Biolife 2(2):579-585
AN INTERNATIONAL QUARTERLY JOURNAL OF BIOLOGY & LIFE SCIENCES
ISSN (online): 2320-4257
www.biolifejournals.com
BIOLIFE RESEARCH ARTICLE

ISOLATION AND IDENTIFICATION OF BIOLARVICIDE FROM SOURSOP (*ANNONA MURICATA* LINN.) AQUEOUS LEAF EXTRACT TO MOSQUITO (*AEDES AEGYPTI* LINN.) LARVAE

Maghdu Nainamohamed Abubacker[1]*, Thiagarajan Deepalakshmi[2] and Chandran Sathya[3]

[1]*PG & Research Department of Biotechnology, National College, Tiruchirappalli, India*
[2]*PG & Research Department of Botany, National College, Tiruchirappalli, India*
[3]*Department of Botany, Seethalakshmi Ramasamy College, Tiruchirappalli, India*

E-mail: abubacker_nct@yahoo.com

ABSTRACT

To assess the biolarvicidal compound and larvicidal potentials of methyl ester of hexadeconic acid isolated from *Annona muricata* Linn. (*A. muricata*) against mosquito larvae *Aedes aegypti* Linn. (*A. aegypti*). The biolarvicidal compound methyl ester of hexadeconic acid was determined using GC-MS, while the larvicidal bioassay was carried out using different concentration of aqueous leaf extract of *A. muricata* against the larvae of *A. aegypti* in accordance with the standard protocol. Isolation and identification of larvicide bioactive compound from soursop (*A. muricata*) leaves against the larvae of *A. aegypti* mosquito, the transmitters of dengue fever has been carried out. The aquatic (rain water) extract of soursop leaves was an active larvicide agent with a lethal concentration (LC 50) varies with the concentration percentage and time. LC 50 for 2.5% = 3.0 h, 2.0% = 3.30 h, 1.5% = 3.15 h, 1.0% = 4.0 h and 0.5% = 5.0 h respectively. Total mortality occurred at 6.0 h for 2.5% concentration, 6.30 h for 2.0%, 7.30 h for both 1.5 and 1.0%, 8.0 h for 0.5% concentration. Removal of exoskeleton (dechitinising property) occurred between 1.0 h to 2.0 h for these concentrations tested for this work. Separation by TLC, analysis and identification with GC-MS showed peaks of 14 compounds, where there three dominant compounds with a retention time relatively close and have the abundance percentage that are large enough, which are identified as methyl ester of hexadecanoic acid, methyl ester of 9-octadecenoic acid (z), and 5-methyl-2-hexanone oxime with the most dominant compound which is methyl ester of hexadecamic acid which has a 44.11% abundance. The results justify the larvicidal potentials of bioactive compound from the leaf aqueous extract of *A. muricata* and the need to incorporate them in vector management and control.

Key words : Soursop, biolarvicide, *Annona muricata*, *Aedes aegypti*, mortality.

INTRODUCTION

Mosquitoes are responsible for more diseases than any other group of Arthropods. To control mosquito population various pesticides are being used widely. Recent reports state that mosquitoes have become genetically and physiologically resistant to many conventional insecticides[1]. These factors have created the need for environmentally safe, biodegradable and target specific insecticides against mosquitoes. The search for such compounds has been directed extensively to the plant kingdom[2,3].

A. aegypti mosquito is a vector of several serious diseases to human, such as malaria, encephalitis, yellow fever, dengue fever, dengue hemorrhagic

fever, filariasis and arbovirus[4]. Dengue Hemorrhagic Fever (DHF) has no medicine or vaccine yet[5]. One way of preventing the spread of DHF is by prevention of dengue virus infection, by controlling and eradicating the vector to break off the disease transmissions[6]. Methods developed by WHO to combat dengue fever is the same as the method used to combat malaria, which is to eradicate the source of transmission, *i.e.* mosquito larvae[7]. Eradication of larvae is the key strategy of vector control programmes around the world[8].

