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Larvicidal and Repellent Properties of *Streptomyces* sp. VITJS4 Crude Extract against *Anopheles stephensi*, *Aedes aegypti* and *Culex quinquefasciatus* (Diptera: *Culicidae*)

S. JEMIMAH NAINE and C. SUBATHRA DEVI*

Industrial Biotechnology Division, School of Biosciences and Technology,
VIT University, Vellore, Tamil Nadu, India

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Abstract

The aim of the present study was to assess the larvicidal and repellent properties of marine *Streptomyces* sp. VITJS4 crude extracts. The marine soil samples were collected from the Puducherry coast, Tamil Nadu, India. The isolate *Streptomyces* sp. VITJS4 was taxonomically characterized and identified. The ethyl acetate crude extract tested for larvicidal property showed 100% mortality for all the 3 species after 24 h exposure against the early fourth instar larvae of malarial vector – *Anopheles stephensi* at 50% and 90% lethal concentration (LC_{50} = 132.86, LC_{90} 396.14 ppm); dengue vector – *Aedes aegypti* (LC_{50} = 112.78, LC_{90} 336.42 ppm) and filariasis vector – *Culex quinquefasciatus* (LC_{50} = 156.53, LC_{90} 468.37 ppm). The *Streptomyces* sp. VITJS4 solvent extracts of hexane, ethyl acetate, benzene, chloroform and methanol were tested for repellent activity against *A. stephensi, A. aegypti* and *C. quinquefasciatus*. The ethyl acetate extract showed complete protection for 210 min at 6 mg/cm^2 against these mosquito bites. The crude extract was analyzed further for Fourier Transform-infrared spectroscopy (FT-IR) analysis. In addition to the importance of bioactive compounds, the utilization of *Streptomyces* sp. VITJS4 crude extracts revealed effective larvicidal and repellent activity against the vectors, which perhaps represents a promising tool in the management of mosquito control.

Key words: *Streptomyces* sp. VITJS4, insecticides, biological control, eco-friendly

Introduction

Mosquitoes are the most important arthropod disease vectors, transmitting dreadful human diseases in over 100 countries, causing the mortality of nearly two million people every year (Kundsen and Slooff, 1992; Klempner *et al.*, 2007). They spread many dreadful diseases such as filariasis, malaria, dengue, yellow fever and Japanese encephalitis, which contribute significantly to disease burden, death, poverty and social debility in tropical countries (Jang *et al.*, 2002). Dengue is transmitted to humans through the bite of the mosquito *Aedes aegypti* (Diptera: *Culicidae*) which causes a severe flu-like illness. Since there is no specific treatment or vaccine for dengue, the only method of controlling or preventing dengue virus transmission is to combat the vector mosquito by using environmental management and chemical methods (Santos *et al.*, 2001). It is estimated that there are between 50 and 100 million cases of dengue fever and about 500,000 cases of dengue hemorrhagic fever each year which require hospitalization (Maheswaran *et al.*, 2008). *Anopheles stephensi* transmits malaria in the plains of rural and urban areas of India. Malaria afflicts 36% of the world population, *i.e.*, ~2 milliard in 107 countries and territories situated in the tropical and subtropical regions. In the South East Asian region of the World Health Organization (WHO), out of about 1.4 billion people, 1.2 billion (85.7%) are exposed to the risk of malaria and most of them live in India. Of the 2.5 million reported cases in the Southeast Asia, India alone contributes about 70% of the total cases (Kondrachine, 1992). Another vector of *Culex quinquefasciatus* has been recorded the year round in different parts of the country (Chand *et al.*, 1988). *C. quinquefasciatus,* the mosquito species commonly found in rural areas is known to cause severe biting nuisance. It is an important vector of lymphatic filariasis caused by nematodes such as *Wuchereria bancrofti* and *Brugiamalayi* in humans in some parts of India. Globally over 100 million individuals are affected due to filariasis annually (Ahmed *et al.*, 1984). As mosquitoes are water breeders their larval stages are attractive targets of pesticides (Rawani *et al.*, 2010). It is known that larvicides play a vital role in controlling

* Corresponding author: C. Subathra Devi, School of Biosciences and Technology, VIT University, Vellore, Tamil Nadu, India; fax. 91-416-2243092; **e-mail: csubathradevi@vit.ac.in**, **subaresearch@rediffmail.com**

mosquitoes in their breeding sites (Amer and Mehlhorn, 2006). Synthetic chemical larvicides continue to be applied for controlling mosquitoes in most parts of the world, especially with organophosphate and pyrethroid larvicides (Rahuman *et al*., 2009). But many of these chemicals are toxic to human, plant, animal life.

