



Received on 05 May, 2016; received in revised form, 15 June, 2016; accepted, 29 June, 2016; published 01 October, 2016

FORMULATION OF NATURAL MOSQUITO PAPER COIL AND EVALUATION OF MOSQUITOCIDAL ACTIVITY

K. Rajya Lakshmi*, M. Mounika and P. Srinivasa Babu

Vignan Pharmacy College, Vadlamudi, Guntur (dt), 522213, A.P., India

Keywords:

Annona squamosa,
leaf extracts, formulation,
Mosquitocidal activity, Paper coil

Correspondence to Author:

Dr. K. Rajya Lakshmi

Associate Professor,
Department of Pharmaceutical
Chemistry, Vignan Pharmacy
College, Vadlamudi, Guntur (Dt),
A.P, India.


Email: drkr13579@gmail.com

ABSTRACT: The main aim of the present study is to formulate low cost natural mosquito paper coil and to evaluate the mosquitocidal activity of *Annona squamosa* leaf extracts. *Annona squamosa* Linn. is the plant belonging to the family of Annonaceae commonly known as custard apple. Several bioactive compounds have been isolated from the *Annona squamosa* leaf extracts reported to possess anti-diabetic activity, Mosquitocidal activity, antioxidant and anti-bacterial activities. Mosquito species are well known vectors for the transmission of vector borne diseases affecting human beings particularly malaria, dengue, yellow fever, Chikungunya fever and filariasis. In recent years development of new synthetic products and their introduction into international usage has become a very costly affair and shows side effects. Hence, a lot of time, money and resources are channelled towards the synthetic formulation. Different extracts of *Annona squamosa* were prepared and they are formulated as a paper coil and evaluated by using commercially obtained paper coil as a standard for its mosquitocidal activity.

INTRODUCTION: Mosquitoes are well known transmitters which transmits various vector-borne diseases like malaria¹⁵, Dengue, Filariasis, Japanese encephalitis, Yellow fever, Chickunguniya etc in humans. These diseases are world's most hazardous diseases caused by mosquitoes. Dengue fever is a mosquito-borne tropical disease caused by the dengue virus. Symptoms typically begin three to fourteen days after infection. Dengue is spread by several species of mosquito of the Aedes type, principally *A. Aegypti*.

Malaria is a mosquito-borne infectious disease of humans and other animals caused by parasitic protozoa belonging to the Plasmodium type. Japanese encephalitis (JE) is formerly known as Japanese B encephalitis to distinguish it between various Economies. Encephalitis is a disease caused by the mosquito-borne Japanese encephalitis virus (JEV). Chikungunya is an infection caused by the chikungunya virus. Lymphatic filariasis, also known as elephantiasis tropica, is caused by parasitic worms of the roundworm family.

The worms spread diseases by the bites of infected mosquitoes. Infections usually begin in childhood. Three types of worms can cause the disease, namely *Wucheraria Bancrofti*, *W. Brugiamalayi*, *W. Brugiatimori*. These worms damage the lymphatic system. Therefore to avoid

<p>QUICK RESPONSE CODE</p> 	<p>DOI: 10.13040/IJPSR.0975-8232.7(10).4140-44</p>
<p>Article can be accessed online on: www.ijpsr.com</p>	
<p>DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.7(10).4140-44</p>	

such type of diseases, measures must be taken to reduce the mortality of human beings due to those diseases.

Annona squamosa Linn is a small ever green tree belonging to the family Annonaceae, cultivated throughout India for its fruits and different parts of the plant which are having high medicinal values. It is used as a folkloric medicine for treatment of various diseases. It is a small shrub or tree which is 7m in height. It is commonly known as sugar apple or custard apple.

The chemical constituents present in *Annona squamosa* leaves are 4-(2-nitro ethyl)-1-((6-O- β -D-xylopyranosyl- β -D-glucopyranosyl)oxy)benzene, Anonaine, Benzyltetrahydro-isoquinoline, Borneol, Camphene, Camphor, Car-3-ene, Carvone, β -Caryophyllene, Eugenol, Farnesol, Geraniol, 16-Hentriacontanone, Hexacontanol, Higenamine, Isocorydine, Limonene, Linalool, Linalool acetate, Menthone, Methylantranilate, Methylsalicylate, Methylheptanone, p-(hydroxyl benzyl)-6,7-(2-hydroxy,4-hydro) isoquinoline, n-Octacosanol, β -Pinene, Rutin, Stigmasterol, β -Sitosterol, Thymol and n-Triacontanol (Gowdhami M et al., 2014; Jayshree et al., 2008; Dinesh K. Yadav et al., 2011). Plant having pharmacological actions includes anti-bacterial⁸ activity, anti-diabetic⁶ activity, anti-genotoxic¹ agent, anti-hyperlipidemic¹ activity, anti-head lice effect, antioxidant^{1, 18} activity, anti-microbial³, anti-neoplastic⁴ activity, pesticidal⁷ activity, insecticidal⁷ activity, Mosquitocidal^{7, 14, 17} activity.