A. aegypti mosquitoes lay eggs in clear water that is not directly affected by land and prefers containers indoor rather than outdoor due to the indoor temperature is relatively stable. A mosquito can lay eggs 4-5 times during her life with an average number of eggs ranges from 10-100 eggs in a single spawn. Thus the total number of eggs produced by a single female mosquito is between 300-700 eggs[9]. Physical control is conducted by managing the environment to prevent mosquito from breeding. Biological control is performed using predators and pathogenic organisms, while chemical control is carried out by applying synthetic insecticides to kill mosquitoes. Genetic control is done by spreading the sterile males into the ecosystem, and integrates control is performed by combining the various existing control techniques[10]. The most widely used mosquito control is the chemical control. The reason for this selection is the prompt results of this control. However, chemical control using synthetic insecticides actually causes adverse side effects, such as the mosquitoes could become resistant, human and livestock poisoning, contamination of vegetables and fruit gardens, as well as environmental pollution[11].

The development of new insecticides that are more environmentally friendly and do not pose hazard needs to be done. The use of bioinsecticides looks promising. Bioinsecticide or biological insecticide is an insecticide which is derived from plant material containing chemicals (bioactive) that are toxic to insects but are easily biodegradable in nature. So, it will not pollute the environment and relatively safe for human. Besides, vegetable insecticides are also selective[12].

Research on bioactive compounds in the Annonaceae family is growing rapidly. Acetogenin compounds from Annonaceae type were reported to have toxicity that is effective against insects of several orders such as Lepidoptera, Coleoptera, Homoptera and Diptera[13,14]. Other studies reported that Annonaceae family contains acetogenin that are larvicidal. Acetogenin also acts as an insecticide, acaricide, antiparasitic and bactericidal[15,16]. *A. muricata* Linn. (Soursop) seed extract contain annonacin, bullatacin, annonin VI, goniothalamin and sylvaticum act as insecticides[17,18].

Preliminary test results indicated that the ethanol extract of soursop seeds is an active agent of larvicide. Phytochemical test shows that ethanol soursop seed extract contains secondary metabolites compounds group of saponin, alkaloids and triterpenoids. These compounds are defense chemical compounds of plant produced in the plant tissue. They are toxic and can also act as the stomach and respiratory poison[19]. The present study will discuss the work in isolating and identifying the bioactive agents of larvicide from the soursop leaf aqueous extract against dengue fever mosquito (*A. aegypti*) larvae as the bioindicator.

MATERIALS AND METHODS

Collection and identification of plant material:

Fresh leaves of *A. muricata* Linn. were collected from a private garden in Tiruchirappalli, Tamil Nadu (Figure a). The taxonomic identities of this plant was confirmed by flora of the Presidency of Madras[20]. The leaf material was washed under running tap water, air dried in shade and then homogenized to fine powder and stored in sterile air tight bottles for the experimental work.

Isolation and identification of bioactive compound - Thin Layer Chromatography (TLC):

Glass plates (4 cm × 12 cm) were used in which 30 gm of silica gel mixed with 60 ml distilled

water slurry was prepared and coated on the glass plate to 0.25 cm thickness and dried for an hour at 110°C in an oven[21].

Preparation of leaf extract for bioactive compound:

The dry powdered leaves (500 mg) of *A. muricata* was mixed with 5.0 ml of chloroform and ground into a paste, dried at room temperature. 1 ml of chloroform was added to the dried samples and spotted on the TLC plates. The TLC plates were kept in several eluent mixture with different polarities to separate the bioactive chemical compounds in chloroform extract has been tried. The eluents used were chloroform : *n*-hexane (8:2), chloroform : ethyl acetate (8:2), chloroform : acetone (8:2), *n*-hexane : acetone (9:1), and chloroform : acetone (9:1). Sample spotting on the TLC plate was done by using a micropipette in which the dot diameter 0.5 mm. The chloroform : acetone (9:1) was the best eluent since it was able to separate the four compounds contained in leaf extract[11].

Gas chromatography and mass spectroscopy (GC-MS) analysis:

GC-MS analyses were performed using a GC Clarus 500 Perkin Elmer equipment equipped with a flame ionization detector and injector MS transfer line temperature of 230°C, fused silica capillary column Elite-5 MS (5% Diphenyl / 95% Dimethyl polysiloxane), 30×0.25 µm df, film thickness, carrier gas Helium at a flow rate of 28 cm/sec was used. 1 ml of extract mixed with methanol (80%) at a split rate of 10:1 was injected. The compound identification was accomplished by comparing the GC relative retention and mass spectra to those of authentic substances analysed under the same conditions, by their Retention Time (RT) and by comparison to reference compounds (Table 2).