Although various biocontrol measures are in vogue, their effective control of larval mosquitoes has not been hitherto highlighted. Microorganisms and microbial products with potential insecticidal activity can play an important role in controlling diseases by interrupting the transmission mechanism by killing insect vectors at the community level (Patil *et al*., 2011). Mosquito control therefore, continues to be an important strategy in preventing mosquito-borne diseases. Microbial control of insect vector populations can be highly effective and generally has advantages over chemical control because many are host specific and safe for non-target organisms (Carlos *et al*., 2011). The common mosquito larvicides nowadays include an organophosphate temephos, methoprene. However, the high amount of chemical larvicides could lead to long-term residual effects to the environment and chronic effects on non-target organisms. Almost 80% of the world's antibiotics are known to come from actinomycetes, mostly from the genera *Streptomyces* and *Micromonospora*. Due to this property, marine actinobacteria have received attention. It has been proposed that these antimicrobials are used in competition between microorganisms, offering an advantage to the producer strains (Jensen *et al*., 2005). Actinobacteria are Gram-positive, filamentous organisms dwelling in the soil (Sanglier *et al*., 1993). They are widespread in distribution (Deepika *et al*., 2009) and produce a vast array of secondary metabolites, including enzymes (Strohl, 2004; Berdy, 2005), antibiotics (Blunt, 2006), anti-helminthics (Bibb, 2005), immunomodulators (Mann, 2001). A new larvicidal antibiotic, aculeximycin was found in the culture broth of an actinomycete identified as *Streptosporangium albidum* which exhibits strong larvicidal activity against mosquito larvae (Ikemoto *et al*., 1983). Hence the present study was aimed to investigate the larvicidal and repellent properties of marine *Streptomyces* sp. VITJS4 crude extract.

Experimental

Materials and Methods

Marine soil collection and isolation. Marine soil samples were collected from the South East coast of India, Puducherry – Thavalakuppam (11.52°N, 79.47°E), at the depth of 10–100 cm at littoral zone and the collected samples were stored at 4°C. The well defined adaptation of marine *Streptomyces* species requires seawater for growth (Macleod, 1965). The isolation was performed on selective media such as actinomycetes isolation agar, Kuster's agars, Bennett agar, Starch casein agar supplemented with 25% marine water and 25% marine soil extract for effective isolation. All the plates were incubated at 30°C for 1–2 weeks. Emerging colonies were sub cultured on ISP2 agar and stored at 4°C.

Taxonomic investigation. The micro-morphological studies and species level characterization was based on morphological, cultural and physiological characteristics following the directions given for the International *Streptomyces* project (ISP) and Bergey's Manual of Determinative Bacteriology (Shirling and Gottlieb., 1966; Lechevalier and Lechevalier, 1970; Buchanan and Gibbons, 1974). Colours were determined according to the scale adopted (Prauser, 1964). The colour of sporulating aerial mycelia and the growth of actinomycetes was determined on starch casein agar plates and compared with key guidelines (Nonomura, 1974)

Fermentation and extraction. The potent isolate *Streptomyces* sp. VITJS4 was inoculated on starch casein broth at a seed concentration of 100 ml in a 250 ml Erlenmeyer flask at an incubation period of 7 days at room temperature and the medium was adjusted to pH 7.2. Various solvents including hexane, chloroform, benzene, methanol and ethyl acetate was used for the extraction process (Remya and Vijayakumar, 2007). All the crude extract powder was weighed and stored for further use.

