WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.084
Volume 9, Issue 8, 2259-2267. Research Article ISSN 2277– 7105

MELIACEAE PLANTS AND VECTOR CONTROL OF MALARIA: LARVICIDAL TOXICITY OF EXTRACTS AND FRACTIONS OF *TRICHILIA MONADELPHA* AND *TRICHILIA EMETICA* ON LARVAE OF *ANOPHELES GAMBIAE*

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Article Received on
24 June 2020,

Revised on 13 July 2020,
Accepted on 03 August 2020,

DOI: 10.20959/wjpr20208-18236

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ABSTRACT

Introduction: Plants will continue to provide novel products as well as chemical models for new drugs in the coming centuries and may be used as sources of alternative agents for control of mosquitoes because of their rich bioactive chemicals. **Aim:** to evaluate the larvicidal toxicity of extracts and fractions of *Trichilia monadelpha* and *Trichilia emetica* on larvae of *anopheles gambiae*. **Materials & Methods:** The methanol extracts of the leaves and seeds of *T. monadelpha* and leaves of *T. emetica* of the Meliaceae family were subjected to toxicity studies on larvae of *Anopheles grambiae*, the malaria vector. The plant materials were extracted by maceration in 100% redistilled methanol for 72 hours and concentrated using a rotatory evaporator. The preliminary phytochemcial screening was done using standard methods. Larval toxicity of crude methanol extracts and fractions were evaluated by exposing fourth instar larvae of *A. gambiae* to varying concentrations (0.0625-1.0000 mg/mL). The larval mortality was recorded after 24 hours of exposure and LC_{50} values determined using the non-linear regression analysis of the graph pad prism® statistical package. **Results**: The methanol crude extract of *T. monadelpha* leaves, seeds and *T. emetica* leaves exhibited % mortalities and had LC_{50} values of >1mg/mL, 0.11mg/mL and 0.22mg/mL respectively. The hexane fractions of

T. monadelpha leaves, seeds and *T. emetica* leaves were the most active of the fractions with LC_{50} values of 0.03mg/mL, 0.05mg/mL and 0.03mg/mL respectively. The reference compound N, N-diethyl-3-toluamide (DEET) had an LC_{50} value of 1.09 mg/mL. Preliminary phytochemical screening revealed the presence of tannins, saponins, cardiac glycosides, flavonoids, anthraquinones and terpenes. **Conclusion**: The results of the study suggest that the extracts and fractions of both *Trichilia* species are promising agents to be considered for development as vector control agents for malaria as the extracts and fractions had overwhelming activities compared to the reference compound.

KEYWORDS: *Anopheles gambiae* larvae, *Trichilia monadelpha*, *Trichilia emetica*, larval toxicity.

INTRODUCTION

Malaria is one of the most devastating infections and represents a great health problem in tropical and subtropical climates, mainly in Sub-Saharan Africa, with no part of the world immune to the risk.[1] Globally, more than 2 billion people live in areas threatened by malaria. The morbidity and mortality associated with the malaria is mostly experienced in Sub-Saharan Africa.[2;3] Annually, there is an estimated 2 million death from malaria which is highest in children under 5 years of age.[4] One of the approaches for control of malaria is the interruption of its transmission, eliminating the malaria vector the female *Anopheles gambiae* mosquito. There has been a serious concern about the use of chemical based mosquitocides in the recent past. The extensive use of these synthetic insecticides have resulted in environmental hazards and development of physiologic resistance in vector species. This has necessitated the need for discovery and development of environmentally safe, biodegradable, economically viable and indigenous methods for vector control. Some herbal products such as nicotine obtained from tobacco leaves, *Nicotiana tabacum*, anabasine and lupinine, two alkaloids extracted from Russian weed *Anabasis aphylla*, rotenone from *Derris eliptica* and pyrethrin from *Chrysanthemum cinerariifolium* flowers have been used as natural insecticides even before the discovery of synthetic insecticides.[5] Several plant extracts are being tested for larvicidal property, particularly on the malaria vector.

T. monadelpha and *T. emetica* are plants of the family Meliaceae indeginous to tropical Africa. *Trichilia monadelpha* is a tree of rain forest especially secondary regrowth and in moist places, sometimes in gallery forest. The bark has analgesic property and is applied in the treatment of intercostals pain in lvory Coast. A leaf decoction is taken for heart problem

in Nigeria.[6] It was reported that the extract of the bark possesses anti-inflammatory and analgesic properties. Aladesanmi and co-workers (2007)[7] reported the antimicrobial and antioxidant activities.

Trichilia. emetica is a small deciduous of evergreen tree of 12–30m high and up to 90 cm in diameter. The plant is considered as emetic in high doses, antiepileptic, general tonic and for bronchial inflammation.[8] It is used as dentifrice[9], for hepatitis, dyspepsia, excessive flatulence, menorrhoea and sterility.

This paper reports the larval toxicity of *T. monadelpha* leaf, seed and *T. emetica* leaf extracts and fractions on the larvae of *Anopheles gambiae* mosquitoes in our continuing studies of the malaria vector control evaluation of Meliaceae plants.