Preparation of leaf extract for biological activity:

500 mg of dried leaf powder was mixed with 100 ml of rain water constitute 0.5% concentration, while 1.0 g, 1.5 g, 2.0 g and 2.5 g constitute 1.0, 1.5, 2.0 and 2.5% concentration of leaf extract was used for the experimental work. The control organisms were maintained in rain water (Figure b).

Toxicity Test:

The various concentration of *A. muricata* leaf extract were tested for its biological activity against *A. aegypti* larvae in petridish. Each concentrations (20 ml) contained 20 larvae including control. Observations were carried out for 24 hours on the death of the larvae. The LC 50 and total mortality for each concentrations were recorded.

.

RESULTS

The larvicidal effect of *A. muricata* leaf aqueous (rain water) extract of LC_{50} and total mortality in terms of time was presented in Table-1.

Table-1. Larvicidal effect of soursop (*A. muricata* Linn) leaf aqueous extract to mosquito (*A. aegypti*) larva

CONSTITUENTS	Concent-ration (%)	No. of larvae tested	Time (h) LC50	No. of larvae alive	No. of larvae death	Time (h)	Total Mortality
Rainwater (Control)	-	20	24.0	20	0	24.0	0
Leaf extract in rain water	0.5	20	5.00	10	10	8.00	10
	1.0	20	4.00	10	10	7.30	10
	1.5	20	3.15	10	10	7.30	10
	2.0	20	3.30	10	10	6.30	10
	2.5	20	3.00	10	10	6.00	10

The maximum concentration of 2.5% tested in this work shows LC_{50} at 3.0 h, 2.0% at 3.30 h, 1.5% at 3.15 h, 1.0% at 4.0 h, 0.5% at 5.0 h (Figure-2d-h), the control organisms alive at 24.0 h and thereafter for all the developmental stages in subsequent days (Figures 1b, c). It is to be noted that in all the concentrations there was 100% mortality occurred with respect to time 6.0 h to 8.0 h (Figures-1j,k). Dechitinising property of larva was observed at 1.0 h (Figure-1i).

DISCUSSION

Larvicidal (Mosquito) efficacies was reported by many workers[22-26]. In all these reports organic solvents were used in the plant extract. In this report the natural habitat of *A. aegypti* rain water source alone tested with *A. muricata* leaf extract. The GC-MS analysis revealed the presence of major bioactive compound methyl ester of hexadoconic acid (Figure 2).

Figure-1. Larvicidal effect of soursop (*Annona muricata* Linn) leaf aqueous extract to mosquito (*Aedes aegypti*) larva

(a-*Annona muricata* Linn. Twig, b-Aedes aegypti larvae in rain water, c-Control (rain water), d-A. muricata aqueous (rain water) leaf extract 0.5%, e-concentration 1.0%, f-1.5%, g-2.0%, h-2.5, i-Dechitinising property of larva (1 hour), j-Morphology of dead larva (3 hours) and k-Morphology of dead larva (6 hours)

