International Journal of Zoology Studies
ISSN: 2455-7269
Impact Factor: RJIF 5.14
www.zoologyjournals.com
Volume 3; Issue 4; July 2018; Page No. 15-25

Antibacterial and mosquitocidal potentials of selected Indian medicinal plants extracts and synthesized silver nanoparticles

K Krishnappa[1*], J Pandiyan[2], J Paramanandham[3], K Elumalai[4]
[1-3] Department of Zoology and Wildlife Biology A.V.C. College (Autonomous), Mannampandal, Mayiladuthurai, Tamil Nadu, India
[1, 4] Department of Advanced Zoology & Biotechnology, Govt. Arts College (Autonomous), Chennai, Tamil Nadu, India

Abstract
In the present study, the antibacterial and mosquitocidal activity of different leaf extracts and silver nanoparticles (SNPs) of *Bridelia montana* and *Litsea chinensis* Indian medicinal plants were analyzed through the zone of inhibition, minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) assays against Gram-positive and Gram-negative bacteria and larvicidal activity of fourth instar larvae of selected human vector mosquitoes. Antibacterial activity of zone of inhibition, MIC and MBC of *B. montana* and *L. chinensis* extracts tested, the methanol extract showed significant activity against the selected bacteria. Moreover, SNPs of both plants showed maximum zone of inhibition, MIC and MBC in among the human pathogenic bacteria. The Larvicidal potential of medicinal plants different extracts tested, methanol extracts provided significant activity. Besides, the larvicidal properties of SNPs provided highest activity and more than 90% of activity recorded at 25µg/mL concentration. Preliminary phytochemical screenings of extracts were observed that the maximum groups of phytochemicals were present in high polar solvents, especially in methanol extracts. In this study we evaluated the antibacterial and mosquitocidal properties of two Indian medicinal plants extracts and SNPs displayed the potential activity which could be a good source for the alternative of synthetic chemical.

Keywords: antibacterial activity, mosquitocidal activity, medicinal plants, silver nanoparticles, gram positive bacteria, gram negative bacteria, vector mosquitoes

1. Introduction
Pathogenic bacteria are major source of morbidity and mortality in humans and cattle's. Even though many pharmaceutical companies produced number of new antibacterial drugs but bacteria develops the resistance against presently using bacterial drugs as well as these drugs has increased and became a global alarm. The excessive usages of synthetic antibiotic drugs are improving the negative health disorder to human and drugs are simultaneously affect the ecosystem and environment [1]. Almost all human pathogenic bacteria capable to obtain the resistance rapidly to the antimicrobial drugs, as a result multiply drug resistant pathogenic bacteria and we get the failure to treatment of infectious diseases [2, 3]. Therefore, it is very essential to search and design the newer and safer alternative approaches to control resistant bacteria. Recently, most of researchers interested to search the eco-friendly and safer antibacterial activity done from phytochemicals. Plants have accumulated variety of secondary metabolites which ability to produce number of phytocompounds like alkaloids, coumarins, flavonoids, glycosides, steroids, saponins, terpenoids, tannins and quinines. These phytocompounds are having high antimicrobial activity and great efficient treatment of bacterial infectious diseases [4-6]. Plants secondary metabolites have been used as medicine for lack of many infectious bacterial diseases that could be a vital source for various antimicrobial agents [7,8]. The use of naturally available phytocompounds for therapeutic purposes has interestingly increased in tropical countries. According to the World Health Organisation medicinal plants and its chemical compositions are best source for obtain a variety of drugs as well as more than half of the people living in developing and undeveloping countries using traditional medicine which are mainly compounds derived from locally available herbal medicinal plants [9-11].
Mosquitoes are small flying blood sucking vectors which transmitting several human diseases such as malaria, dengue fever, yellow fever, filariasis, schistosomiasis and Japanese encephalitis. Mosquitoes are more responsible for the spreading of many dreadful human diseases. In particularly, tropical and subtropical countries mosquito-borne diseases have created an economic impact and loss in commercial and labor outputs [12]. Mosquito borne diseases are common and spread in half of the world which infecting more than 700,000,000 people annually worldwide and 40,000,000 of the Indian population [13-16]. *Aedes aegypti* generally day biting mosquito which is transmitting several human diseases such as dengue fever, chikungunya ferver, yellow fever, West Nile virus infection, etc., [17]. *Ae. aegypti* is a cosmotropical species that breed in household water containers [18]. In worldwide around 2.5 billion people are risk due to the dengue fever, dengue hemorrhagic fever and dengue shock syndrome [19]. Malaria is transmitted by *Anopheles stephensi* basically which are night biting mosquitoes. Malaria is one of the most important diseases in India which causing all life stages of human being as well as more than two to three million new cases arising every year. Estimated more than 300 million