Insect rearing. *A. aegypti*, *A. stephensi* and *C. quinquefasciatus* larvae were collected from rice field and stagnant water areas of Vellore and identified in Zonal Entomological Research Centre, Chennai, Tamil Nadu, India. The larvae were kept in plastic and enamel trays containing tap water. They were maintained and reared in the laboratory as per the method described (Kamaraj *et al*., 2009). All the experiments were carried out at 27°C. Larvae were fed a diet of brewer's yeast, dog biscuits and algae collected from ponds in a ratio of 3:1:1, respectively. Pupae were transferred from the trays to a cup containing tap water and were maintained (45 × 45 × 40 cm) till adults emerged and were maintained in glass cages provided with 10% sucrose solution in a jar with a cotton wick. On day 5, the adults were given a blood meal from a pigeon placed in resting cages overnight for blood feeding by females.

Larvicidal bioassay. During preliminary screening with the laboratory trial, the larvae of *A. aegypti*, *A. stephensi* and *C. quinquefasciatus* were collected from the insect-rearing cage. The larvicidal activity was assessed by the procedure of WHO with some modification (Rahuman *et al*., 2009). For bioassay test, larvae were taken in five batches of 20 in 249 ml of water and 1 ml of the desired *Streptomyces* sp. VITJS4 ethyl acetate extract. The numbers of dead larvae were counted

after 24 h of exposure and the percentage of mortality was reported from the average of five replicates. The experimental set up at which 100% mortality of larvae occurred were selected for dose response bioassay.

Repellent bioassay. The stock solutions of the extracts were diluted with polysorbate 80 and distilled water to obtain test solutions of 1.0, 3.0 and 6.0 mg/cm^2 hexane, ethyl acetate, benzene, chloroform and methanol prepared separately. For repellent experiment, 50 laboratory reared blood-starved adult female mosquitoes between 3 and 10 days old were placed into laboratory cages (45 × 45 × 40 cm). Before each test, the forearm and hand of a human subject were washed with unscented neutral soap, thoroughly rinsed and allowed to dry for 10 min before extract application. The different *Streptomyces* sp. VITJS4 extracts were applied from the elbow to the fingertips. The arm was left undisturbed. An arm treated with acetone and polysorbate 80 served as a control. The control and treated arms were introduced simultaneously into the cage. The number of bites was counted over 15 min, every 30 min and from 180 to 210 min. Protection time was recorded as the time elapsed between repellent application and the observation period immediately once a confirmed bite was obtained. If no bites were confirmed at 210 min, tests were discontinued and protection time was recorded as 210 min. The number of mosquitoes attempting to bite the control arm during the observation period was recorded. The experiments were conducted five times in separate cages. The percentage protection was calculated by using the following formula (Fradin and Day, 2002).

Protection = ({No. of bites received by control arm} – {No. of bites received by treated arm}) / No. of bites received by control arm X 100

Infrared spectroscopy. FT-IR has proven to be a valuable tool for the characterization and identification of compounds or chemical bonds present in an unknown mixture (Eberhardt *et al*., 2007; Hazra *et al*., 2007). The FT-IR spectra of the ethyl acetate crude extract samples were recorded on a Thermo Nicolet, Avatar 370 spectrometer equipped with a Deuterated triglycine sulphate detector (DTGS) over the 4000–400 cm^{-1} range at the resolution of 4 cm^{-1} and a maximum source aperture and the infrared spectra of the crude extracts were measured (as KBr discs).

Data analysis. The average parasite mortality data were subjected to probit analysis for calculating LC_{50} and other statistics at 95% fiducial limits of upper confidence limit (UCL) and lower confidence limit (LCL) were calculated.

Results

The present investigation was carried out with an aim to develop a safe and eco-friendly strategy and to explore the larvicidal and repellent activity of marine *Streptomyces* as a sustainable source for the biocontrol of vectors. A set of experimental tests was performed to determine the safe and effective dose of the extracts to be used for each test. The larvicidal bioassays performed on early fourth instar larvae of *A. stephensi*, *A. aegypti* and *C. quinquefasciatus* with ethyl acetate extract of *Streptomyces* sp. VITJS4 (Table I). The mortality rate

Table I
Larvicidal activity of ethyl acetate extract of *Streptomyces* sp. VITJS4 against the fourth-instar larvae of *A. stephensi*, *A. aegypti* and *C. quinquefasciatus*

