

Biosynthesis of silver nanoparticles from mangrove plant (*Avicennia marina*) extract and their potential mosquito larvicidal property

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Abstract To identify the larvicidal activities of silver nanoparticles synthesised with *Avicennia marina* leaf extract against the larvae of *Aedes aegypti* and *Anopheles stephensi*, in vitro larvicidal activities such as LC₅₀ and LC₉₀ were assessed. Further, characterisation such as UV and FTIR analysis were carried out for the synthesised silver nanoparticles. The LC₅₀ value of the synthesised silver nanoparticles was identified as 4.374 and 7.406 mg/L for *An. stephensi* and *Ae. aegypti* larvae respectively. Further, the LC₉₀ values are also identified as 4.928 and 9.865 mg/L for *An. stephensi* and *Ae. aegypti* species respectively. The synthesised silver nanoparticles have maximum absorption at 420 nm with the average size of 60–95 nm. The FTIR data showed prominent peaks in (3940.57, 3929.00, 3803.63, 3712.97, 2918.30, 2231.64, 1610.50, 1377.17, 1257.59, 1041.59, 1041.56, 775.38, 667.37 and 503.21) different ranges. The biosynthesis of silver nanoparticles with leaf aqueous extract of *A. marina* provides potential source for the larvicidal activity against mosquito borne diseases. The present study proved the mosquitocidal properties of silver nanoparticles synthesised from mangroves of Vellar estuary. This is an ideal eco-friendly approach for the vector control programs.

Keywords Biosynthesis · AgNO₃ · Mangrove · Larvicidal · FTIR

Introduction

Mosquitoes are the most important single group of insects in terms of public health importance, which transmit a number of diseases, such as malaria, filariasis, dengue, Japanese encephalitis, etc. causing millions of deaths every year (Service MV 1983). Repeated use of synthetic insecticides for mosquito control has disrupted natural biological control systems and led to resurgences in mosquito populations (Dhanasekaran et al. 2010). Finding a new safe and efficient drug for treatment of viral respiratory disease, in particular retrovirus infection has been the field of research for many scientist and significant attention has been paid to natural compounds (Zandi et al. 2009). *Avicennia marina* (Forssk.) Vierh, has been traditionally used for treatment of rheumatism, small pox, ulcers and other ailments (Bandaranayake 2002). Its antiviral activity on poliovirus and herpes simplex virus type-1 has not been reported yet from anywhere. Balakrishnan et al. (2013) analyzed the samples collected from the mangrove forests of Parangipettai, Vellar estuary yielded a mosquitocidal *Bacillus*, whose extracellular metabolite(s) exhibited mosquito larvicidal and pupicidal activity. Some studies were done about the anti parasitic, antifungal and antibacterial activity of *A. marina* such as in vitro antimalarial and anticandidal activities as well as cytotoxicity of *A. marina* (Premanathan et al. 1999; Khafagi et al. 2003; Abeyasinghe et al. 2006). The leaves of *A. marina* have no effect on behavioral changes, morbidity or mortality in rats (Ali and Bashir 1998). Synthesis of nanoparticles by using chemical and physical methods requires high pressure, energy,

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temperature and toxic chemicals. Plant extracts are suitably scaled up for large scale biosynthesis of silver nanoparticles in a controlled manner according to their size, shape and sensitivity. But the present study is the first attempt for the biosynthesis of nanoparticles by using mangrove plant extract. Among the mangrove plants *Rhizophora mucronata* is previously proved to have antibacterial, antiplasmodial and antiviral activities (Premanathan et al. 1999; Ravikumar et al. 2009; Ravikumar et al. 2011). Nanosized silver can also be used to treat immunologic and inflammatory diseases (Shin et al. 2007). Currently, silver sulfadiazine is listed as an essential anti-infective topical medicine by the World Health Organization (WHO 2010). In the present study, an attempt has been made to identify the larvicidal activity of silver nanoparticles biosynthesised with *A. marina* leaf extract.

