducted in five replicates at 25± 2°C and 72-80 per cent relative humidity[18]. The observed percentage mortality was corrected using Abbott's formula[19].

Larvicidal bio-assay: The infusion (powdery form) of the leaf and root of *Clerodendrum infortunatum* samples was mixed with the larval food in different ratios of 1:1, 1:3: and 1:7. Pots were prepared with plastic tray (45 × 30 cm) having cubic wells of 3cm^2 (13 × 8 wells) with a thin layer of plaster of Paris at the bottom. Fourth instar larvae (n=10) were exposed in different rearing pots and were fed on food with plant infusions at different concentrations with negative (without insecticide) and positive controls[20]. Larval mortality was examined under microscope and corrected mortality was calculated[19].

Phytochemical analysis:

Phase separation - Hexane extract of *C. infortunatum* leaves was dissolved in hexane: methanol (1:1, v/v) for phase separation (polar soluble and non-polar soluble) on the basis of polarity and each phase was collected and dried at 40° C separately.

Thin layer chromatography (TLC) - Thin layer chromatography plate was prepared on micro glass slides (5 × 1.5 cm) by making slurry of silica gel and gypsum in dichloromethane. Spots of the methanol and hexane phase of *C. infortunatum* leaves (hexane extract) were marked with the help of capillary tube on the plate. The slides were placed in solvent (chloroform: methanol:: 93:7, v/v) and left the loaded sample to resolve. The slide was taken out after complete resolution and allowed to dry. Sulphuric acid (20%) was sprayed, observed under ultra-violet light to see appearance of purple spot[21].

Column chromatography - Silica gel column chromatography was performed using hexane (100%) and followed by hexane : ethyl acetate (95: 5, 90:10,85:15, 80:20, 75:25, 70:30, 65:35, 60:40, v/v) solvent[21]. Nine eluents were taken out and bio-assay was conducted for the different eluents to find out the molecule(s) responsible for the adulticidal effect. TLC was performed again for the effective eluent with the solvent (chloroform: methanol, 93:7, v/v) and 20 per cent sulphuric acid was sprayed to observe for appearance of any distinct purple spot. The eluent showing the distinct spot was kept for further analysis of molecule responsible for the insecticidal effect. Absorbance was noted at 300 nm using ultra-violet visible ray (UV-VIS) spectrophotometer[21].

High performance liquid chromatography (HPLC) - The sample showing the purple spot in TLC indicating the presence of diterpenoid was sent for HPLC to validate the active molecule at Sophisticated Analytical Instrument Facility (SAIF), Central Drug Research Institute, Lucknow[21].

Results & Discussion

Different plant extracts were tested for their adulticidal and insecticidal effect on sandflies. Highest mortality of adult sandflies (100%) were observed with the crude aqueous extract of the leaf of *Nicotiana tabacum* (Voucher No.PK1) followed by the hexane extract (77.7%) of the root of *C. infortunatum* (Voucher No. PK90) (Table). The experiments conducted earlier with petroleum ether (3600 mg/l), ethyl acetate (2800 mg/l) and methanol (4500 mg/l) extracts of *Lantana camara* leaves showed 39.4, 13.33 and 87.5 per cent, mortality of *P. argentipes*, respectively[22]. The methanol extract of *Tarchonanthus camphoratus* was found to be effective against *P. duboscqi*, but there was no effect of ethyl acetate extract on the same species[17]. Crude extracts from dried aerial parts of *T. camphoratus*, *Acalypha fruticosa* and *Tagetes minuta* were found to reduce the fecundity of *P. duboscqi* significantly ($P<0.05$) and vectorial capacity of sandflies in Kenya[23]. The essential oils of *Eucalyptus* spp. were effective against the egg, larval and adult phases of *Lutzomyia longipalpis*[24]. Comparable LD50 (50% lethal dose) values were found with n-hexane, dichloromethane, ethyl acetate and methanol extracts of *T. minuta* and *A. fruticosa* extracts[20]. The insecticidal action of myrtle oil against sandflies was 62.2 per cent at a concentration of 1 mg/cm^2 (Ref. 25). *Antonia ovata* and *Derris amazonica* displayed significant insecticidal effect against *L. longipalpis*[26]. Lemon oil applied to human skin was found to be 70 per cent protective against sandfly bites[27]. Neem oil (2%) mixed with coconut or mustard oil was found 100 per cent protective against the bite of *P. argentipes*[28]. No larval mortality was observed with infusion of *C. infortunatum* added in larval food in the present study. However, 100 per cent larvicidal effect of aqueous crude extract of *N. tabacum* leaf was observed in IV instar larvae of sandflies. The leaf extract of *C. infortunatum* has been found to be non-toxic to Swiss Albino mice[29]. The toxicity of *N. tabacum* plant has already been reported against African catfish (*Clarias gariepinus*)[30], thereby restricting its further analysis as a future insecticide.

