



## MOSQUITO REPELLENT ACTIVITY OF THE LEAF EXTRACTS OF *LAWSONIA INERMIS* (L)

<sup>1</sup>Datti,\*Y. and Idris, M.M.

<sup>1</sup>Chemistry Department, School of Science Education, Federal College of Education (Technical) Bichi, Kano,

<sup>2</sup>Department of Pure and Applied Chemistry, Bayero University, PMB 3011, Kano, Nigeria

Correspondence author: [whydee@yahoo.co.uk](mailto:whydee@yahoo.co.uk)

### ABSTRACT

**The ethanolic extract of the leaves of *Lawsonia inermis* (commonly known as *lalle* in Hausa) was sequentially extracted with petroleum ether, chloroform, ethyl acetate and methanol. The extracts were respectively labeled LI1, LI2, LI3, and LI4, with the ethanolic extract labeled LI, and 12.5% and 25% of the test extracts were tested for repellency against *Anopheles gambiae*, and the repellent activity was assessed using the human-bait technique. Five volunteers participated in the laboratory tests, one each for the extracts. The chloroform extract labeled LI1-02 was found to be the most active, (100% repellency), while the extract labeled LI1-03 (25%) recorded the least activity (15.4%). It can be concluded that the chloroform extract labeled LI1-02 is responsible for repellent activity of *Lawsonia inermis*.**

**Keywords:** Repellency, *Anopheles gambiae*, human-bait technique, *Lawsonia inermis* extracts.

### INTRODUCTION

Vector-borne diseases are among the most important public health problems and obstacles to socioeconomic development of developing countries, particularly in the tropics, with malaria alone causing an estimated 1.5–2.7 million deaths and 300–500 million cases per year (WHO, 1997). To date, no method of malaria control has proven effective enough to control the high transmission intensities found in sub-Saharan Africa (Beier *et al.*, 1999; Lengeler *et al.*, 1998).

Over two billion people, primarily in tropical countries, are at risk from mosquito-borne diseases, such as dengue hemorrhagic fever, malaria and filariasis (Service, 1993). The search for effective vaccines against these diseases is still in progress. Mosquito control and personal protection from mosquito bites are currently the most important measures to control these diseases. The use of repellents is an obvious practical and economical means of preventing the transmission of these diseases to humans (Coleman *et al.*, 1993). However, human toxicity reactions after the applications of synthetic repellents vary from mild to severe (Zadikoff, 1979; Edwards and Johnson, 1987). To avoid these adverse effects, research on repellents that are derived from plant extracts to replace synthetic repellents with such adverse effect has been conducted in many laboratories (Edwards and Johnson, 1987). Recently, extracts of several plants, including neem (*Azadirachta indica*), basil (*Ocimum basilicum*), citronella grass (*Cymbopogon nardus*), galingale (*Alpinia galanga*), clove (*Syzygium aromaticum*) and thyme (*Thymus vulgaris*), have been studied as possible mosquito repellents (Sharma *et al.*, 1993; Barnard, 1999).

Repellents are substances that are designed to make surfaces unpleasant or unattractive to organisms like insects, animals and plants. They typically contain an active ingredient that repels unwanted organisms as well as secondary ingredients, which aid in delivery and cosmetic appeal. Repellency

is known to play an important role in preventing the vector borne diseases by reducing man-vector contact. Synthetic chemicals and insecticides used for control of vectors are causing irreversible damage to the eco-system, as some of them are non-degradable in nature (Das *et al.*, 2003). Thus the need to find repellents of natural origin arises.

Aklilu *et al.*, (2002) were able to quantify the repellent activity of a live-potted plants against *Anopheles gambiae*. The repellent effect of some six aboriginal plant species was confirmed against *Trogoderma granarium* by Dwivedi and Shekhwat (2004). While in 2008, Shah and his colleagues were able to ascertain the repellent effect of some plants' extract against saw-toothed grain beetle (*Oryzaephilus surinamensis* (L)). In 2001, the potential of volatile oils derived from some plant species, turmeric, citronella and hairy basil, for use as topical repellents against both diurnal and nocturnal mosquitoes, was demonstrated by Tawatsin *et al.* The oviposition deterrent and skin repellent activities of *Solanum trilobatum* leaf extract was confirmed against the malarial vector *Anopheles stephensis*, by Rajkumar and Jebanesan (2005). Similarly, in 2006, Tawatsin *et al.*, were able to evaluate the repellency effect of essential oils extracted from some plants in Thailand against mosquito vectors, diptera and culicidae, as well as the extracts oviposition deterrent effects against *Aedes aegypti*. Also, in 2009, Pugazhvendan *et al.*, tested and ascertained the repellent activity of the leaves of three plant species; *Argemone mexicana*, *Tephrosia purpurea* and *Prosopis juliflora*, against *Tribolium castaneum*, the red flour beetle, which is a major pest of grain based products (e.g. flour mills, ware houses and retail stores). Maharaj and Gayaram (2008), showed the repellent activities of some plant species against mosquito, using the time lag trials. Lastly, the effectiveness of an essential oil derived from *Zanthoxylum piperatum* was confirmed to be an alternative to some standard synthetic repellents in a research conducted by Kamsuk *et al.*, (2006).