Materials and methods

Collection of plant material

Fresh matured leaves of *A. marina* were collected from artificially developed mangrove forest (Lat. 9°38'N and Long. 78°57'E) located on the shores of Vellar estuary, Parangipettai, Southeast coast of India (Tamil Nadu, India). The authentication of the plant species was developed by Prof. K. Kathiresan, Dean & Director, Centre of Advanced Study in Marine Biology, Annamalai University, Parangipettai, Tamil Nadu, India.

Biosynthesis of silver nanoparticles

The collected leaf sample was washed with tap water and distilled water to remove the adhering salts and other associated animals. About 10 g of finely cut leaves was placed with 100 mL of sterilized double distilled water and then boiled the mixture for 5 min. The boiled extract was filtered with Whatman No. 1 filter paper. A total of 10 mL

Characterisation of biosynthesised nanoparticles

About 1 mL of solution (Diluted with 1:20v/v Milli Q water) was monitored in UV–vis spectrophotometer (between 300–700 nm with 5 nm intervals) with different time intervals (15, 30 min, 4, 6 and 8 h). After 8 h of incubation, the solution was centrifuged at 12,000 rpm for 20 min and their pellets were redispersed in sterile distilled water. The centrifugation and redispersion was repeated three times to ensure the complete separation of nanoparticles. The purified pellet was dried and subjected to the FTIR spectroscopy measurement in the diffuse reflectance mode at a resolution of 4 cm⁻¹ in KBr pellets.

Larvicidal bioassay (preliminary screening)

The eggs and egg rafts of *Aedes aegypti* and *Anopheles stephensi* were procured from Vector Control Research Centre (VCRC), Puducherry, India. Filter paper with attached eggs was dipped into a plastic tray containing 500 mL of dechlorinated water for 30–40 min, to hatch out larvae. The reared larvae were maintained for 5 days in standard environment (27 ± 1).

Larval/pupal toxicity test

Laboratory colonies of mosquito larvae/pupae were used for the larvicidal/pupicidal activity. Twenty five numbers of first to fourth instars larvae and pupae were introduced into 500 mL glass beaker containing 249 mL of dechlorinated water and 1 mL of desired concentrations (plant extract and silver nanoparticles). Larval food was given for the test larvae. At each concentration, two to five trials were made and each trial consisted of five replicates. The control was made by mixing 1 mL of acetone with 249 mL of dechlorinated water. The larvae and pupae were exposed to dechlorinated water without acetone served as control. The control mortalities were calculated by using Abbott's formula (Abbott 1925).

$$\text{Corrected mortality} = \frac{\text{Observed mortality in treatment} - \text{Observed mortality in control}}{100 - \text{Control mortality}} \times 100$$

of collected filtrate was treated with 90 mL of silver nitrate aqueous solution (21.2 g of AgNO₃ powder in 125 mL of Milli Q water) and incubated at room temperature for 10 min, resulting in the formation of brownish yellow solution indicating the synthesis of silver nanoparticles (Parashar et al. 2009).

$$\text{Percentage mortality} = \frac{\text{No. of dead larvae/pupae}}{\text{No. of larvae/pupae introduced}} \times 100$$

The LC₅₀ and LC₉₀ were calculated from toxicity data by using Probit analysis (Finney 1971).

Statistical analysis

The average larval mortality data were subjected to probit analysis for calculating LC_{50} and LC_{90} , and other statistics at 95 % confidence limit (LCL-UCL) and Chi square values were calculated using the SPSS 16.0 version (Statistical software package - SPSS Inc., Chicago, IL, USA) to find the regression equation values. Results with $p \leq 0.05$ were considered to be statistically significant.

