Iranian Journal of Pharmaceutical Sciences
Spring 2006: 2(2): 97-102
www.ijps.ir

Original Article

Fatty Acid Composition and Toxicity of *Melia azedarach* L. Fruits against Malaria Vector *Anopheles stephensi*

Abbas Hadjiakhoondi [a, *], Hassan Vatandoost[b], Mahnaz Khanavi[a],
Hamid Reza Sadeghipour-Roodsari[c], Mohsen Vosoughi[d], Motahareh Kazemi[a],
Mohammad Reza Abai[b]

[a]*Department of Pharmacognosy, Medicinal Plant Research Center and Pharmaceutical Sciences Research Center, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran*
[b]*Department of Medical Entomology, School of Public Health and Institute of Health Research, Tehran University of Medical Sciences, Tehran, Iran*
[c]*Department of Physiology , Faculty of Medicine , Tehran University of Medical Sciences, Tehran, Iran*
[d]*Department of Chemistry, Medicinal Plant Research Center and Pharmaceutical Sciences Research Center, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran*

Abstract

The fruit of *Melia azedarach* L. (Meliaceae) was extracted with hexane, and its fatty acids methyl ester was analyzed using GC/MS. Thirteen components representing 85.1% of the total extract were identified. The major components identified were methyl palmitate (18.8%), methyl linolenate (16.1%) and methyl linoleate (9.8%). The fruits' extract had a LC_{50} of 5.5 ppm against the larvae of *Anophles stephensi*.

Keywords: Anophles stephensi; Fatty acid; *Melia azedarach* L.; Methyl linoleate; Methyl linolenate; Methyl palmitate.
Received: January 1, 2005; *Accepted:* September 12, 2006

1. Introduction

The high rate of mortality of malaria disease on one hand and the side effects of chemical insecticide on the other hand has guided the new research fields toward the production of insecticides effective on larvae of the main malaria vector, *Anophles stephensi*. The extract of Neem or *Melia indica* (Meliaceae) fruit has recently been shown to have satisfactory larvicidal effects. This plant is native to India and has been studied for its antimalaria [1, 2], as well as spermicidal [3], antifungal [4] and antidiabetes effects [5].

Meliaceae contains 45 genus and over 750 species in tropical regions [6]. Different parts of the species of this family have been used as medicine and insecticides for pest control [7].

Melia azedarach L. (Meliaceae) is a short-lived deciduous tree, native in southern Asia and Australia, which reaches a maximum height of 50 feet. It has been cultivated since the sixteenth century, chiefly for ornamental purposes and has become naturalized in most tropical and subtropical countries [8]. The

*Corresponding author: Dr. Abbas Hadjiakhoondi, Department of Pharmacognosy, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, P.O.Box: 14155/6451, Iran.
Tel: (+98)21-66494996, Fax: (+98)21-66461178
Email: abbhadji@sina.tums.ac.ir

Table 1. Chemical composition of the fatty acids methyl ester extracts of the fruits of *Melia azedarach* from Iran.

Peak No.	Compounds	RI [a]	Amount (%)
1	Methyl caproate	904	5.2
2	Dimethyl fumarate	989	7.9
3	Methyl plamitoleate	1878	1.6
4	Methyl palmitate [b]	1905	18.8
5	Methyl linoleate [b]	2063	9.8
6	Methyl linolenate [b]	2073	16.1
7	Methyl oleate [b]	2079	2.9
8	Menthy stearate [b]	2102	3.0
9	Docosane [b]	2198	4.0
10	Pentacosane [b]	2497	6.1
11	Hexacosane [b]	2596	4.4
12	Heptacosane [b]	2698	3.7
13	Nonacosane [b]	2898	1.6
	Total		**85.1**

[a]RI: relative retention indices as determined on a DB-1 column using the homologous series of *n*-alkanes.
[b]This compound was compared with an authentic sample.

leaves' extracts of this plant have insecticidal properties [7]. Due to the existence of *M. azedarach* L. in the north of Iran especially in the coastal parts of Caspian-sea [8], which have never been under research so far, we decided to study the plant considering its toxicity on larvae of the main malaria vector, *Anophles stephensi*.

2. Materials and methods

2.1. Plant Materials

The fruits of *M. azedarach* L. were collected in September 2003, 8th km Toskestan, around Gorgan, Golestan, Iran at an altitude of 160 m. Voucher specimen has been deposited at the Herbarium of the Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran (No. 6640 TEH).

Air-dried and powdered fruits (500 g) were extracted using percolator with 2 liters of *n*-hexane for 3 times at the room temperature for two weeks. Fatty acids extract was concentrated in a Buchi Rota evaporator Model RE 120 at 50 °C, separately.