*Lawsonia inermis* is a shrub of Asian origin. The plant is extensively used in the West and North African ethnomedical practice for the treatment of jaundice, hysteria and nervous disorder (Kumar et al., 2005). The leaves are utilized locally for cosmetic purposes especially as tint for hands, feet and nails (Singh et al., 2005). Henna also acts as an anti-fungal (Bosoglu et al., 1998) and a preservative for leather and cloth. It was listed in the medical texts of the Ebers Papyrus (Bryan and Smith, 1774) and by Ibn Qayyim al-Jawziyya (14th c CE (Syria and Egypt) as a medicinal herb (Ibnil Qayyim). The use of *Lawsonia inermis* (henna) in the management of burn wound infections has also been reported by Muhammad and Muhammad (2005).

### MATERIALS AND METHODS

Three hundred gram of the dried and ground form of the leaves *Lawsonia inermis*, was put in 2.5L brown capacity brown bottle, and 1L of 90% ethanol was added. The set up was left for two weeks with constant shaking after which the mixture was filtered and then concentrated using rotavapor, to get the crude residues labeled LI. Twenty gram of the crude residue was dissolved in aqueous methanol and then extracted with 100cm<sup>3</sup> of petroleum ether (3 times). The petroleum ether extract was concentrated, dried, weighed and labeled LI1-01. The aqueous methanol portion was again extracted with 100cm<sup>3</sup> chloroform (3 times), and the extract was concentrated, dried, weighed and labeled as LI1-02. The aqueous methanol portion was again extracted with 100cm<sup>3</sup> ethyl acetate (3 times), and the extract was concentrated, dried, weighed and labeled as LI1-03. The aqueous methanol portion was finally concentrated, dried, weighed and labeled as LI1-04. Each of the five extracts LI1, LI1-01, LI1-02, LI1-03 and LI1-04, was each tested for repellency against *Anopheles gambiae*.

### Test mosquitoes and the Repellency test

The mosquitoes used in this study were laboratory-reared *Anopheles gambiae* (age 3-5 days). These were reared at the Department of Biological Sciences of Bayero University Kano, who willingly supplied the researchers with the larvae used in this research. Mosquito larvae have a well-developed head with mouth brushes used for feeding, a large thorax, and a segmented abdomen. They have no legs. In contrast to other mosquitoes, *Anopheles* larvae lack a respiratory siphon and for this reason position themselves so that their body is parallel to the surface of the water. Larvae breathe through spiracles located on the 8th abdominal segment and therefore must come to the surface frequently. The larvae occur in a wide range of habitats but most species prefer clean, unpolluted water. Larvae of *Anopheles* mosquitoes have been found in fresh- or salt-water marshes, mangrove swamps, rice fields, grassy ditches, the edges of streams and rivers, and small, temporary

rain pools. Many species prefer habitats with vegetation.

The repellency of the five extracts from *Lawsonia inermis*, were all assessed in the laboratory using a human-bait technique (WHO, 1996). Five volunteers (age 21-34 years) participated in the laboratory tests, with each volunteer exposed to only one of the extracts at a time. The testing period lasted up to eight hours spread over 14 days, depending on the efficacy of repellent. The timing of the tests depended on the fact that *Anopheles gambiae* are night-biters. Evaluations were carried out in a large room, at room temperature. An area of 3x10 cm on each forearm of each of the human volunteers was marked out with a permanent marker. The test extract 12.5% and 25% W/V (extract/ethanol) was applied to the marked area of one forearm of each volunteer, while the other forearm was treated with only ethanol free from the extracts, to serve as a control. During the test, the forearm was covered by a paper sleeve with a hole corresponding to the marked area. Each volunteer put the test forearm in a mosquito cage (80x40x40 cm<sup>3</sup>), containing 50 female mosquitoes (3-5 days old), for the first three minutes of every half-hour exposure. However, before the start of each exposure, the bare hand, used as control area of each volunteer, was exposed for up to 30 seconds. If at least two mosquitoes landed on the hand, the repellency test was then continued. This was done to ensure that the mosquitoes were host seeking. The number of mosquitoes probing the treated area of each volunteer was noted for half-hour (Tawatsin et al, 2001).