The column separated ninth fraction of the hexane extract-methanol phase of *C. infortunatum* leaf was found most effective (63%) in the bio-assay test. A purple spot appeared on TLC with the same fraction indicating the presence of a diterpenoid. This fraction contained nine compounds. High peaks were observed for compounds 1 and 5 with retention time of 2.439 and 5.182 min and peak area 20.44 and 56.52 per cent, respectively. One of these or both might be the active molecule(s) showing insecticidal property. Presence of the diterpene clerodane cannot be ignored. It has been previously reported that clerodane is a biologically active molecule in *C. infortunatum*[31]. Further purification is required for confirmation and presence of other compounds by employing further bio-assay tests. Once the structure of the active molecule is identified and validated, it can be further synthesised and formulated as product.

The insecticidal effect of plants varies according to the distribution of plant species in different localities, seasonality, part of the plant used and the solvent used for extraction. Majority of the reports available are on

Table. Susceptibility status of adult sandflies (*P.argentipes*) exposed to different extracts of plants

Sl. No.	Plant species	Family	Plant part used	Concentration (mg/l)	Solvent	% Corrected mortality ± SE (n=10) for each replicate
1	*Nicotiana tabacum*	Solanaceae	Leaf	7900	Aqueous	100 ± 0.001
2	*Jatropha gossypifolia*	Euphorbiaceae	Leaf	7000	Aqueous	10.6 ± 0.016
3	*Physalis minima*	Solanaceae	Leaf	800	Aqueous	0 ± 0.000
			Flower	-	Aqueous	0 ± 0.000
4	*Datura alba*	Solanaceae	Leaf	400	Aqueous	0 ± 0.000
			Flower	-	Aqueous	0 ± 0.000
5	*Ipomoea fistulosa*	Convolvulaceae	Leaf	3600	Aqueous	0 ± 0.000
			Flower	-	Aqueous	0 ± 0.000
6	*Vitex negundo*	Verbenaceae	Leaf	3600	Aqueous	0 ± 0.000
7	*Tamarindus indica*	Caesalpinaceae	Leaf	4300	Aqueous	20 ± 0.002
			Leaf	6900	Aqueous	0 ± 0.000
8	*Evolvulus alsinoides*	Convolvulaceae	Stem	5300	Aqueous	0 ± 0.000
9	*Cynotis axillaris*	Commelinaceae	Root	1900	Aqueous	0 ± 0.000
			Leaf	5400	Aqueous	0 ± 0.000
			Stem	16000	Aqueous	0 ± 0.000
10	*Clerodendrum infortunatum*	Verbenaceae	Whole plant	-	Ethyl Acetate	44.4 ± 0.008
			Whole plant	-	Acetone	0 ± 0.000
			Whole plant	-	Hexane	22.2 ± 0.002
			Leaf	100	Hexane	36.1 ± 0.065
			Bark	6900	Hexane	22.2 ± 0.002
			Root	15257	Hexane	77.7 ± 0.086
			Leaf	5000	Methanol	44.5 ± 0.085
			Bark	1600	Methanol	0 ± 0.000
			Root	3100	Methanol	0 ± 0.000

insecticidal effect of plant extracts on different insects other than sandflies. The column separated ninth fraction of *C. infortunatum* leaf extract was found to be 63 per cent effective against adult *P. argentipes*. Further identification and characterization of the active molecule(s) is required to formulate a potential insecticide against sandflies.