Results

The present study was tested with mangrove leaf extracts from *A. marina* against the vector borne disease causing mosquito like *An. stephensi* and *Ae. aegypti*. It revealed that, mangrove leaf extracts of *A. marina* showed various ranges of larvicidal activities and the maximum percentage of larvicidal activity. The color change was noted by visual observation in the mangrove leaf extracts when incubated with $AgNO_3$ solution (Fig. 1a, b). Absorption spectrum of leaf extracts at different wavelengths ranging from 200 to 800 nm revealed a peak at 420 nm. Both callus and leaf extracts without $AgNO_3$ did not show any change in colour. The colour intensity of the synthesised silver nanoparticles was increased with increased time duration and the maximum intensity was observed with 420 nm wavelength (Fig. 2). The results of the present study suggest that, the maximum (100 %) percentage of mosquito larvicidal activity was identified with 5 and 10 mg/L *An. stephensi* and *Ae. aegypti* fourth instar larvae (Table 1). The results of the dose dependent assay suggested that, the value LC_{50} was identified as 4.374 and 7.406 mg/L *An.*

stephensi and *Ae. aegypti* respectively. Further, the LC_{90} values were identified as 4.928 and 9.865 mg/L for *An. stephensi* and *Ae. aegypti* respectively. The results of 95 % fiducial limits (FL), lower confidence limit (LCL), upper confidence limit (UCL) and Chi square (X^2) values are mentioned in Table 2. The results of the FTIR spectra of the synthesised silver nanoparticles exhibited prominent peaks with (3940.57, 3929.00, 3803.63, 3712.97, 3423.65, 2918.30, 2231.64, 1610.58, 1377.17, 1257.59, 1041.50, 775.38 and 667.37) different values (Fig. 3).

Discussion

Many marine plants synthesized a variety of chemically diverse secondary metabolites in response to selection pressures from herbivores and microorganisms and some of these compounds are recognized as insecticides. The bio-activity of phytochemicals against mosquito larvae can vary significantly depending on plant species, plant parts, age of plant parts, solvent used in extraction and mosquito species (Syed Ali et al. 2012). Most studies on phytochemicals focus on herbs and other medicinal plants. This may due to historical experiment knowledge and some scientific studies have shown that they are particularly active against certain organisms. Several studies have focused specifically on medicinal plants in different geographical regions. Commonly a connection is extrapolated between plant activity against disease agents based on traditional experience and insecticidal activity against mosquitoes. Mosquito control management including periodic larviciding (WHO 1975; Becker et al. 2003) is very helpful in favorable conditions. Larval breeding



Fig. 1 Conical flask with silver nitrate ($Ag-100$ ppm) before (1) and after (2) exposure to *A. marina* showing $AgNO_3$ a silver nanoparticles after 6 h and b 72 h

Fig. 2 UV-vis absorption spectra of silver nanoparticles synthesized by *A. marina* leaf extract with different time intervals

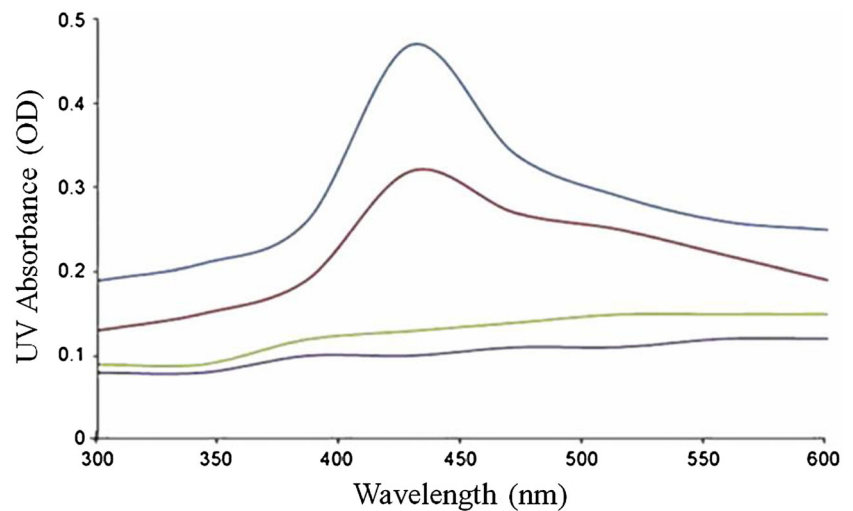


Table 1 Larvicidal activity of the *A. marina* synthesised silver nanoparticles against *Ae. aegypti* and *An. stephensi* larvae