MATERIALS AND METHODS

Collection and preparation

The leaves of *T. monadelpha* were collected from Arulogun village in Akinyele Local Government Area in June 2010, the seeds of *T. monadelpha* in September 2010 from the main campus, University of Ibadan and *T. emetica* leaves in August 2010 from Oluwoyin locality in Ido Local Government Area, all in Oyo State, Nigeria. Plant materials were identified and authenticated by Mr. Seun Osiyemi of forest herbarium, Forest Research Institute of Nigeria, Ibadan. Voucher specimens of *T. monadelpha* leaves, seeds and *T. emetica* leaves were deposited in Forest Herbarium Ibadan under FHI 108931, FHI 108931 and FHI 108932 respectively.

Extraction of plant materials

Powdered leaves (2,350g), seeds (1,608g) of *T. monadelpha* and leaves (2,450g) of *T. emetica* were air-dried, pulverized, weighed and macerated in 100% redistilled methanol for 72 hours, and concentrated in a rotatory evaporator. The extracts were kept in refrigerator (4^{o}C) after estimation of percentage yields.

Phytochemical Screening

The phytochemical screening was done using standard methods.[10]

Larval collection

Larvae were collected from breeding sites at Ojoo area, Ibadan, Oyo State, Nigeria and reared in plastic bowls containing clean well water. They were fed with dog biscuit.

Larval toxicity assay

Stock solutions of both the methanol crude extracts and fractions were prepared at 100mL with 1mL of ethanol and 99mL of untreated clean well water. This was serially diluted to the final test concentration of 0.0625-1.0000 mg/mL. Twenty fourth-instar larvae were released into each cup of 100mL solution and toxicity of extracts was estimated by percentage (%) mortality. After 24 hours of exposure, the number of dead larvae in the cups were counted. Control experiments, without the extract, but with 1% ethanol and N, N-diethyl -3–toluamide (DEET) were run in parallel. All the experiments were done in triplicates.

Bioassay-guided fractionation of crude extracts

The active methanol crude extracts (40g) were dissolved separately in methanol–water in the ratio 3:1 and partitioned successively with n-hexane, chloroform, and ethylacetate. All the fractions were concentrated to dryness and percentage yields obtained.

Statistical Analysis

Results were expressed as means ± SEM of three independent experiments. Larval toxicities were reported as LC_{50} obtained from Graph Pad Prism® Statistical Software.

RESULTS

Table 1: Extraction yields of methanol crude extracts and fractions.

Plants	Quantity Extracted (G)	Percentage Yield Of Crude Extracts	Quantity Partitioned (G)	Percentage(%) Yield Of Fractions N-Hexane Chloroform Ethylacetate Aqueous
T. monadelpha				
Leaf	2350	2.6	40	41.9 22.0 13.8 21.2
Seed	1608	13.9	40	22.5 22.9 12.5 26.0
T. emetica				
leaf	2450	2.5	40	42.3 9.3 26.7 3.2

Table 2: Preliminary phytochemical screening.

Tests	*Trichilia monadelpha* leaf	seed	*Trichilia emetica* Leaf
Alkaloids	-	-	-
Tannins	+	+	+
Saponins	+	+	+
Cardiac glycosides	+	+	+
Flavonoids	+	+	+
Anthraquinones	+	-	+
Terpenes	-	+	-

Key: - = absence of metabolite, + = present of metabolite

Table 3: Larvicidal effect of methanol crude extract of *Trichilia monadelpha* and *Trichilia emetic.*

Concentration (mg/mL)	*T. monadelpha* % mortality ± SEM		*T. emetic* % mortality ± SEM	DEET % mortality ± SEM (Positive control)
	Leaf	Seed	Leaf	
10	100±0.0	100±0.0	100±0.0	
5	100±0.0	100±0.0	100±0.0	
2.5 **A**	100±0.0	100±0.0	100±0.0	
1.25	100±0.0	100±0.0	100±0.0	
0.625	86.5±0.7	93.4±0.3	9.0±0.0	
2				100±0.0
1	45±0.6	100±0.0	100±0.0	45.0±1.0
0.5	41±0.3	100±0.0	80±1.2	37.5±2.0
0.25 **B**	20±0.0	98.5±0.3	68.5±1.9	30.0±2.5
0.125	15±0.0	75.0±2.5.	40±1.2	20.0±0.5
0.0625	8.5±0.0	0±0.0	26.5±1.5	
1% Ethanol (Negative control)				5±0.0

Results were expressed as the mean ± SEM of three independent experiments

Table 4: LC50 of methanol crude extracts of *Trichilia monadelpha* and *Trichilia emetica*.