Percentage repellency (% repellency) in the field evaluation was analyzed according to the formula described by Yap et al., (1998).

$$\% \text{ Repellency} = \frac{C - T}{C} \times 100$$

Where C is the number of mosquitoes that landed on the controls and T is the number of mosquitoes that landed on the treated volunteers.

### RESULTS AND DISCUSSION

From the result obtained, it can be seen that different extracts show certain level of repellency, with LI1-02, which is the chloroform extract showing the highest degree of repellency of 100%, while LI1-03 showed the least degree of repellency of 15.4%, while other extracts have also shown some degree of repellency ranging from 15.6% to 93.5%. Going back to the technique employed by Tawatsin et al, 2001, where the number of mosquitoes probing the treated hand is counted, and the repellency evaluation technique adopted by Yap et al., (1998), the most active of the four extracts will be the one with the least number of mosquitoes probing the hand, which will consequently be the one with the highest degree of repellency, we can say that the most active of these extract is the chloroform extract LI1-02.

**Table 1: Physical features of the five extracts from *Lawsonia inermis***

S/N	EXTRACT	WEIGHT (g)	COLOUR
1	LI1	29.24	Dark green
2	LI1-01	6.46	Green
3	LI1-02	7.84	Green
4	LI1-03	5.63	Green
5	LI1-04	4.87	Black

**Table 2: Result of Bioactivity Test**

Fraction	Concentration (%)	No of Mosquitoes	% Repellency
LI1	12.5	5	83.9
	25.0	2	93.5
CONTROL	0	31	
LI1-01	12.5	21	34.4
	25.0	27	15.6
CONTROL	0	32	
LI1-02	12.5	0	100
	25.0	0	100
CONTROL	0	28	
LI1-03	12.5	23	41.0
	25.0	33	15.4
CONTROL	0	39	
LI1-04	12.5	19	44.1
	25.0	27	20.6
CONTROL	0	34	

**CONCLUSION**

From the result obtained from this research, it can be concluded that extract LI1-02 is the most active, and is more effective in repelling mosquitoes, while LI1-03 is less effective. The repellent activity of the chloroform extract from the leaves of *Lawsonia*

*inermis* is an important discovery in our struggle to find a lasting solution to the menace of mosquitoes in particular, and insects in general. Based on this result, further work should be geared towards isolating and characterizing the active compound in LI1-02.

**REFERENCES**

- Aklilu, S., Kabiru, E. W., Wande, W. L., Killeen, F., and Ali, A. H. (2002). Repellency of live potted plants against *Anopheles gambiae* from human baits in semi-field experimental huts. *Am. J. Trop. Med. Hyg.* 67(2) 191-195
- Anand, K. K., Singh, B., Chand, D., Chandan, B. K. (1992). An evaluation of *Lawsonia alba* extract as hepatoprotective agent. *Planta Med.* 58:22-25.
- Barnard, D. R. (1999). Repellency of essential oils to mosquitoes (Diptera: Culicidae). *J. Med. Entomol.* 36 625-629
- Beieret, J. C., Killeen, GF., Githure, J. (1999). Short report: entomologic inoculation rates and *Plasmodium falciparum* malaria prevalence in Africa. *Am J Trop Med Hyg* 61: 109-113.
- Bhandarkar, M., Khan, A. (2003). Protective effect of *Lawsonia alba* Lam. against carbontetrachloride induced hepatic damage in albino rats. *Indian J Exp Biol.* 41(1):85-87
- Bosoglu A, Birdane F, Solmaz, H. (1998). "The effect of Henna (Folium lawsoniae) paste in ringworm in calves". *Indian Veterinary Journal* 75 (1): 83-84.
- Bryan, C. P., Elliot G. S. (1974) [1930]. *Ancient Egyptian medicine: the Papyrus Ebers*. Chicago: Ares Publishers.
- Chaudhary G., Sandeep G., Priyanka P. (2010). *Lawsonia inermis* Linnaeus: A Phytopharmacological Review *IJPSDR April-June, Vol 2, Issue 2 (91-98)* 97
- Das, N. G., Baruah, I., Talukdar, P. K., and Das, S. C. (2003). Evaluation of botanicals as repellents against mosquitoes. *J. Vect. Borne Dis.* 40 49-53
- Dikshit, V., Dikshit, J., Saraf, M., Thakur, V., Sainis, K. (2000). Immunomodulatory activity of naphthoquinone fraction of *Lawsonia inermis* Linn. *Phytomedicine (Jena)*. 7:102-103.
- Dixit, S. N., Srivastava, H. S., Tripathi, R. D. (1980). Lawsons, the antifungal antibiotic from the leaves of *Lawsonia inermis* and some aspects of its mode of action. *Indian Phytopathol.* 31:131-133
- Dwivedi, S. C., and Shekhwat, N. B. (2004). Repellent effect of some indigenous plant extracts against *Trogoderma granarium*. *Asian J. Exp. Sci.* 18:1&2 47-51
- Edwards, D. L., and Johnson, C. E. (1987). Insect-repellent-induced toxic encephalopathy in a child. *Clin. Pharm.* 6 496-498.
- Hemalatha, K., Natraj, H. N., Kiran, A. S. (2004). Hepatoprotective activity of leaves of *Lawsonia alba*. *Indian J Nat Prod.* 20(4):14-17.