MATERIALS AND METHODS:

Collection of plant materials: The fresh leaves of *Annona squamosa* were collected at Guntur, Andhra Pradesh during the month of November, 2015 and the species were identified and authenticated by Prof. Satyanarayana Raju, Head of the Department of Botany and Microbiology, Acharya Nagarjuna University, Guntur.

Preparation of Plant Extracts:

The collected leaves were washed and dried under shade at room temperature. The dried leaves were powdered. The powdered leaves were weighed and stored in the container. The powdered plant material was subjected to Soxhlet extraction^{9, 13}

and cold maceration using methanol and chloroform solvents respectively. The excess solvent from the extract was removed and stored in a desiccator. Extractive values of methanolic and chloroformic extracts were found to be 5.05 and 3.23 % w/w respectively.

Preliminary Phytochemical Screening of Leaf extracts of *A. Squamosa*: Methanolic and chloroformic extracts of *Annona Squamosa* were subjected to preliminary phytochemical screening for the detection of secondary metabolites like alkaloids, steroids, flavonoids, tannins, Saponins, etc. as they are responsible for therapeutic effects. results are shown in the **Table 1**.

Screening of Phytoconstituents by TLC:

Methanolic and Chloroformic leaf extracts of *Annona squamosa* were subjected to Thin Layer Chromatographic studies to identify the active constituents. Results are given in the **Table 2**.

Formulation of Paper coil: The paper coils were formulated by taking the 7X7 dimensional What'smann filter paper and those are impregnated in various concentrations of methanol and chloroform extracts(1%, 2%, 3% w/v) respectively. Then the paper coils were dried at room temperature and collected.

Mosquito culture: Mosquito larvae were collected from the stagnant water from the garden in Guntur. The collected larvae were cultivated in the large tub¹⁶ containing fresh water and 1% sucrose solution is added as a nutrients to the larvae. After a few days, larvae were developed into pupae and further grown into adult mosquitoes. These developed mosquitoes were introduced into different chambers¹⁹.

Mosquitocidal activity: The Mosquitocidal activity was done by slight modification of the standard procedures of WHO and (Subrata Mallick et al 2015). Adult Mosquitoes were introduced into the 09 different chambers, measuring about 14.5 X 12.2 cm, each chamber containing 10 mosquitoes. The prepared paper coil was rolled into like a cone, which was inserted into the iron mesh that was placed at the top of the each chamber containing mosquitoes. This mesh was completely closed in

order to avoid escaping of the smoke from the chamber. Paper coil was lighted and the mortality of mosquitoes are observed at different time intervals.

Statistical analysis: The percentage of corrected mortality was analyzed by Handerson-Tiltons formula.

$$\text{Corrected\%} = 1 - \left(\frac{n \text{ in } C_0 \text{ before treatment} \times n \text{ in treatment after}}{n \text{ in } C_0 \text{ after treatment} \times n \text{ in treatment before}} \right) \times 100$$

RESULTS: It indicates that methanolic extract gave the positive results to alkaloids, flavonoids, tannins, saponins and carbohydrates. Besides the chlorofomic extract showed the positive results to steroids and flavonoids.

TABLE 1: PRELIMINARY PHYTOCHEMICAL SCREENING OF THE LEAF EXTRACT

S.no	Name of the Constituent	Chloroform	Methanol
1	Tannins	---	+++
2	Saponins	+++	+++
3	Terpinoids	---	+++
4	Flavonoids	+++	+++
5	Glycosides	---	+++
6	Alkaloids	---	+++
7	Steroids	+++	---

'+' – Indicates presence of constituents

'-' – Indicates absence of constituents

TABLE 2: THIN LAYER CHROMATOGRAPHY

S. no.	Leaf extract	Solvent System	No. of Spots appeared	R _f Values
01	Methanol	Chloroform : Methanol 8:2	04	0.05, 0.15, 0.21, 0.35
02	Chloroform	Chloroform : Methanol 9:1	03	0.57, 0.75, 0.84

TABLE 3: RESULTS OBTAINED AFTER BURNING OF PAPER COIL

Time (min)	No of dropped down mosquitoes							No of mosquitoes dead										
	T ₁	T ₂	T ₃	T ₄	T ₅	T ₆	S	C ₀	C ₁	T ₁	T ₂	T ₃	T ₄	T ₅	T ₆	S	C ₀	C ₁
15	0	0	0	0	0	0	1	0	0	0	0	1	0	1	1	1	0	0
30	2	1	1	1	1	2	4	0	0	1	2	2	1	1	2	3	0	0
45	4	4	4	4	3	7	10	0	0	3	4	5	3	2	6	5	1	1
60	5	7	6	5	7	10	10	2	2	7	8	9	5	6	9	10	2	2