Acknowledgment

Authors thank Miss Aarti Rama, Shriyut N. K. Sinha, S. A. Khan, A. K. Mandal of the Division of Vector Biology and Control of Rajendra Memorial Research Institute of Medical Sciences, Patna, for their support in searching of literature, conducting experiment and preparation of manuscript.

***Conflicts of Interest*:** None.

References

1. Dinesh DS, Das ML, Picado A, Roy L, Rijal S, Singh SP, *et al*. Insecticide susceptibility of *Phlebotomus argentipes* in visceral leishmaniasis endemic districts in India and Nepal. *PLoS Negl Trop Dis* 2010; *4* : e859.
2. Dinesh DS, Das P, Picado A, Davies C, Speybroeck N, Ostyn B, *et al*. Long-lasting insecticidal nets fail at household level to reduce abundance of sandfly vector *Phlebotomus argentipes* in treated houses in Bihar (India). *Trop Med Int Health* 2008; *13* : 953-8.
3. Singh RK, Mittal PK, Dhiman RC. Insecticide susceptibility status of *Phlebotomus argentipes*, a vector of visceral leishmaniasis in different foci in three states of India. *J Vector Borne Dis* 2012; *49* : 254-7.
4. Picado A, Das ML, Kumar V, Kesari S, Dinesh DS, Roy L, *et al*. Effect of village-wide use of long-lasting insecticidal nets on visceral leishmaniasis vectors in India and Nepal: a cluster randomized trial. *PLoS Negl Trop Dis* 2010; *4* : e587.
5. Isman MB. Neem and other botanical insecticides: barriers to commercialization. *Phytoparasitica* 1997; *25* : 339-44.
6. Sukumar K, Perich MJ, Boobar LR. Botanical derivatives in mosquito control: a review. *J Am Mosq Control Assoc* 1991; *7* : 210-37.
7. Renbold H. Secondary plant compounds in insect control with special reference to azadirachtins. *Adv Invert Reprod* 1984; *3* : 481-91.
8. Franzen H. Need for development of new strategies for locust control. In: Rembold H, Bonn, editor. *New strategies for locust control*, vol. 89. Bonn, Germany: ATSAF: 1993. p. 9-13.
9. Food and Agriculture Organization (FAO). *Pesticide residues in food*, Report FAO Plant production and protection paper No. 116, Rome: FAO; 1992.
10. Raji JI, Akinkurolere RO. The Toxicity of some indigenous plant extracts on the developmental stages of mosquito (*Anopheles gambiae*). *Nigerian Bioscientist* 2010. Available from: *http://nigerianbioscientist.com*, accessed on December 8, 2015.
11. Ciccia G, Coussio J, Mongelli E. Insecticidal activity against *Aedes aegypti* larvae of some medicinal South American plants. *J Ethnopharmacol* 2000; *72* : 185-9.
12. Kimball JW. Kimball's biology pages. Online biology textbook. Available from: *http://users.rcn.com/jkimball.ma.ultranet/BiologyPages/2003*, accessed on August 19, 2003.
13. Yang YC, Lee SG, Lee HK, Kim MK, Lee SH, Lee HS. A piperidine amide extracted from *Piper longum* L. fruit shows activity against *Aedes aegypti* mosquito larvae. *J Agric Food Chem* 2002; *50* : 3765-7.
14. Varma J, Dubey NK. Prospectives of botanical and microbial products as pesticides of tomorrow. *Curr Sci* 1999; *26* : 172-9.
15. Roark RC. Some promising insecticidal plants. *Econ Bot* 1947; *1* : 437-45.
16. Shaalan EA, Canyon D, Younes MW, Abdel-Wahab H, Mansour AH. A review of botanical phytochemicals with mosquitocidal potential. *Environ Int* 2005; *31* : 1149-66.
17. Ireri LN, Kongoro J, Ngure P, Mutai C, Langat B, Tonui W, *et al*. The potential of the extracts of *Tagetes minuta* Linnaeus (Asteraceae), *Acalypha fruticosa* Forssk (Euphorbiaceae) and *Tarchonanthus camphoratus* L. (Compositae) against *Phlebotomus duboscqi* Neveu Lemaire (Diptera: Psychodidae), the vector for *Leishmania major* Yakimoff and Schokhor. *J Vector Borne Dis* 2010; *47* : 168-74.
18. World Health Organization (WHO). *Control of the leishmaniases*. Report of a WHO Expert Committee. WHO Tech Rep Ser No. 793; Geneva: WHO; 1990. p. 1-158.
19. Abbott WS. A method of computing the effectiveness of an insecticide. *J Econ Entomol* 1925; *18* : 265-7.
20. Ireri LN, Kongoro J, Ngure PK, Tonui W. Laboratory evaluation of selected medicinal plant extracts in sugar baits and larval food against *Phlebotomus Duboscqi* Neveu Lemaire (diptera: Psychodidae), a vector for cutaneous leishmaniasis in Kenya. *Web Med Central Parasitol* 2011; *2* : 1-10.
21. Harborne JB. *Phytochemical methods: a guide to modern techniques of plant analysis*. 2nd ed. New York: Chapman and Hall; 1984.
22. Kumari S, Dinesh DS, Kumar A, Kumar V, Das P. Insecticidal and fungicidal effect of plant extract- a laboratory based study. *Int J Agric Sci Res* 2013; *3* : 17-24.
23. Samuel M, Zipporah N, Rosebella M, Zipporah O, Peter N, Philip N, *et al*. Effect of leaf crude extracts of *Tarchonanthus Camphoratus* (Asteraceae), *Acalypha fruticosa* (Fabaceae) and *tagetes minuta* (Asteraceae) on fecundity of *Phlebotomus duboscqi. Am Int J Contem Res* 2012; *2* : 194-200.
24. Maciel MV, Morais SM, Bevilaqua CML, Silva RA, Barros RS, Sousa RN, *et al*. Chemical composition of *Eucalyptus* spp. essential oils and their insecticidal effects on *Lutzomyia longipalpis. Vet Parasitol* 2010; *167* : 1-7.
25. Yaghoobi-Ershadi MR, Akhavan AA, Jahanifard E, Vatandoost H, Amin GH, Moosavi L, *et al*. Repellency effect of Myrtle essential oil and DEET against *Phlebotomus papatasi*, under laboratory conditions. *Iran J Public Health* 2006; *35* : 7-13.