Composition of fatty acids was estimated by gas chromatography. They were separated in the form of methyl ester derivatives. Preparation of the samples was done from 0.15 g of extracted fat, by saponification with 2 ml 0.5 N potassium hydroxide solution in 2 ml of methanol at 75 °C for half an hour, and following esterification with 1 ml of sulfuric acid solution in 5 ml of methanol (2:10 v/v) and 5 ml 0.5 N potassium hydroxide solution at 75 °C. After washing the solution 3 times with 20 ml of *n*-hexane, the fatty acids methyl ester (FAME) was transferred to the *n*-hexane layer [9].

2.2. Analytical technique

Gas chromatography (GC) analysis was carried out using a Thermoquest 2000 GC with capillary column DB-1 (30 m x 0.25 mm x 0.25 mm); carrier gas, He; split ratio, 1:25, and a flame ionization detector. The column temperature was programmed at 50 °C for 1 min. and then heated to 265 °C with a 2.5 °C/min. rate and then kept constant at 265 °C for 20 min. Gas chromatography-Mass spectroscopy (GC-MS) was performed on a Thermoquest 2000 with a quadrupole detector, on capillary column DB-1; carrier gas, He; flow rate, 1.5 ml/min. The column held at 50 °C for 1 min. and programmed up to 265 °C at a rate of 2.5 °C/min and then kept constant at 265 °C for 20 min. The MS operated at 70 eV ionization energy. Retention indices were calculated using retention times

Table 2. Larvicidal effects of *Melia azedarach* fruit extracts against *Anopheles stephensi* larvae.

Concentration of extract (mg/l)	No. tested	No. dead	Mortality (%)
None (control)	100	4	4
1	100	30	30
2	100	49	49
4	100	63	63
8	100	64	64
16	99	70	70

of *n*-alkanes that were injected after the oil at the same chromatographic conditions. The compounds were identified by comparison of retention indices (RI, DB-1) with those reported in the literature and the authentic samples and by comparison of their mass spectra with the Wiley library [10] or with the published mass spectra [11].

2.3. Bioassay test

The fatty acids extract from *M. azedarach* fruits were evaluated against late 3rd and early 4th instar larvae of *An. stephensi* under laboratory condition. The mosquito was collected from malarious area of Iran and then maintained at School of Public Health and Institute of Health Research, Tehran University of Medical Sciences. Different concentrations of extract were prepared in a DMSO and then larvae were exposed to different concentrations of the solutions. The minimum concentration was 1 mg/l and maximum 16 mg/l. The method was according to that described by WHO [12]. Mortality was determined after 24 h of exposure. All of the tests were conduced at 25 °C in a laboratory condition. In each concentration at least 100 mosquito larvae were tested. Dosage-mortality regression line was determined by the probit analysis method of Finney [13], using the Probit 79 program. Goodness of fit of the points to a straight line was tested by Chi-square (X^2) analysis.

3. Results and discussions

The constituents of the fatty acids methyl ester (FAME) extraction of *M. azedarach* are shown in Table 1. The FAME was light green with a distinct sharp coffee odor, in a yield of 0.2% (w/w). Thirteen components were detected in the FAME of *M. azedarach*, representing 86.84% of the total extract. Some compounds were compared with authentic samples (Table 1). The major constituents were methyl palmitate (18.8%), methyl linolenate (16.1%) and methyl linoleate (9.8%).

In a previous study on oil of *M. indica* seeds from forty-two samples from different areas of India, methyl ester of oleic, stearic, palmitic, linoleic, myristic, arachidic (eicosanoic), and behenic (docosanoic) acids were detected. Between 90% and 99% of the total FAME was oleic acid (48.6-69.0%), palmitic acid (14.5-25.0%), and stearic acid (13.4-27.5%), respectively. Other fatty acids methyl ester (myristic, arachidic, linoleic, and behenic) were present in negligible or nondetectable amounts. A wide variation in the contents of different fatty acids is, thus, evident [14].

Mortality rate of *A. stephensi* by different concentration of *M. azedarach* is shown in Table 2. The mortality data were subjected to probit analysis using Finney method [13]. From the regression line between logarithmic dose and probit mortality, all of the parameters including LC_{50} and 95% confidence interval, LC_{90} and 95% confidence interval, were determined. Table 3 indicates parameters of regression line. In another study, neemarin an AZ-rich formulation was tested against mosquito larvae both under laboratory and field conditions. LC_{50} and LC_{90} values for *A. stephensi,* the main malaria vector in Iran, was calculated 0.2 and 0.9 mg/l [15].

Neem products are capable of producing

Table 3. Probit regression line parameters of *Anopheles stephensi* exposed to *Melia azedarach*.