T₁ = Chloroform extract of Annona paper coil (1% w/v), T₂ = Chloroform extract of Annona paper coil (2% w/v), T₃ = Chloroform extract of Annona paper coil (3% w/v), T₄ = Methanolic extract of Annona paper coil (1% w/v), T₅ = Methanolic extract of Annona paper coil (2% w/v), T₆ = Methanolic extract of Annona paper coil (3% w/v), S = Good night paper coil, C₀ = Control of chloroform, C₁ = Control of methanol

TABLE 4: PERCENTAGE OF MOSQUITOES DEAD

S.no	Time(Min)	% of Mosquitoes Dead						Standard Coil
		T ₁	T ₂	T ₃	T ₄	T ₅	T ₆	
1.	15	0	0	10	0	0	10	
2.	30	10	20	20	10	10	30	
3.	45	22	33	44	22	11	44	
4.	60	68.5	75	87.5	37.3	50	87.5	

DISCUSSION: Preliminary phytochemical screening of methanolic and chloroformic extracts of *Annona squamosa* leaves revealed the presence of different primary and secondary metabolites. These two extracts were subjected to TLC studies (Table 2) in order to identify the phytoconstituents present in the leaf extract. Preliminary tests revealed that *Annona squamosa* leaves contains

steroids, flavonoids, carbohydrates, saponins, alkaloids and tannins. With the help of two extracts, six different paper coils were formulated with different concentrations. Then the mosquitocidal activity evaluated for formulated paper coils by comparing with that of the marketed paper coil (Table 3 and 4). The data obtained was

statistically analyzed by using Henderson-Tilton's formula then the graph (Fig. 1) was plotted.

Fig.1 shows that T₃ and T₆ are having highest mortality with 87.5% when compared with that of the Standard commercial coil, having 80% mortality. Significant activity was reported by T₂ and T₁ (75% and 68.5% mortality) and very less activity was seen with the T₄ and T₅.

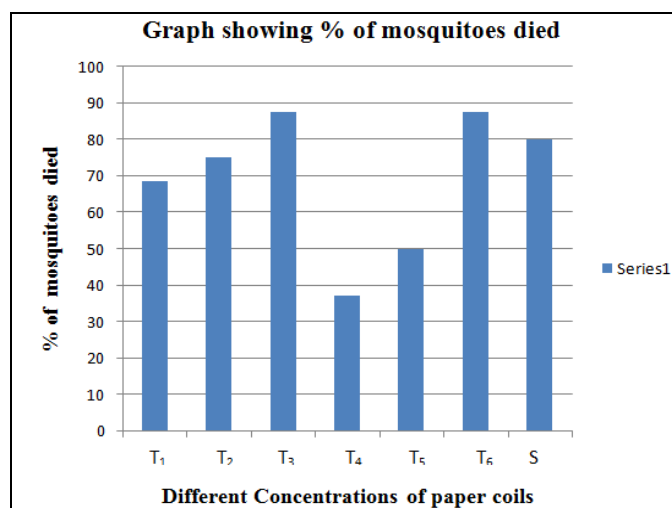


FIG. 1: GRAPH SHOWING % OF MOSQUITOES DIED

CONCLUSION: The practice of synthetic mosquito coils to control mosquitoes proved hazardous due to its adverse impact on ecospheres, non biodegradability nature, toxicity and resistance among different species of mosquitoes. So, it is necessary to resolve another way to overcome these problems. Insecticides of plant origin which are biodegradable, eco- friendly, target specific and moreover have no toxic effects on environment. *Annona squamosa* Linn is a multipurpose tree with edible fruits with medicinal and industrial products. The present study was done for the formulation of low cost natural paper coil from *Annona Squamosa* Linn and its mosquitocidal activity was evaluated. The significant activity demonstrated by extract of *Annona squamosa* suggests that the plant has high killing effects on mosquitoes. A 3% w/v methanolic and chloroformic leaf extracts of *Annona squamosa* showed highest mortality of about 87.5%.

CONFLICT OF INTEREST: We declare that there is no conflict of interests regarding the publication of this paper.

ACKNOWLEDGEMENT: We are very thankful to the Management of Vignan Pharmacy College, vadlamudi, for providing laboratory facilities for this research work.

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How to cite this article:

Lakshmi KR, Mounika M and Babu PS: Formulation of Natural Mosquito Paper Coil and Evaluation of Mosquitocidal Activity. Int J Pharm Sci Res 2016; 7(10): 4140-44.doi: 10.13040/IJPSR.0975-8232.7(10).4140-44.

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