Species	Concentration (ppm)	Percent[a] mortality ± SD	LC_{50} (ppm) (LCL-UCL)	LC_{90} (ppm) (LCL-UCL)	r^2	χ2 (df = 4)
A. stephensi	300	100 ± 0.000	132.86 (126.61–139.48)	396.14 (275.02–412.31)	0.992	9.678
	240	84 ± 0.327				
	180	62 ± 0.894				
	120	41 ± 0.521				
	60	29 ± 0.438				
A. aegypti	300	98 ± 0.521	112.78(106.67–119.04)	336.42(319.47–360.26)	0.987	7.376
	240	81 ± 0.905				
	180	59 ± 0.848				
	120	39 ± 0.394				
	60	28 ± 0.486				
C. quinquefasciatus	300	100 ± 0.000	156.53(150.28–163.43)	468.37(419.19–486.09)	0.993	8.800
	240	86 ± 0.198				
	180	63 ± 0.637				
	120	44 ± 0.984				
	60	30 ± 1.091				

Control (distilled water) – nil mortality. LC_{50} and LC_{90} lethal concentration that kills 50 and 90 % of the exposed larvae, UCL upper confidence limit, LCL lower confidence limit, r^2 regression coefficient.

of 100, 84, 62, 41 and 29% were found against the fourth instar larvae of *A. stephensi*; 98, 81, 59, 39 and 28% against *A. aegypti*; and 100, 86, 63, 44 and 30% against *C. quinquefasciatus* in the concentrations of 300, 240, 180, 120 and 60 ppm respectively. The ethyl acetate extract of *Streptomyces* sp. VITJS4 showed highest mortality rate against the larvae of *A. stephensi* (LC_{50} = 132.86 and LC_{90} = 396.14; $\chi 2$ = 9.678), *A. aegypti* (LC_{50} = 112.78 and LC_{90} = 336.42; $\chi 2$ = 7.376), *C. quinquefasciatus* with (LC_{50} = 146.24 and LC_{90} = 468.37; $\chi 2$ = 8.800). The hexane, ethyl acetate, benzene, chloroform and methanol extracts of *Streptomyces* sp. VITJS4 showed significant repellence against *A. stephensi*, *A. aegypti* and *C. quinquefasciatus* (Tables II, III and IV). In this observation, the *Streptomyces* sp. VITJS4 extracts gave protection against mosquito bites without

Table II
Repellence of different extracts of *Streptomyces* sp. VITJS4 against the fourth-instar larvae of *A. stephensi*

Solvents	Concentration (mg/cm²)	% of Repellency ± SD Time after application of repellent (min)							
		15	30	60	90	120	150	180	210
Hexane	1.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	74.5 ± 0.4	78.3 ± 1.1	79.2 ± 0.5
	3.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	81.1 ± 1.8	86.6 ± 1.8	81.7 ± 1.9
	6.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	87.9 ± 1.4	89.5 ± 0.6	82.9 ± 0.3
Ethylacetate	1.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	94.1 ± 1.4	90.4 ± 0.5	88.6 ± 1.3
	3.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	97.3 ± 1.8	93.2 ± 0.4	91.4 ± 1.5
	6.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	99.7 ± 1.0	93.6 ± 0.8
Benzene	1.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	90 ± 0.0	82.2 ± 1.1	81.5 ± 1.2
	3.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	90.4 ± 0.7	87.1 ± 0.4	84.4 ± 0.8
	6.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	91.6 ± 1.6	83.0 ± 1.6	86.7 ± 1.7
Chloroform	1.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	89.5 ± 1.0	82.2 ± 1.6	80.9 ± 0.4
	3.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	90.7 ± 0.6	83.1 ± 0.2	83.3 ± 0.9
	6.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	92.2 ± 1.2	86.0 ± 1.0	85.0 ± 0.8
Methanol	1.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	90.1 ± 0.0	76.4 ± 0.8	79.9 ± 0.2
	3.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	92.1 ± 0.0	78.8 ± 1.2	81.3 ± 1.3
	6.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	96.3 ± 0.5	78.0 ± 1.6	83.0 ± 0.4

Table III
Repellence of different extracts of Streptomyces sp. VITJS4 against the fourth-instar larvae of *A. aegypti*