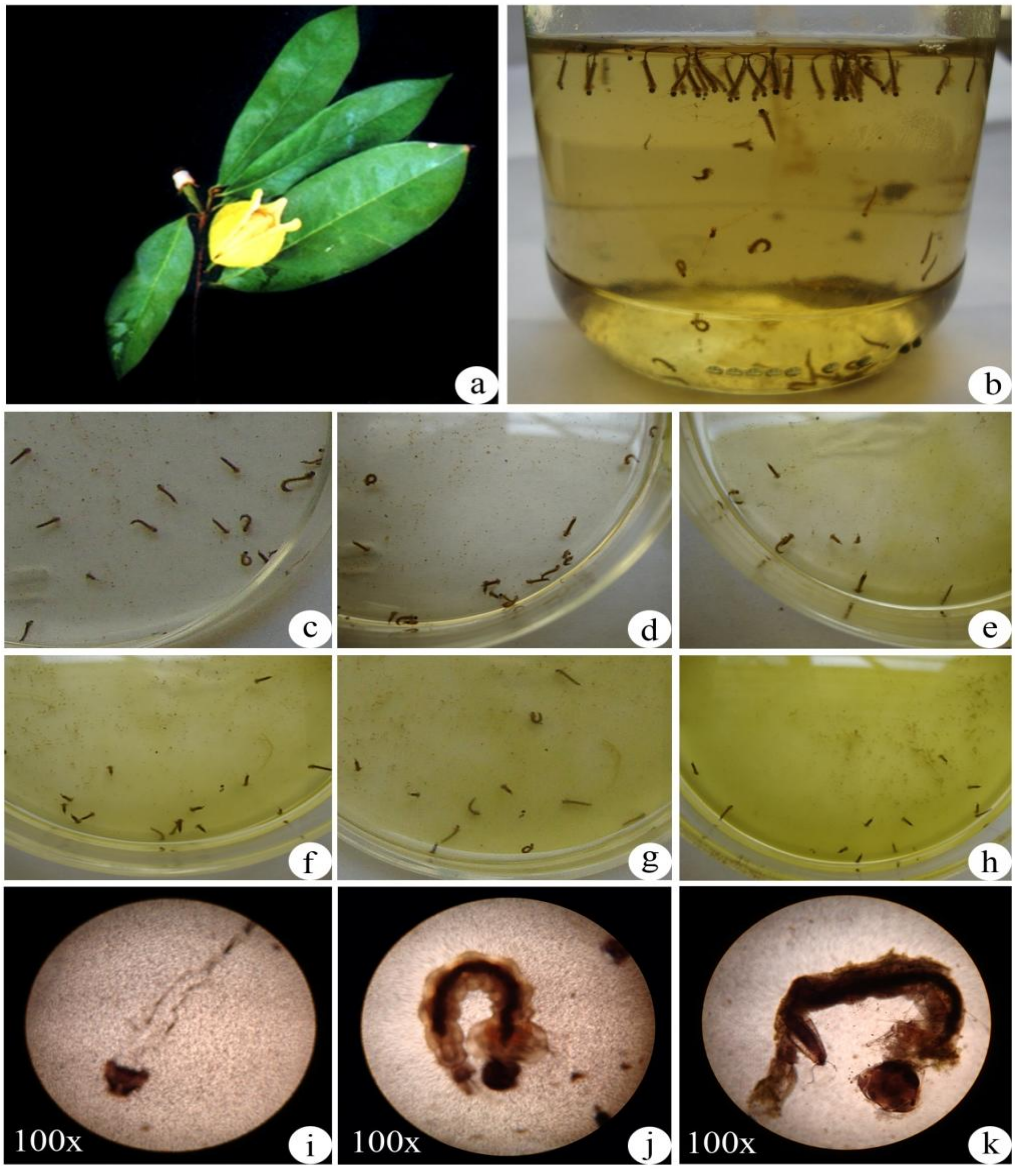
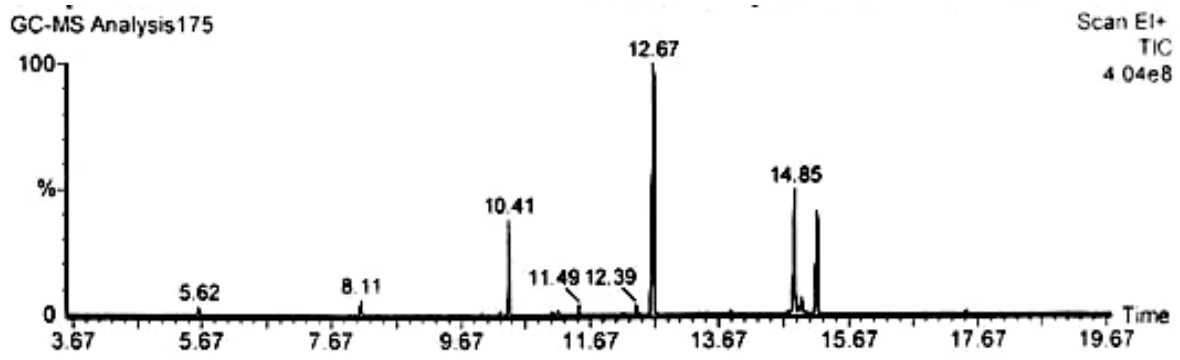