Intercept	Slope ± SD	LC_{50} (95% Confidence interval)	LC_{90} (95% Confidence interval)	X^2	*P*
-1.1835	1.598±0.324	2.8581	13.6435	13.386	0.001
		5.5047	34.90		
		14.5473	172.08		

multiple effects on a number of insect species, such as anti-feeding effect, growth regulation, fecundity suppression, and sterilization, oviposition repellency or attractancy and changes in biological fitness [16]. For example neem extracts have been shown to have repellent activity against *Mansonia* spp., mosquitoes in Ethiopia [17]. In mosquitoes, compounds extracted from neem tree showed mortality for 4th instar larvae of *A. stephensi* with LC_{50} value of 60 ppm [18].

In conclusion, the extraction of *M. azedarach* showed biological effects against the larvae of *A. stephensi*. Similar activities in *M. azedarach* and other species of *Melia* also are shown by other researchers around the world [16-19]. Therefore, extracts of this plant offer a valuable candidate for potential development of a botanical insecticide for malaria vectors control.

References

[1] Sastry BS, Suresh Babu K, Hari Babu T, Chandrasekhar S, Srinivas PV, Saxena AK, Rao MJ. Synthesis and biological activity of amide derivatives of nimbolide. *Bioorg Med Chem Lett* 2006; 16: 4391-4.

[2] Wandscheer CB, Duque JE, da Silva MAN, Fukuyama Y, Wohlke JL, Adelmann J, Fontana JD. Larvicidal action of ethanolic extracts from fruit endocarps of *M. azedarach* and *Azadirachta indica* against the dengue mosquito *Aedes aegypti*. *Toxicon* 2004; 44: 829-35.

[3] Khillare B, Shrivastav TG. Spermicidal activity of *Azadirachta indica* (neem) leaf extract. *Contraception* 2003; 68: 225-9.

[4] SaiRam M, Ilavazhagan G, Sharma SK, Dhanraj S A, Suresh B, Parida MM, Jana AM, Devendra K, Selvamurthy W. Anti-microbial activity of a new vaginal contraceptive NIM-76 from neem oil *(Azadirachta indica)*. *J Ethnopharmacol* 2000; 71: 377-82.

[5] Yanpallewar S, Rai S, Kumar M, Chauhan S, Acharya SB. Neuroprotective effect of *Azadirachta indica* on cerebral post-ischemic reperfusion and hypoperfusion in rats. *Life Sci* 2005; 76: 1325-38.

[6] Ghahreman A. *Chromophytes of Iran*. 2nd edition. Tehran: Tehran University Publication, 1994; pp. 342-4.

[7] Méndez MC, Aragão M, Elias F, Riet-Correa F, Gimeno EJ. Experimental intoxication by the leaves of *M. azedarach* (Meliaceae) in cattle. *Pesq Vet Bras* 2002; 22:19-24.

[8] Sabeti H. *The forests and bushes of Iran*. Yazd: Yazd University Publication, 1994; p. 810.

[9] Wasowicz E, Zawirska-Wostasiak R, Rudzinska M. Estimation of fatty acids and *trans*-fatty acids composition in chosen dry food mixes. *Food Sci Technol* 2001; 4: 1-14.

[10] Massada Y. *Analysis of essential oil by gas chromatography and spectrometry*. NewYork: Wiley, 1976.

[11] Adams RP. *Identification of essential oil components by gas chromatography/ mass spectroscopy*. Allured: Carol Stream, IL, 1995.

[12] Insecticide resistance and vector control, 17th report of WHO expert committee on insecticides. World Health Organization. *Technical Report Series*; 1970; 43.

[13] Finney DJ. *Probit analysis*. 3rd edition. Cambridge: Cambridge University Press, 1971.

[14] Kumar J, Parmar BS. Physicochemical and chemical variation in neem oils and some bioactivity leads against *Spodoptera litura* F. *J Agric Food Chem* 1996; 44: 2137-43.

[15] Vatandoost H, MoinVaziri V. Larvicidal activity of neem extract (*Azadirachta indica*) against mosquito larvae in Iran. *Eastern Mediterranean Health J* 2004; 10: 573-81.

[16] Mulla MS, Tsu T. Activity and biological effects of neem products against arthropods of medical and veterinary importance. *J Am Mosquito Control Association* 1999; 15: 133-52.

[17] Hadis M, Lulu FM, Mekonnen Y, Asfaw T. Field trials on the repellent activity of four plant product against mainly mansonia population in western Ethiopia. *Phytother Res* 2003; 17: 202-5.

[18] Ruskin FR. *Neem a tree for solving global problems*. Washington: National Academy Press,

1992.
[19] Sharma VP, Dhiman RC. Neem oil as a sandfly (Diptera: Psycodidae) repellent. *J Am Mosquito Control Association* 1993; 9: 364-66.