Concentrations (mg/L)	% of mortality in <i>Ae. aegypti</i>	% of mortality in <i>An. stephensi</i>
20.00	100.00 ± 0.00	100.00 ± 0.00
10.00	100.00 ± 0.00	100.00 ± 0.00
5.00	100.00 ± 0.00	98.00 ± 15.88
2.50	90.15 ± 8.79	87.58 ± 5.85
1.25	88.68 ± 9.72	68.13 ± 3.58

habitat is a successful and safer way to interrupt larval stages of vectors rather than the infective adult stage (Thangam and Kathiresan 1991). Many plant derived natural compounds was tested for mosquito control (Oladimeji et al. 2007, 2008; Vinayagam et al. 2008; Chakkaravarthy et al. 2011; Almeahadi 2011). Ishibashi et al. (1993) reported the extracts of *Aglaia elliptifolia* showed

insecticidal activity. Early studies envisaged that the Indian marine plant extracts possessed potential larvicidal activity (Thangam et al. 1993; Rao et al. 1995).

The production of the silver nanoparticles synthesized from callus and leaf extracts of *A. marina* was evaluated through spectrophotometer in a range of wavelength from 300 to 600 nm. This revealed a peak at 420 nm in leaf extracts of *A. marina* indicating the production of silver nanoparticles. This is similar to the surface plasmon vibrations with characteristic peaks of silver nanoparticles prepared by chemical reduction (Petit et al. 1993; Kong and Jang 2006). Due to the excitation of plasma resonances or inter band transitions, some metallic nanoparticle dispersions exhibit the unique bands/peaks (Kuber and D'Souza 2006). A similar result was obtained by other researchers (Ahmad et al. 2003; Kathiresan et al. 2009). The results of the FTIR used to identify the possible bio molecules responsible for the stabilization of the

Table 2 Dose dependant larvicidal activity of silver nanoparticles synthesized by *A. marina* leaf extract against *Ae. aegypti* and *An. stephensi* larvae

Species	Concentrations (mg/L)	% of mortality	LC ₅₀ (mg/L)	95 % FL LCL–UCL	LC ₉₀ (mg/L)	95 % FL LCL–UCL	X ²
<i>Ae. aegypti</i>	20.00	100.00 ± 0.00	–	–	–	–	–
	10.00	85.22 ± 2.05	–	–	–	–	–
	5.00	70.49 ± 2.84	–	–	–	–	–
	2.50	65.58 ± 6.27	4.374	6.674–12.280	4.928	6.080–10.679	0.619*
	1.25	50.47 ± 5.23	–	–	–	–	–
<i>An. stephensi</i>	20.00	100.00 ± 0.00	–	–	–	–	–
	10.00	87.83 ± 2.51	–	–	–	–	–
	5.00	69.43 ± 2.13	–	–	–	–	–
	2.50	63.24 ± 5.38	7.406	7.029–13.626	9.865	7.982–26.926	1.148*
	1.25	47.74 ± 5.08	–	–	–	–	–

FL fiducial limits, LCL lower confidence limit, UCL upper confidence limit, X² Chi square value