Table-2. Bioactive compounds identified in *Annona muricata* leaf extract

No.	RT	Name of the Compound	Molecular formula	MW	Peak Area %
1.	5.62	Hexanal, O-methyloxime	$C_7H_{15}NO$	129	1.07
2.	8.11	Butanal, O-methyloxime	$C_5H_{11}NO$	101	1.68
3.	10.00	2-Propanone,oxime	C_3H_7NO	73	0.12
4.	10.41	5-Methyl-2-hexonone oxime	$C_7H_{15}NO$	129	10.73
5.	11.09	1,2-Ethanediamine, N-[2-aminoethyl)	$C_4H_{13}N_3$	103	0.24
6.	11.17	Butanamide, 4-cyano-N-methyl	$C_6H_{10}N_2O$	126	0.46
7.	12.39	1,13-Tridecanediol, diacetate	$C_{17}H_{32}O_4$	300	1.10
8.	12.67	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270	44.11
9.	14.85	9-Octadecenoic acid (Z)-,methyl ester	$C_{19}H_{36}O_2$	296	19.60
10.	14.95	10-Undecen-1-yl acetate	$C_{13}H_{24}O_2$	212	2.42
11.	15.19	Acetamide, N-acetyl-N-methyl	$C_5H_9NO_2$	115	17.98
12.	15.43	3-Cyclohepten-1-one	$C_7H_{10}O$	110	0.09
13.	17.49	5-Hexen-2-one, O-methyloxime	$C_7H_{13}NO$	127	0.37
14.	17.95	Acetic acid, 2-methylpropyl ester	$C_6H_{12}O_2$	116	0.03

Figure-2. GC-MS Chromatogram of *Annona sp*.



The total mortality of *A. aegypti* larvae between 6.0 to 8.0 h might be due to this bioactive compound. The present work will help to incorporate this bioactive compound in vector management and control.

ACKNOWLEDGEMENTS

Author (MNA) wish to thank DST-FIST, Government of India, New Delhi for providing the infrastructure facilities to the Department of Botany, National College, Tiruchirappalli, Tamil Nadu. Authors also expresses thanks to Padmavibhushan Dr. V. Krishnamurthy, President, Sri. K. Ragunathan, Secretary and Dr. K. Anbarasu, Principal, National College, Tiruchirappalli for all the supports and encouragement given to PG and Research Department of Biotechnology to carry over the research work.