Solvents	Concentration (mg/cm²)	% of Repellency ± SD Time after application of repellent (min)							
		15	30	60	90	120	150	180	210
Hexane	1.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	97.2 ± 1.0	91.3 ± 0.6	76.2 ± 0.7
	3.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	95.1 ± 1.2	87.6 ± 0.4	76.7 ± 1.2
	6.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	91.4 ± 0.6	82.5 ± 0.6	76.9 ± 0.4
Ethyl acetate	1.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	97.1 ± 1.4	90.2 ± 1.1	80.5 ± 1.2
	3.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	99.3 ± 1.8	92.1 ± 0.4	84.4 ± 0.8
	6.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	99.0 ± 1.6	88.7 ± 1.7
Benzene	1.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	96.2 ± 0.4	80.1 ± 1.1	72.3 ± 0.7
	3.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	92.1 ± 1.2	86.1 ± 0.4	74.5 ± 0.8
	6.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	89.7 ± 0.4	89.0 ± 1.6	79 ± 1.0
Chloroform	1.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	95.2 ± 0.6	80.2 ± 1.0	76.3 ± 0.3
	3.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	93.5 ± 1.0	80.5 ± 0.2	78.9 ± 0.7
	6.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	90.1 ± 1.2	83.7 ± 0.4	82.0 ± 1.2
Methanol	1.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	90 ± 0.0	86.1 ± 0.4	79.3 ± 0.7
	3.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	90.1 ± 0.5	92.3 ± 0.6	81.6 ± 1.2
	6.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	94.8 ± 1.0	93.0 ± 0.2	83.5 ± 0.2

Table IV
Repellence of different extracts of *Streptomyces* sp. VITJS4 against the fourth-instar larvae of *C. quinquefasciatus*

Solvents	Concentration (mg/cm²)	% of Repellency ± SD Time after application of repellent (min)							
		15	30	60	90	120	150	180	210
Hexane	1.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	96.2 ± 0.6	80.2 ± 1.0	76.3 ± 0.3
	3.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	93.5 ± 1.0	83.5 ± 0.2	76.9 ± 0.7
	6.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	91.1 ± 1.2	83.7 ± 0.4	82.0 ± 1.2
Ethyl acetate	1.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	92.4 ± 0.8	90.2 ± 0.7
	3.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.6	96.7 ± 0.6	91.6 ± 1.2
	6.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 1.2	99.0 ± 1.2	96.7 ± 0.4
Benzene	1.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	98.2 ± 1.0	81.3 ± 0.6	71.2 ± 0.7
	3.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	95.1 ± 1.2	87.6 ± 0.4	74.7 ± 1.8
	6.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	91.4 ± 0.6	90.5 ± 0.6	76.9 ± 0.4
Chloroform	1.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	98.3 ± 0.4	82.2 ± 1.6	76.3 ± 0.4
	3.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	94.7 ± 0.6	86.1 ± 0.2	80.9 ± 0.3
	6.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	90.6 ± 1.0	92.3 ± 0.6	86.8 ± 0.8
Methanol	1.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	97.2 ± 0.6	80.2 ± 1.2	72.3 ± 0.6
	3.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	94.2 ± 0.3	86.5 ± 0.6	75.4 ± 0.4
	6.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0	90.9 ± 1.7	90.7 ± 0.4	82.1 ± 1.2

any allergic reaction to the test person and also, the repellent activity was dependent on the strength of the crude extract. The highest repellence at 150, 180 and 210 min. was observed with *Streptomyces* sp. VITJS4 ethyl acetate crude extract at the dosage of 6 mg/cm² against *A. stephensi*, *A. aegypti* and *C. quinquefasciatus.* The partial characterization of the crude extracts showed the presence of functional groups which was revealed by IR spectra. The FTIR spectral analyses of ethyl acetate crude extracts showed certain common absorption bands at 3359 and 3251 cm^{-1} which are characteristics of the hydroxyl group (O-H). The vibrational peaks at 2116 cm^{-1} and 1630 cm^{-1} were alkynes (C≡C) and alkene (C=C) functional groups, respectively (Fig. 1). The research work is ongoing with the goal of isolating the identified compounds and characterizing the structure of the compounds in the most active crude extract.