26. Luitgards-Moura JF, Castellon Bermudez EG, Rocha AF, Tsouris P, Rosa-Freitas MG. Preliminary assays indicate that *Antonia ovata* (Loganiaceae) and *Derris amazonica* (Papilionaceae), Ichthyotoxic plants used for fishing in Roraima, Brazil, have an insecticide effect on *Lutzomyia longipalpis* (Diptera: Psychodidae: Phlebotominae). *Mem Inst Oswaldo Cruz* 2002; *97* : 737-42.

27. Rojas E, Scorza JV. The use of lemon essential oil as a sandfly repellent. *Trans R Soc Trop Med Hyg* 1991; *85* : 803.

28. Sharma VP, Dhiman RC. Neem oil as a sandfly (Diptera: Psychodidae) repellent. *J Am Mosq Control Assoc* 1993; *9* : 364-6.

29. Das S, Bhattacharya S, Biswas M, Kar B, Suresh Kumar RB, Pramanik G, *et al*. Acute and sub-chronic toxicity study of *Clerodendron infortunatum* leaf in adult male Albino mice. *Am-Eurasian J Sci Res* 2011; *6* : 188-91.

30. Kori-Siakpere O, Oviroh EO. Acute toxicity of tobacco (*Nicotiana tabacum*) leaf dust on the African catfish: *Clarias gariepinus* (Burchell, 1822). *Arch Appl Sci Res* 2011; *3* : 1-7.

31. Abbaszadeh G, Srivastava C, Walia S. Insect growth inhibitory activity of clerodane diterpenoids isolated from *Clerodendron infortunatum* L. on the cotton bollworm, *Helicoverpa armigera* (Hubner). *Nat Acad Sci Lett* 2012; *35* : 457-64.

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