Journal of Coastal Life Medicine

journal homepage: www.jclmm.com

Original article https://doi.org/10.12980/jclm.4.2016J6-174

Chemical composition and laboratory investigation of *Melissa officinalis* essential oil against human malarial vector mosquito, *Anopheles stephensi* L. (Diptera: Culicidae)

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ARTICLE INFO

Article history:
Received 2 Sep 2016
Received in revised form 22 Sep, 2nd revised form 30 Sep 2016
Accepted 8 Oct 2016
Available online 21 Nov 2016

Keywords:
Melissa officinalis
Anopheles stephensi
Larvicidal activity
Ovicidal activity
Repellent activity

ABSTRACT

Objective: To decide the larvicides, ovicidal, pupicidal and repellent activity of *Melissa officinalis* (*M. officinalis*) chemical compositions against important mosquito *Anopheles stephensi* (*An. stephensi*) (Diptera: Culicidae).

Methods: A chemical constituent of 24 compounds was identified in the oils of *M. officinalis* compounds representing to 98.73%. A total of 25 3rd instar larvae of *An. stephensi* were showed to a variety of concentrations (30–300 mg/L) in laboratory by means of utilizing the standard procedure portrayed by World Health Organization (2005). The larvae were exposed for 24 h and mortalities were subjected to probit analysis. The ovicidal activity was strong-minded against *An. stephensi* mosquito to a variety of concentrations ranging from 15–90 mg/L under the laboratory circumstances. The repellent activity of *M. officinalis* chemical compositions tested at concentrations of 0.75 and 1.50 mg/cm^2 was evaluated in a net cage (45 cm × 45 cm × 40 cm) including 100 blood starving female mosquitoes of *An. stephensi* using the methods of World Health Organization (1996).

Results: The LC_{50} and LC_{90} values of citronellal compound against *An. stephensi* larvae were 85.44 and 159.73 mg/L, respectively. Mean percent hatchability of the ovicidal action was observed 48 h post-treatment. Similarly, the citronellal compound and other compositions were found to be mainly effective against eggs of *An. stephensi*. Citronellal compound exerted 45, 60, 75 and 90 mg/L against *An. stephensi*, respectively. The repellent activity of citronellal compound was contained to be mainly effective and the maximum action was observed at 0.75 and 1.50 mg/cm^2 concentrations giving 100% protection up to 210 min against *An. stephensi*.

Conclusions: This current study was undertaken to evaluate the larvicidal, ovicidal, repellent potential of compounds from the *M. officinalis* essential oil against *An. stephensi*. This is initial statement on the mosquito larvicidal, ovicidal and repellent activity of *M. officinalis* chemical compositions.

1. Introduction

Mosquitoes are bothers and noteworthy vector for the broadcast of a few living debilitating illnesses. Mosquitoes are real vectors for broadcast of dengue fever, malaria, filariasis, yellow fever and a few different illnesses, thus they have been declared Open Enemy Number 1[1-3]. *Anopheles stephensi* L. (*An. stephensi*) is more important vector of malaria fever within urban district of India than other West Asian countries. Malaria remains one of the majority widespread diseases in tropical world. In India, malaria is transmitted by *Anopheles* mosquitoes. Malaria is still the most critical reason for horribleness and mortality with around a few million new cases emerging each year[4,5]. Human malaria is the foremost reason of morbidity and transience in the global. In Asia, the mosquito is the most important vector of malaria[6]. Malaria troubles 36% of the worldwide people *i.e.* 2 020 million in 107 countries and lands located in tropical and subtropical regions[7]. As indicated by the most recent evaluations, there was around 198 millions of people suffering from cases of malarial fever in 2013 and expected 584 000 passing. Most passings happen in children who live in Africa, which are child's the bucket each minutes from malaria. The death rate of malaria amongst kids in Africa has been decreased by an expected 58% ever since 2000[8]. As per the most recent evaluations from World Health Organization, there were 214 million new instances of malaria worldwide in 2015 and there were expected 438 000 cases of malaria passing. Children under five are especially helpless to malaria ailment, disease and demise. Malaria

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Foundation Project: Supported by University Grants Commission, New Delhi (Ref. No F 42-597/2013SR).
The journal implements double-blind peer review practiced by specially invited international editorial board members.

slaughtered expected 306 000 children under five comprehensively, incorporating 292 000 kids in the African Region[9]. The control of mosquito and human being security are now the most critical aspects to control these infections[10,11].