Discussion

Vector borne diseases are the major treats to human which has led to tremendous economic impacts such as malaria, filariasis, dengue, yellow fever and encephalitis are continuing to be major health problems for the people (Das and Ansari, 2003). The exposure to pesticides among humans has been linked to immune dysfunction, various forms of cancer and birth defects (Nigam and Venkatakrishna Bhatt, 2001). As to control the injudicious use of pesticides, it is therefore necessary to identify a safe, eco-friendly alternate source of larvicide in order to reduce mosquito menace (Nascimento *et al*., 2000). Natural products contribute as the source of novel bioactive metabolites. The discovery of new active metabolites must be followed by adequate biological testing (Jemimah Naine *et al*., 2012). A few numbers of larvicidal and repellent drugs have been discovered from natural products in the past and new ones are being developed. Recently turning back to nature, the marine environment represents an inexhaustible resource of new compounds with unique structures from wide range of microorganisms. The larvicidal activity of extracellular metabolites of keratinophilic fungus *Trichophyton mentagrophytes* was found effective against 3rd instar larvae of *A. aegypti* LC_{50} and LC_{90} being 110 ± 11.5 and 200 ± 20.7 respectively after 2 days (Murugesan *et al*., 2009). A novel polyketide metabolite from marine *Streptomyces* sp. AP-123 was found to exhibit high larvicidal activities against *Helicoverpa armigera* (63.11%) and *Spodoptera litura* (58.22%) and the LC_{50} values were 645.25 ppm and 806.54 ppm respectively (Arasu *et al*., 2013). The spinosad from the actinomycete, *Saccharopolyspora. spinosa*, showed LC_{50} and LC_{90} values of first, second, third and fourth-instar larvae at 0.001, 0.031, 0.034, 0.036 and 0.0113, 0.102, 0.111, 0.113 ppm (Das and Ansari., 2003). Twenty three isolates of actinomycetes showed positive larvicidal activity against *A. aegypti*. Among the 23 isolates, four isolates were *Streptomyces* (A14, A21, A49 and A63) and found to be more active against the larvae of *A. aegypti* with LC_{50} value ranging between 15.83 and 68.06 µg/ml. Three new alpha-class milbemycins (named milbemycins alpha-28, alpha-29 and alpha-30) isolated from