- Ibn Qayyim al-Jawziyah, Muhammad ibn Abi Bakr. *Medicine of the Prophet*. trans. Penelope Johnstone. Cambridge: Islamic Texts Society (1998) pp 223.
- Innocent, E., Gikonyo N. K., and Nkunya M. H. H. (2008). Repellency property of long chain aliphatic methyl ketones against *Anopheles gambiae*. *Tanzania j. Health Research* 10:1
- Kumar, S., Singh Y. V., and Singh, M. (2005). "Agro-History, Uses, Ecology and Distribution of Henna (*Lawsonia inermis* L. syn. *Alba Lam*)". *Henna: Cultivation, Improvement, and Trade*. pp. 11–12.
- Latha, P. G., Suja, S. R., Shyamal, S., Rajasekharan, S. (2005). Some hepatoprotective garden plants. *Natural Product Radiance*. 4(4):278-279.
- Lengeler, C., Smith, T. A., Armstrong-Schellenberg, J. (1997). Focus on the effect of bednets on malaria morbidity and mortality. *Parasitol Today* 13: 123–124.
- Maharaj, R., and Gayaram, R. (2008). Testing plant samples for mosquito repellency, time lag trials. *Malaria research lead programme* 1:96
- Muhammad, H. S., Muhammad, S. (2005). The use of *Lawsonia inermis* Linn. (henna) in the management of burn wound infections. *African Journal of Biotechnology* 4:934-937.
- Rajkumar, S., and Jebanesan, A. (2005). Bioactivity of flavonoids compounds from *Poncirus trifoliata* against the dengue vector, *Aedes aegypti*. *J. Parasitology Research* 104:1 19-25
- Service, M. W. (1993). Mosquitoes (*Culicidae*). In: R.P. Lane and R.W. Crosskey (Eds.), *Medical Insects and Arachnids* Chapman & Hall, London, pp 723
- Shah, M. M. R., Prodhan, M. D. H., Siddique, M. N. A., Mamun, M. A. A., and Shahjahan, M. (2008). Repellent effect of some indigenous plant extracts against saw-toothed grain beetle *Oryzaephilus surinamensis*, *Int. J. Sustain Crop Prod*, 3(5): 51-52
- Sharma, V. P., Ansari, M. A., and Razdan, R. K. (1993). Mosquito repellent action of neem (*A. indica*) oil. *J. Am. Mosq. Contr. Assoc.* 9 359-360
- Singh, M., Jindal, S. K., & Singh, D. (2005). "Natural Variability, Propagation, Phenology and Reproductive Biology of Henna". *Henna: Cultivation, Improvement, and Trade*. pp. 13–18.
- Tawatsin, A., Wratten, S. D., Thavara U., and Scott, R. R. (2001). Repellency of volatile oils from plants against three mosquito vectors. *J. Vector Ecology* 26(1) 76-82
- Tripathi, R. D., Srivastava, H. S., Dixit S. N. (1978). A fungi toxic principle from the leaves of *Lawsonia inermis*. *Experientia* (Basel). 34:51-52.
- WHO, (1996). Report of the WHO informal consultation on the evaluation and testing of insecticides. CTD/WHOPES/IC/96.1, Control of Tropical Diseases Division. World Health Organization, Geneva, pp 69
- Zadikoff, C. M. (1979). Toxic encephalopathy associated with use of insect repellent. *J. Ped.* 95:140-142