REFERENCES

1. Sukumar K, Perich MJ, Boobar LR. Botanical derivatives in mosquito control: A review. *J Am Mosq Control Assoc.* 1991; **7**: 210-237.
2. Srinivasa RM, Sunil M, Suryanarayana MV, Venkateshwarlu Y, Srinivasalu M. Effect of lamellarin alkaloid mixture obtained from marine ascidian, *Didemrum obscurum* and its synergistic action with *Beanveria bassiana* on *Culex quinquefasciatus* larvae. *Nat. Prod. Rad.* 2005; **4**: 460-465.
3. Thomas TG, Raghavendra K, Saxena Shivlal VK. Mosquito larvicidal properties of latex from unripe fruits of *Carica papaya* (Caricaceae). *J Commun Dis.* 2004; **36**: 290-292.
4. Ndione RD, Faye O, Ndiaye M, Dieyl A, Afoutou JM. Toxic effects of neem products (*Azadirachta indica* A. Juss) on *Aedes aegypti* Linnaeus 1762 larvae. *Affric J Biotechnol.* 2007; **6**: 2846-2854.
5. Daniel. "When larvae and mosquitoes are resistant to insecticides". *Farmacia*. **7**: 44.
6. WHO. *Prevention and control of dengue and dengue hemorrhagic fever*. Jakarta: Penebit Buku Kedokteran (EGC). 2005.
7. Manuel FB, Douglas KA. *Human medical agent from plant*. Washington DC, American Chemical Society. 1992.
8. Okumu FO, Knols BJG, Fillinger U. Larvacidal effect of a neem (*Azadirachta indica*) oil formulation on the malaria vector *Anopheles gambiae. Malaria J.* 2007; **6**: 63.
9. Lee HL. Breeding habitats and factors affecting breeding of *Aedes* larvae in urban town of Peninsular, Malaysia. *J Biosci.* 1990; **1**: 107-112.
10. Hadi UK, Soviana S. *Ektoparasit: Pengenalan, Diagnosis dan Pengendaliannya.* Bogor: IPB. 2000.
11. Komansilan A, Abadi AL, Yanuwiadi B, Kaligis DA. Isolation and identification of biolarvicide from soursop (*Annona muricata* Linn) seeds to mosquito (*Aedes aegypti*) larvae. *International Journal of Engineering and Technology*. 2012; **12**: 28-32.
12. Moehammade N. "*Potensi biolarvasida ekstrak herba Ageratum conyzoides Linn. dan daun Saccopetalum horsfieldii Benn. terhadap larva nyamuk Aedes aegypty". Jurnal Berkala Penelitian Hayati* (in Indonesian). 2005; **10**: 1-4.
13. Hui YH, Rupprecht JK, Anderson JE, Wood KV, McLanghlin. "Bullatalicinone, a new potent bioactive acetogenin and squamocin from *Annona bullata* (Annonaceae)". *Phytother Res.* 1991; **5**: 124-129.
14. Li XH, Hui YH, Rupprecht JK, Lin YM, Wood KV, Smith DL, Chang CJ, McLaughin JL. "Bullatacin, Bullatacinone, Sqnamone, a new bioactive Acetogenin from the bark of *Annona squamosa." J Natur Prod*. 1991; **53**: 81-86.
15. Alali FQ, Lin XX, McLanghlin JL. "Annonaceous acetogenins: recent progress". *J Nat Prod*. 1999; **62**: 504-540.
16. Abubacker MN, Deepalakshmi T. Antioxidant and antibacterial activity of (*Annona muricata* L.) leaf aqueous extract. *International Journal of Plant Sciences*. 2012; **7**: 301-306.
17. Wurangian FL. Penentuan kadar senyawa Annonasin pada ekstrak biji sirsak (Annona muricata L.) untuk formula pestisida secare kromatograft cair kinerja tinggi (KCKT). Master thesis, Universitas Padjadjaran, Bandung, (in Indonesian). 2001.
18. Rupprecht JK, Hui YH, Mc Laughlin. Annonaceous acetogenins: A review. *J Nat Prod.* 1990; **53**: 237-278.
19. Yeni. Efektivitas ekstrak daun Babandotan (*Ageratum conyzoides* Linn.) terhadap larva *Anopheles sundacius* Linn. *di Desa Babakan Pangandaran Jawa Barat.* Jurusan Biologi FMIPA, Universitas Lampung, Bandar Lampung, Laporan Kerja Praktik, (in Indonesian). 2008.
20. Gamble JS. *Flora of the Presidency of Madras*. Botanical Survey of India, Calcutta (WB), India. 1967.
21. Bothast RJ, Hesseltine CW. Bright greenish yellow fluorescence and aflatoxin in agricultural commodities. *Appl Microbiol.* 1975; **30**: 337-338.
22. Fayemiwo KA, Adeleke MA, Okoro OP, Awojide SH, Awoniyi IO. Larvicidal

efficacies and chemical composition of essential oils of *Pinus sylvestries* and *Syzygium aromaticum* against mosquitoes. *Asian Pacific Journal of Tropical Biomedicine*. 2014; **4**: 30-34,
23. Periyanayagam K, Sundara Saravanan K, Ismail M. Dechitinising property of *Caesalpinia bonduc* (Linn.) Roxb. against *Culex quinquefasciatus*. *Natural Product Radiance*. 2007; **6**: 290-292.
24. Mariath IR, Heloina de S Falco, Barbosa-Filho JM, Layanna CF de Sousa, Anna Claudia de A Tomaz, Batista LM, Margareth de Fatima FM Oiniz, Athayde-Filho PF, Tavares JF, Silva MS, Emidio Vasconcelos L da Chnha. Plants of the American continent with antimalarial activity. *Brazilian Journal of Pharmacognosy*. 2009; **19**: 158-192.
25. Promsiri S, Naksathit A, Kruatachue M, Thavara U. Evaluations of larvicidal activity of medicinal plant extracts to *Aedes aegypti* (Diptera: Culicidae) and other effects on a non-target fish. *Insect Science*. 2006; **13**: 179-188.
26. Rajeshwar Y and Lalitha R. Preliminary phytochemical screening and *in vitro* anthelmintic effects of *Acmella paniculata* plant extracts. 2013, Biolife, **1(3);** 106-112.

Conflict of interest statement:
We declare that we have no conflict of interest.

DOI: https://dx.doi.org/10.5281/zenodo.7209383
Received: 7 April 2014;
Accepted; 22 May 2014;
Available online : 14 June 2014