Concentration (mg/mL)	*T.monadelpha*(LC50)		*T.emetica*(LC50) Leaf	DEET (mg/mL)
	leaf	seed		
0.625-10.0000	0.04	0.09	0.19	
0.0625-1.0000	>1.00	0.11	0.22	1.09

Table 5: Larvicidal effect of the most active fractions(n-hexane)

Concentration (mg/mL)	*T.monadelpha* % mortality ± SEM		*T.emetica* % mortality± SEM (n-hexane)
	leaf (n-hexane)	seed (n-hexane)	
0.1	100 ± 0.0	100± 0.0	100 ± 0.0
0.5	100 ± 0.0	100± 0.0	100± 0.0
0.25	100 ± 0.0	100± 0.0	100± 0.0
0.125	100 ± 0.0	98 ± 0.3	96.7± 0.7
0.0625	83.4 ± 0.0	58 ± 0.9	65 ± 0.6

Table 6: LC_{50} of most active fractions.

Concentration (mg/mL)	*T.monadelpha*(LC50)		*T.emetica*(LC50) Leaf (n-hexane)
	Leaf (n-hexane)	seed (n-hexane)	
0.0625-1.0000	**0.03**	**0.05**	**0.03**

The percentage yields of crude methanol extracts and fractions of *T. monadelpha* and *T. emetica* are displayed in Table 1. Yields ranged from 2.5% to 41.9%.

Preliminary phytochemical screening revealed the presence of tannins, saponins, cardiac glycosides, flavonoids, anthraquinones and terpenes (Table 2).

The methanol crude extracts of *T. monadelpha* leaf, seed and *T. emetic* leaf at the concentration range of 0.625-10.0000 mg/mL exhibited percentage mortality ±SEM as shown in table 3A with their LC_{50} of 0.04mg/mL, 0.09mg/mL and 0.19mg/mL respectively (Table 4).

At 0.0625-1.0000mg/mg/mL, the % mortalities were as shown in Table 3B with their LC_{50} >1mg/mL, 0.11mg/mL and 0.22mg/mL respectively (Table 4)

The n-hexane fractions at a concentration range of 0.0625-1.0000 mg/mL exhibited % mortalities (Table 5) with their LC_{50} 0.03mg/mL, 0.05mg/mL and 0.03 mg/mL respectively as stated in Table 6.

DISCUSSION

The percentage yields of crude methanol extracts and fractions of *T. monadelpha* and *T. emetica* are displayed in Table 1. Yields ranged from 2.5% to 41.9%.

Preliminary phytochemical screening revealed the presence of tannins, saponins, cardiac glycosides, flavonoids, anthraquinones and terpenes (Table 2).

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The reference compound (positive control) was DEET and it had LC_{50} 1.09mg/mL, while 1% ethanol was included as negative control.

Malaria reduces work capacities, impairs physical and mental development in man especially in children, diminishes the returns achieved through education and limits their potential to contribute fully to the social and economic growth of the countries. Malaria affects the health and wealth of nations and individuals alike. In Africa today, malaria is understood to be both a disease of poverty and a cause of poverty and it is estimated to result in growth penalty of 1.3% per person, per year in the region.[11;12] In some countries with very high malaria incidence, the disease may account for as much as 40% of the public health expenditure, 30–50% of impatient admissions and up to 60% out patient visits.[13]

Furthermore, it has been reported that malaria account for 25% of infant and 30% of childhood mortality.[14] Among pregnant women, malaria is estimated to cause as many as 10,000 deaths each year (RBM 2001–2010), contributing to approximately 2 to 15% of maternal anaemia, 8 to 14% low birth weight infants (an important contributor to infant mortality) and 3 to 8% of all infant deaths.[15]

However, the synergistic activity of phytochemical constituents of *T. monadelpha* leaf, seed and *T. emetica* leaf extracts and n-hexane fractions exhibited intrinsic larval toxicities and may serve as good alternative to vector control.

Kumar, and Maneemegalai[16] reported that the larvicidal activity of leaves and flowers of *Lantana camara* L (Verbenaceae) was observed to be attributed to the phytochemicals such as cardiac glycosides, flavonoids, terpenoids and saponins when tested on 3rd and 4th instar larvae of *Aedes aegypti* and *Culex quinquefasciatus* after 24 hours.

Also reported by Hadjikhoondi *et al*,[17] that in a biochemical investigation of different extracts and larvicidal activity of *Tagetes minuta* L on *Anopheles stephensi* larvae, the aglycone flavonoids and saponins might have been the effective components responsible for the larvicidal effect on the *Anopheles Stephensi* larvae.

Reported by Ho *et al*.,[18] was meliternatin, a flavonoid isolated from *Melicope subunifoliolata* stapf (Rutaceae) called anti-insects compound, when screened against two speices of insect; was shown to have strong feeding detterent activity against *Sitophilus zeamais* and very good larvicidal activity against *Aedes aegypti*. Moreover, identification of

novel effective mosquitocidal compounds is imperative because of increasing resistance of mosquitoes to currently used insecticides, concern for the environment and food safety, the unacceptability of many organophosphates and organochlorines and the high cost of synthetic pyrethroids.[19]

CONCLUSION

Activities displayed by methanol crude extracts and n-hexane fractions were concentration dependent and the results better than the toxicities exhibited by DEET, indicating that they could be considered for development as vector control agents for malaria.

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