In addition, artificial pesticides area unit is toxic and adversely has an effect on the environment by pollution water, soil and air[12,13]. New studies excited the research of pesticidal properties of plant crude extracts and essential oils could also be an alternate of artificial pesticides which is, as a result, effective, eco-friendly, simply perishable, target specific and cheap[14,15]. Biological science pesticides are more and more used for mosquito and pest management as a result of the effectuality in addition to non-toxic special effects on non-target organisms and several other studies have centered on phytochemicals against pesticides that are conducted around the worldwide. However, the majority of them are controlled to preliminary screening[16-18]. The essential oils are obtained nearly completely from vegetable organs: leaves, flowers, seeds, woods, barks, fruits, roots and rhizomes[19]. *Melissa officinalis (M. officinalis)* (lemon balm) is a crucial herb among the autochthonous medicinal plants of genus *Melissa*. *M. officinalis* grows widely in central, Southern Europe and Asia. It's a well known herb accustomed providing fragrance to totally different food and food product. This too, has been used as ancient drugs to cure nervousness, headaches, rheumatism and duct disorders[20]. Plant origin pesticides are newly inflated curiosity as alternative measures for controlling pest. This present study was undertaken to assess the insecticidal activities with particular regard to larvicidal, ovicidal and repellent potential of compounds from *M. officinalis* essential oil against medically necessary species of malarial vector, *An. stephensi*.

2. Materials and methods

2.1. Mosquito rearing

The protozoal infection vector *An. stephensi* mosquito was noted in the Department of Zoology, Annamalai University. The larvae were exhausted dog biscuits and yeast powder within the 3:1 magnitude relation. Adults were given 10% sucrose solution and one week old chick for blood meal. Mosquitoes were commanded at (28 ± 2) ˚C, 70%–85% ratio of relative humidity, with a photograph amount of 14 h light-weight and 10 h dark.

2.2. Plant material and essential oil extraction

The recent aerial elements of *M. officinalis* were collected from the forest region of Malappuram District, Kerala and placed at the foothills of Western Ghats of Southern India. The recent aerial elements were collected throughout Jan-June 2010 and plant elements were dried and small-grained mistreatment electrical liquidizer and essential oil was obtained by hydrodistillation of 5 kg of recent elements using Clevenger equipment for 4 h. The distilled oils were dried out over anhydrous sodium sulphate and then kept in a sterile amber bottle vials at 4 ˚C in the refrigerator.

2.3. Gas chromatography analysis

Examination was carried out on a Varian gas chromatograph outfitted with fire ionization locator and a BPI (100% dimethyl polysiloxane) narrow segment. He at a stream rate of 1.0 mL/min and 8 psi channel weight were utilized as a bearer air. Temperature was modified from 60 to 220 ˚C at 5 ˚C/min with a last hold time of 6 min. The injector and indicator temperatures were kept up at 250 and 300 ˚C, respectively. The example (0.2 μL) was infused with 1:20 split proportion.

2.4. Gas chromatography-mass spectrometry (GC-MS) analysis

Gas chromatography-mass spectroscopic examination was executed on an Agilent 6890 GC outfitted with 5973 N mass particular identifier and a HP-5 (5% phenyl methylpolysiloxane) slender segment. The stove temperatures were customized from 50 to 280 ˚C at the rate of 4 ˚C/min and commanded at this temperature for 5 min. The delta and interface temperatures were 250 and 280 ˚C, individually. The transporter gas was He at a stream rate of 1.0 mL/min (consistent stream). The example (0.2 μL) was infused with a split of 20:1. Electron sway mass spectrometry was conveyed at 70 eV. Particle resource and fourfold temperature were kept up at 230 and 150 ˚C separately.