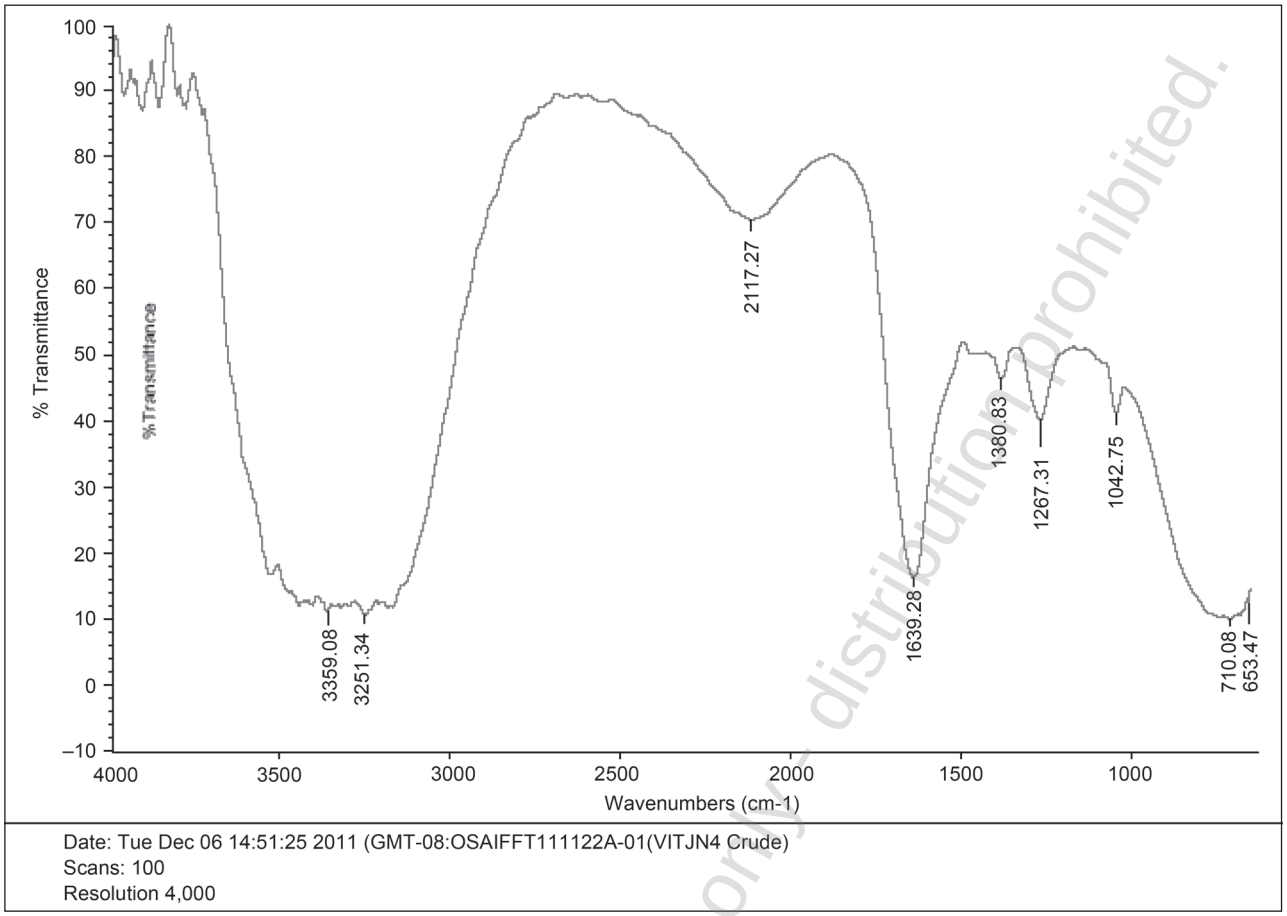

Fig. 1. FTIR analysis of *Streptomyces* sp. VITJS4 ethyl acetate crude extract.

Streptomyces bingchenggensis have been shown to possess potent acaricidal and nematocidal activity (Xiang *et al*., 2007). Recent studies have indicated that spinosad, a mixture of two tetracyclic macrolide compounds produced during the fermentation of a soil actinomycete, may be suitable for controlling a number of medically important mosquito species, including the dengue vector, *A. aegypti* (Antonio *et al*., 2009). The relevant studies of a novel isolated compound 5-(2,4-dimethylbenzyl) pyrrolidin-2-one from marine *Streptomyces* VITSVK5 sp. was found to have complete larvicidal activity at 1000 ppm against *Rhipicephalus* (*Boophilus*) *microplus*, *A. stephensi* and *Culex tritaeniorhynchus* and the LC_{50} values were 210.39 ppm, 169.38 ppm, 198.75 ppm, respectively (Saurav *et al*., 2013). In the present study, isolate *Streptomyces* sp. VITJS4 ethyl acetate crude extract exhibited LC_{50} and LC_{90} value of 132.86 and 396.14 against *A. stephensi* larvae, 112.78 and 336.42 against *A. aegypti* larvae, 156.53 and 468.37 against *C. quinquefasciatus* larvae which is comparable with supporting report showing 35 isolates with larvicidal activity against *C. quinquefasciatus*, *A. stephensi* and *A. aegypti* (Vijayan *et al*., 1991). Interestingly, the data on repellent assay of *Streptomyces* sp. VITJS4 ethylacetate crude extracts showed complete protection for 210 min at the dosage of 6 mg/cm^2 against mosquito bites of *A. stephensi*, *A. aegypti* and *C. quinquefasciatus*. The larvicidal compound (2S,5R,6R)-2-hydroxy-3,5,6-trimethyloctan-4-one from *Streptomyces* sp. against blood-sucking parasites was found to have complete larvicidal activity at 250 ppm against *R. microplus*, *A. subpictus* and *C. quinquefasciatus* and the LC_{50} values were 94.49 ppm, 69.65 ppm, 82.82 ppm, respectively (Deepika *et al*., 2012). The crude extracts of LK-3 and LK-1 with the highest concentrations of 1,000 ppm provided over 120 and 90 min protection against *Culex gelidus* bites (Karthik *et al*., 2011). The results of the present study support previous observation that marine sediments are the sources of metabolically active *Streptomyces* (Moran *et al*., 1995). The crude extracts are attributed with complex mixtures of several active compounds and may facilitate the future application of biotechnological procedures for cost-effective production. The significant properties of bioactive potential perhaps serve as a promising source and could act as a new mode of controlling mosquitoes.

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