* $p \leq 0.05$ level values are represents as mean ± SD values

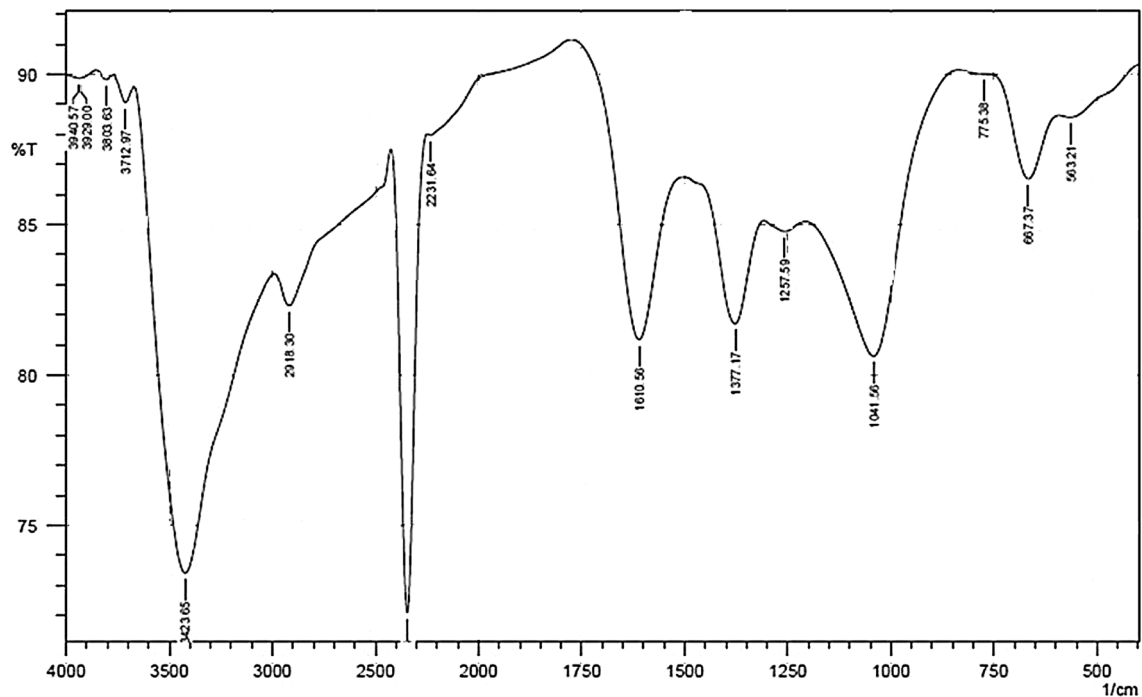


Fig. 3 FT-IR spectrum of silver nanoparticles synthesised by *A. marina* leaf extract

synthesised silver nanoparticles. The prominent peaks of the FTIR results are showing the correspond values to the amide group (N-H stretching-3 426.89), aliphatic group (Cyclic CH_2 -2 925.49), methyl group (CH_3 -2 869.56), alkane group (CH -2 346.95) alkene (CC-1 631.49 and 669.178) and ether groups (COC-1 031.73). The observed peaks are considered as major functional groups in different chemical classes such as flavonoids, triterpenoids and polyphenols (Asmathunisha et al. 2010). Hence, the terpenoids are proved to have good potential activity to convert the aldehyde groups to carboxylic acids in the metal ions. Further, amide groups are also responsible for the presence of the enzymes and these enzymes are responsible for the reduction synthesis and stabilization of the metal ions, further, polyphenols are also proved to have potential reducing agent in the synthesis of the silver nanoparticles (Asmathunisha et al. 2010; Prasad and Elumalai 2011; Mukunthan et al. 2011). Previously, Baun et al. (2008) reported that, the toxicity of C60-carbon nanotubes and titanium dioxide to an aquatic invertebrate *Daphnia magna*. The coastal plants are generally rich in polyphenolic compounds (Kathiresan and Veera Ravi 1990). Thus several factors may determine the nanoparticle synthesis by saltmarsh plant and however, the exact mechanism is yet to be elucidated. It is concluded from the present findings that, the biosynthesised silver nanoparticles of leaf aqueous extract of *A. marina* provided potential killing effect on mosquito larvae's which could be used for prevention of several dreadful diseases.