2.5. Larvicidal activity

The larvicidal activities of chosen medicinal plants concentrates were assessed according to the convention beforehand portrayed[21]. In view of the wide range and thin range tests, all concentrates tried going from 30–300 mg/L was readied. Moreover, they were tried against newly shed (0–6 h) third instar hatchlings of chose mosquitoes. The plants concentrates were disintegrated in 1 mL dimethyl sulfoxide (DMSO) and afterward weakened in 249 mL of dechlorinated faucet water toward acquire each of the fancied focuses. The control was readied utilizing 1 mL of DMSO as a part of 249 mL of dechlorinated water. The hatchlings of test species (25) were presented in 250 mL plastic glass including 250 mL of fluid medium (249 mL of dechlorinated water + 1 mL of DMSO) and the necessary measure of compound syntheses was included. The larvae mortality was watched and evidenced after 24 h of post treatment. For every examination, 5 replicates were kept up at once. Percent mortality was rectified for controlling mortality[22].

2.6. Ovicidal activity

The method of Su and Mulla was to some extent changed and wont to take a look at the ovicidal action[23]. The varied concentrations which were explicit within the earlier experiments were readied as of the stock resolution. Earlier than the treatment, the eggs of hand-picked mosquitoes were counted on an individual basis by the assistance of hand lens. Newly hatched eggs (100) were bare to every concentration of oil chemical compositions till they emerged or expired. Eggs exposed to DMSO in water served as management. During the treatment, the eggs from every concentration were on an individual basis transferred to H_2O cups for emerging assessment when investigating the eggs beneath a magnifier. Each experiment was replicated 5 times. The hatchability was evaluated 48 h post treatment.

2.7. Repellent activity

The repellent study was following the strategies of World Health Organization[24]. About 3 to 4 days, recent blood-starved females of hand-picked mosquito species (100) were unbroken in a very netting cage (45 cm × 45 cm × 40 cm). The unpaid assistant had no contact with moisturizers, fragrances or perfumed cleansers upon the arrival of the measure. The arm of test person was cleaned with isopropyl alcohol. After air drying the arm, solely 25 cm^2 of dorsal facet of the skin on every arm were exposed and the residual space was lined with plastic gloves. The major chemical compositions of *M. officinalis* were dissolved in one metric capacity unit and DMSO served as management. The chosen chemical compositions of volatile oil at 0.75 and 1.50 mg/cm^2 focus were connected. The administration and care for arms were introduced at the same time into the cage. Lots of bites were calculated over 5 min each 30 min. The research was carried

out for 5 times. It absolutely was ascertained that there was not skin annoyance from chemical compositions.

2.8. Statistical analysis

The normal mortality information was focused to probe investigation for ascertaining LC_{50} and LC_{90}[25]. *Chi*-square values were figured by utilizing SPSS rendition 16.0 for windows and significant level was set at $P < 0.05$.

3. Results

3.1. Chemical component of M. officinalis

The chemical constituents of essential oil from *M. officinalis* along with the retention indices was summarized in Table 1. The vital oil was obtained as contemporary aerial elements. *M. officinalis* hydro was distilled in an exceedingly Clevenger equipment and was studied by GC-MS. A total of 24 components were detected representing to 98.73%. The main elements in essential oil were citronellal (22.46%), β-citronellol (14.69%), geraniol (17.74%), geranial (11.55%) and geranyl acetate (12.47%). The proportion compositions of remaining 19 compounds ranged from 0.07%–2.82%.