Conclusion

The biosynthesized silver nanoparticles using *A. marina* leaves extract proved excellent mosquitocidal activity. Hence, the biological approach appears to be cost efficient alternative to conventional physical and chemical methods of silver nanoparticles synthesis and would be suitable for developing a biological process for large-scale production.

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Conflict of interest All authors have read the manuscript and have agreed to submit it in its current form for consideration for publication in the Journal. We declare that we have no conflict of interest.

References

- Abbott WS (1925) A method of computing the effectiveness of an insecticide. *J Econ Entomol* 18:265–267
- Abeyasinghe PD, Wanigatunge RP, Pathirana RN (2006) Evaluation of antibacterial activity of different mangrove plant extracts. *Ruhuna J Sci* 1:108–116
- Ahmad A, Mukherjee P, Senapati S (2003) Extracellular biosynthesis of silver nanoparticles using the fungus *Fusarium oxysporum*. *Colloids Surf B* 28:313–318
- Ali BH, Bashir AK (1998) Toxicological studies on the leaves of *Avicennia marina* (mangrove) in rats. *J Appl Toxicol* 18:111–116

- Almehmadi RM (2011) Larvicidal, histopathological and ultra-structure studies of *Matricharia chamomella* extracts against the rift valley fever mosquito *Culex quinquefasciatus* (Diptera: Diptera). *J Entomol* 8:63–72
- Asmathunisha N, Kathiresan K, Anburaj K (2010) Synthesis of antimicrobial silver nanoparticles by callus leaf extracts from saltmarsh plant *Sesuvium portulacastrum* L. *Colloids Surf B Biointer* 79:488–493
- Balakrishnan S, Indira K, Srinivasan M (2013) Mosquitocidal properties of *Bacillus* species isolated from mangroves of the Vellar estuary, Southeast coast of India. *J Para Dis*. doi: 10.1007/s12639-013-0371-9
- Bandaranayake WM (2002) Bioactivities, bioactive compounds and chemical constituents of mangrove plants. *Wetl Ecol Manag* 10:421–452
- Baun A, Hartmann NB, Grieger K (2008) Ecotoxicity of engineered nanoparticles to aquatic invertebrates: a brief review and recommendations for future toxicity testing. *Ecotoxicology* 17:387–396
- Becker N, Petric D, Zgomba M (2003) Mosquitoes and their control. Kluwer Academic/Plenum Publishers, New York, pp 5–23
- Chakkaravarthy VM, Ambrose T, Vincent S (2011) Bioefficacy of *Azadirachta indica* (A. juss) and *Datura metel* (Linn.) leaves extracts in controlling *Culex quinquefasciatus* (Diptera: Culicidae). *Trends Appl Sci Res* 8:191–197
- Dhanasekaran D, Sakthi V, Thajuddin N (2010) Preliminary evaluation of *Anopheles* mosquito larvicidal efficacy of mangrove actinobacteria. *Int J Appl Biol Pharm Tech* 1(2):374–381
- Finney DJ (1971) Probit analysis. Cambridge University Press, Cambridge
- Ishibashi F, Satasook C, Isman MB (1993) Insecticidal 1H-Cyclopentetrahydro[b] Benzofurans from *Aglaia odorata*. *Phytochemistry* 32:307–310
- Kathiresan K, Veera Ravi A (1990) Seasonal changes in tannin content of mangrove leaves. *Indian For* 116(5):390–392
- Kathiresan K, Manivannan S, Nabeel MA, Dhivya B (2009) Studies on silver nanoparticles synthesized by a marine fungus *Penicillium fellutanum* isolated from coastal mangrove sediment. *Colloids Surf B* 7:133–137
- Khafagi I, Gab-Alla A, Salama W (2003) Biological activities and phytochemical constituents of the gray mangrove *Avicennia marina* (Forssk.) Vierh. *Egypt J Bot* 5:62–69