Table 1
Chemical constituents of the essential oil from aerial parts of *M. officinalis.*

Peak	Compounds	RT (min)	Concentration of aerial parts (%)
1	α-Pinene	938	0.24
2	Camphene	955	0.35
3	β-Pinene	978	0.08
4	Limonene	1 030	1.54
5	γ-Terpinene	1 061	0.07
6	Linalool	1 096	1.22
7	Citronellal	1 153	22.46
8	α-Terpineol	1 188	0.52
9	Estragol	1 194	0.38
10	β-Citronellol	1 227	14.69
11	Geraniol	1 254	17.74
12	Geranial	1 272	11.55
13	Neryl acetate	1 364	1.22
14	Geranyl acetate	1 381	12.47
15	β-Elemene	1 391	1.35
16	α-Cedrene	1 408	0.84
17	α-Amorphene	1 442	1.25
18	γ-Muurolene	1 480	2.82
19	Germacrene D	1 484	1.03
20	γ-Gurjunene	1 473	0.59
21	α-Muurolene	1 501	1.74
22	α-Elemol	1 549	2.03
23	α-Cadinol	1 653	1.92
24	Farnesol	1 713	0.44

Value were expressed as mean ± SD of five replications. Mortality of the larvae was observed after 24 h of RT. RT: Retention time (min).

3.2. Larvicidal activity of M. officinalis

The eventual outcomes of the larvicidal activity of chemical compounds of *M. officinalis* tested against *An. stephensi* were exhibited in Table 2. Among 5 compounds tried, the most extreme larvicidal action was seen in citronellal against *An. stephensi*, among which LC_{50} and LC_{90} qualities were 85.44 and 159.73 mg/L, separately. The information was factually noteworthy at $P < 0.05$ and the *Chi*-square values in the assays showed 21.722.

3.3. Ovicidal activity of M. officinalis

The hatchability of eggs of *An. stephensi* was tried with 5 distinct compounds of *M. officinalis* at various fixations and outcomes were recorded in Table 3. The percent hatchabilities were contrarily corresponding to the centralizations of compound and straightforwardly relative to eggs. Amongst the 5 compounds tried for the ovicidal action against *An. stephensi*, a citronellal compound was observed to be better than different structures against eggs of *An. stephensi* vector mosquitoes. The citronellal compound applied 100% mortality (0 hatchability) at 45, 60, 75 and 90 mg/L against *An. stephensi*, separately. Control bunch demonstrated no eggs mortality (100% hatchability).

Table 2
Larvicidal activities of *M. officinalis* chemical compositions against third instar larvae of *An. stephensi.*

Major compounds	LC_{50} (ppm)	95% confidence limit (LCL–UCL)	LC_{90} (ppm)	95% confidence limit (LCL-UCL)	*Chi*-square value (*df*)*
Citronellal	85.44	(62.32–102.22)	159.73	(138.48–192.83)	21.722(5)*
β-Citronellol	109.07	(88.53–134.62)	185.36	(162.72–257.22)	22.351(5)*
Geraniol	98.72	(79.48–125.37)	172.62	(145.84–238.77)	24.591(5)*
Geranial	145.32	(129.47–169.86)	248.33	(228.51–287.62)	22.541(5)*
Geranyl acetate	126.73	(105.26–142.85)	213.72	(195.84–253.21)	24.225(5)*

Value were expressed as mean ± SD of five replications. Mortality of the larvae was observed after 24 h of exposure period. *: Statistically significant at $P < 0.05$ level; LCL: Lower confidence limit; UCL: Upper confidence limit.

Table 3
Ovicidal activity of *M. officinalis* chemical compositions against eggs of *An. stephensi.*

Concentrations (mg/L)	Percentage of hatchability of egg				
	Citronellal	β-Citronellol	Geraniol	Geranial	Geranyl acetate
Control	100.0 ± 0.00[a]	100.0 ± 0.00[a]	100.0 ± 0.00[a]	100.0 ± 0.00[a]	100.0 ± 0.0[a]
15	39.4 ± 1.4[b]	55.2 ± 1.2[b]	45.2 ± 1.2[b]	73.2 ± 1.2[b]	65.2 ± 1.2[b]
30	23.8 ± 1.2[c]	39.6 ± 1.8[c]	37.6 ± 1.8[c]	64.3 ± 1.5[c]	51.5 ± 1.8[c]
45	NH	28.2 ± 1.2[d]	24.2 ± 1.4[d]	45.8 ± 1.3[d]	34.2 ± 1.5[d]
60	NH	NH	NH	32.6 ± 1.4[e]	18.3 ± 1.2[e]
75	NH	NH	NH	25.2 ± 1.8[f]	NH
90	NH	NH	NH	NH	NH

Values were expressed as mean ± SD of five replications. Different alphabets in the column were statistically significant at $P < 0.05$ level of Duncan's new multiple range method test. Eggs in control groups were sprayed with no phytochemicals.

Table 4
Repellent activity of chemical compositions of *M. officinalis* against *An. stephensi.*

Major compounds	Concentrations (mg/cm^2)	Percentage of repellency							
		30 min	60 min	90 min	120 min	150 min	180 min	210 min	240 min
Citronellal	0.75	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	89.7 ± 3.9
	1.50	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	93.9 ± 2.6
β-Citronellol	0.75	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	84.9 ± 2.6	69.8 ± 2.9
	1.50	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	94.3 ± 2.5	75.6 ± 2.2
Geraniol	0.75	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	93.2 ± 2.4	71.5 ± 2.7
	1.50	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	96.7 ± 2.2	83.1 ± 2.5
Geranial	0.75	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	89.4 ± 2.5	80.6 ± 2.2	69.4 ± 2.9	53.5 ± 2.8
	1.50	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	93.5 ± 2.2	93.4 ± 2.8	72.6 ± 2.2	68.4 ± 2.1
Geranyl acetate	0.75	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	85.6 ± 2.2	69.6 ± 2.9	61.7 ± 2.1
	1.50	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	93.5 ± 2.9	82.5 ± 2.2	73.4 ± 2.5

Values were expressed as mean ± SD of five replications.

3.4. Repellent activity of *M. officinalis*

The data referring to the repellent activity of chemical compounds of *M. officinalis* were additionally portrayed within the Table 4. Among 5 compounds tested, the most repellent activity was discovered in citronellal at 0.75 and 1.50 mg/cm^2 concentration giving 100% insurance up to 210 min against *An. stephensi*.

4. Discussion

Our outcomes demonstrated that the chemical composition of *M. officinalis* essential oil have noteworthy larvicidal and ovicidal repellent actions against *An. stephensi*. The outcomes are practically identical with an earlier report by the chemical composition and larval action of essential oil, *Zanthoxylum gilletii* against *Anopheles gambiae* (*An. gambiae*). The important oils were extracted by hydro-distillation and it was chemical components strong-minded by GC-MS. The important oil was under enemy control by means of sesquiterpenes and monoterpense which had reported in 38.30% and 34.00%, respectively. The important oil showed great action against *An. gambiae* and the evidence of LC_{50} and LC_{90} values of 57.73 and 140.24 mg/mL, respectively. The eggs of mosquito species were the majority affected by *n*-hexane. About 0.00% hatchability and chloroform and 3.67% fractions evaluated to the *Culex quinquefasciatus* (*Cx. quinquefasciatus*) were recorded at 2000 mg/L[26]. The dichloromethane fractions of *Spondias mombin* was the majority effective fraction by LC_{50} value of 2172.815 μg/mL. Compounds identified were mainly 1-*O*-galloyl-6-*O*-luteoyl-α-D-glucose and ellagic acid[27]. GC-MS and larvicides action of important oil were obtained from *Mentha* and *Pulegium* against *Cx. quinquefasciatus*. *Mentha suaveolens* and *Mentha longifolia* essential oil, which are once containing a most divide of piperitenone oxide, showed the highest results. The LD_{50} value was likely at 17 mg/L for both essential oil and LD_{90} value was estimated at 28 mg/L[28].