- Kong H, Jang J (2006) One-step fabrication of silver nanoparticle embedded polymer nano fibers by radical-mediated dispersion polymerization. *Chem Commun* 28:3010–3012
- Kuber CB, D'Souza SF (2006) Extracellular biosynthesis of silver nanoparticles using the fungus *Aspergillus fumigatus*. *Colloids Surf B* 47:160–164
- Mukunthan KS, Elumalai EK, Patel TN (2011) *Catharanthus roseus*: a natural source for the synthesis of silver nanoparticles. *Asian Pac J Trop Biomed* 1(4):270–274
- Oladimeji HO, Nia R, Kalu N (2007) In vitro biological activities of *Carica papaya*. *Res J Med Plant* 1:92–99
- Oladimeji HO, Ubulom PM, Akpabio EI (2008) Larvicidal and antimicrobial potentials of *Nymphaea odorata*. *J Pharmacol Toxicol* 3:357–362
- Parashar UK, Saxena PS, Srivastava A (2009) Bio inspired synthesis of silver nanoparticles. *Dig J Nanomater Biostruct* 4:159–166
- Petit C, Lixon MP, Pileni MP (1993) In situ synthesis of silver nanocluster in AOT reverse micelles. *J Phys Chem* 97:12974–12983
- Prasad TNVKV, Elumalai EK (2011) Biofabrication of Ag nanoparticles using *Moringa oleifera* leaf extract and their antimicrobial activity. *Asian Pac J Trop Biomed* 1(6):439–443
- Premanathan M, Kathiresan K, Yamamoto N (1999) In vitro anti-human immunodeficiency virus activity of polysaccharide from *Rhizophora mucronata* Poir. *Biosci Biotechnol Biochem* 63(7):1187–1191
- Rao DR, Mani TR, Rajendran R (1995) Development of high level of resistance to *Bacillus sphaericus* in a field population of *C. quinquefasciatus* Say (Diptera: Culicidae). *Afr J Biomed Res* 8:31–33
- Ravikumar S, Ramanathan G, Subhakaran M (2009) Antimicrobial compounds from marine halophytes for silkworm disease treatment. *Int J Med Sci* 5:184–191
- Ravikumar S, Ramanathan G, Jacob Inbaneson S (2011) Antiplasmodial activity of two marine polyherbal preparations from *Chaetomorpha antennina* and *Aegiceras corniculatum* against *Plasmodium falciparum*. *Parasitol Res* 108(1):107–113
- Service MV (1983) Biological control of mosquitoes has it a future? *Mosq News* 43:113–120
- Shin SH, Ye MK, Kim HS (2007) The effects of nanosilver on the proliferation and cytokine expression by peripheral blood mononuclear cells. *Int Immuno pharmacol* 7:1813–1818
- Syed Ali M, Ravikumar S, Margaret Beula J (2012) Bioactivity of seagrass against the dengue fever mosquito *Aedes aegypti* larvae. *Asian Pac J Trop Biomed* 2:570–573
- Thangam TS, Kathiresan K (1991) Mosquito larvicidal activity of marine plant extracts with synthetic insecticides. *Bot Mar* 34:537–539
- Thangam TS, Kathiresan K, Mabbett T (1993) Mosquito larvicidal activity of seaweed extract against *A. aegypti* and *C. quinquefasciatus*. *Int Pest Control* 35:94–95
- Vinayagam A, Senthilkumar N, Umamaheshwari A (2008) Larvicidal activity of seaweed extract against *A. aegypti* and *C. quinquefasciatus*. *Int Pest Control* 35:94–95
- WHO (1975) Instructions for determining the susceptibility of resistance mosquito larvae to insecticides. Mimeographed Document WHO/VBC/75 583
- WHO (2010) Antiretroviral treatment working group treatment white paper
- Zandi K, Taherzadeh M, Yaghoubi R (2009) Antiviral activity of *Avicennia marina* against herpes simplex virus type 1 and vaccine strain of poliovirus (An in vitro study). *J Med Plan Res* 3(10):771–775