Santos *et al.* investigated that eucalyptol showed the lowly action value (1.419 mg/L) among 21 tested components against *Aedes aegypti* (*Ae. aegypti*)[29]. Specifically, 12 pure components extract of 2 *Eucalyptus* species leaf essential oil were experimented for larvicide action against IV instar larva of *Ae. aegypti* and *Aedes albopictus*. Fascinatingly, the *Melaleuca alternifolia* essential oil was investigated and the percentages of the aforementioned components were significantly different as a number of extra accessions of this essential oil[30-32]. The mosquitocidal activity of *Salvia sclarea* essential oil was detected against *Ae. aegypti*, *An. stephensi* and *Cx. quinquefasciatus*. The LC_{50} and LC_{90} values of essential oil from *Salvia sclarea* were tested against *Ae. aegypti*, *An. stephensi* and *Cx. quinquefasciatus* larvae which were 66.13, 71.47 and 76.06 mg/L, 130.19, 142.63 and 144.28 mg/L, respectively. The repellent action of essential oil from *Salvia sclarea* was found to be valuable against *Ae. aegypti* followed by *An. stephensi*, *Cx. quinquefasciatus* and the highest concentration of 6 mg/cm^2 provides 100% protection up to 280, 240 and 160 min against *Ae. aegypti*, *An. stephensi* and *Cx. quinquefasciatus*[16]. Among mosquitoes, the essential oils from tea tree have been recognized due to the larvicides action against *Cx. quinquefasciatus* (LC_{50} = 204.1 mg/L)[33]. The ovicidal and oviposition reaction of 10 plants changeable oils were assessed in conditions against *Cx. quinquefasciatus*. Among the 10 clove oils, cinnamon oil and aniseed enlisted showed most astounding ovicidal acvitity (100%) at 200 mg/L. Most extreme oviposition reaction was acquired in clove oil (100%)[34].

Synthetic elements of 15 components were recognized in oil from *Pogostemon cablin* components representing to 98.96%. The repellent activity of patchouli alcohol compound was observed to be better for repellent action than 2 mg/cm^2 fixation giving 100% security up to 280 min against *Ae. aegypti*, *An. stephensi* and *Cx. quinquefasciatus*[35]. Vilasininoid and two havanensinoids from the chloroform fractions and methanol extracts of root barks of *Turraea wakefieldii* and *Turraea floribunda* demonstrated LD_{50} values of 7.1, 4.0 and 3.6 mg/L, respectively, against *An. gambiae* larvae[36]. The larvicidal activity of chloroform extract was likely additional determined, when its A7 fraction displayed powerful toxic effective against *Ae. aegypti* (LC_{50} values of 4.7 μg/mL) and *Aedes albopictus* (LC_{50} values of 5.3 μg/mL). Liquid chromatography-mass spectrometry analysis of the chloroform extract provided a hesitant identification of 13 components. Bis-(3-oxaundecyl) tetrasulfide was known as main compound in A7 fraction. The methanol extract displayed power repellent action against oviposition, together by weak adulticides action against mosquitoes[37]. Attendance of metabolites similar to alkaloids, flavonoids, anthocyanins and anthroquinones in the proved extract power is the reasoning for the larvicides and pupicides action of the crude extracts and fractions of water hyacinth. Repellent action didn't show by these plant extracts at the investigated concentrations. The potential elements present in the aquatic plant, *Eichhornia crassipes* helping in controlling of the filarial vector, *Cx. quinquefasciatus*[38]. To identify the larvicide action of compounds of important oils against mosquitoes, the (+)-limonene, monoterpenes β-asatone, myrcene, ρ-cymene, α-phellandrene, linaly acetate, (+)-β-pinene, (-)-β-pinene, γ-terpinene and α-terpinene, phenylpropenes safrole, terpinolene,and eugenol and the sulfur including components diallyl disulfide have powerful larvicides activity on mosquitoes[39]. In conclusion, this research discloses that *M. officinalis* essential oil had notable mosquitocidal property. In India, vast potential medicinal plant diversity is elements or components which can help in controlling mosquitoes. This research was take on to evaluate the mosquitocidal activities with special reference to larvicidal, ovicidal and repellent potential of compounds from the essential oil of medicinal plant *M. officinalis* against medically important species of human malarial vector, *An. stephensi*. These outcomes obtained are positive in research of choosier, biodegradable and naturally produced mosquitocidal compounds.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgments

The authors are appreciative to superior power for stipend of money related help with Award of University Research Fellowship, furthermore Professor and Head, Department of Zoology, Annamalai University for the laboratory facilities provided and University Grants Commission, New Delhi, for their financial assistance (Ref. No. F 42-597/2013SR).

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