people suffered by malaria as the results one million deaths per year [20]. Lymphatic filariasis is transmitted by *Culex quinquefasciatus* which is common in tropical zones. Around 120 million people suffered globally and 44 million people having common chronic manifestation [21].

Most common one techniques available for the control of human vector mosquitoes is the use of synthetic chemical insecticides but synthetic insecticides are high toxic on non-target organisms and give the negative effect on environment as well as over long time use of synthetic chemical pesticides leads mosquitoes to develop the resistance against chemical pesticides [22-23]. Therefore, most of the researcher would like to search the newer pesticides which should be less toxic to non-target organism, low cost, easy preparation, biodegradable, species specificity etc.,. So, must urgent to find the safer pesticides which would be prepared from botanical because they are promising effectiveness on target pest, environment – friendly and easily biodegradable. Botanical pesticides are traditional method by prepared locally available medicinal plants and which are practiced many parts of the human communities testing successfully with pest species [24-26]. In view of an increasing interest and significance of developing phytochemicals research are safer and alternative to synthetic chemical mosquitocidal and antibacterial properties, this study was undertaken to assess the antibacterial and mosquitocidal activity of the plants extracts and silver nanoparticles against human pathogenic bacteria and important human vector mosquitoes.

2. Materials and Methods

2.1 Collection and processing of plants

In the present study, survey of literature reports led to the selection of *Bridelia montana* and *Litsea chinensis* for their antibacterial and mosquitocidal efficacy against the selected human pathogenic bacteria and vector mosquitoes. Samplings were carried out during the flowering season (January – June) from 2016 to 2017 at different places of Salem District, Tamilnadu, India. Bulk samples (leaves) were collected, air-dried in the shade at room temperature and after drying, each sample was separately ground to a fine powder.

2.2 Method for crude extraction

Powders of selected indian medicinal plants *B. montana* and *L. chinensis* were extracted with five different organic solvents (hexane, diethyl ether, dichloromethane, ethyl acetate and methanol with ascending order of polarity) in a sequential manner, in order to produce crude extracts containing a wide range of active compounds. About 200g of powder plant material were placed in an extraction cylinder of soxhlet apparatus and nearly 7 orders were run 12 hours of continuous extraction. The residual plant material was extracted in an extraction flask with the corresponding solvent one more time using 1000 mL of the same solvent. The two filtrates were combined and the solvent was condensed under reduced pressure 22–26 mmHg at 45^{0}C to yield the respective solvent extract using rotary vacuum evaporator (SUPERFIT, PBU -6 Model). The residual plant material was then sequentially extracted with other solvents, using the same procedure described for hexane and the semi solid residues were collected in the Petri dishes individually and allowed complete dry in a desiccator.

2.3 Antibacterial activity

In vitro antimicrobial evaluation of hexane, diethyl ether, dichloromethane, ethyl acetate and methanol crude extracts were carried out against 8 bacterial strains, which includes 4 Gram-positive bacteria (*Bacillus subtilis* (MTCC:441), *Micrococcus luteus* (MTCC:1538), *Staphylococcus aureus* (MTCC:96) and *Streptococcus mutans* (MTCC:497) and 4 Gram-negative bacteria *Escherichia coli* (MTCC:443), *Klebsiella pneumoniae* (MTCC:109), *Proteus vulgaris* (MTCC:426) and *Schigella flexneri* (MTCC:1457). The bacterial strains were obtained from the Institute of Basic Medical Sciences (IBMS), University of Madras, Taramani Campus, and Chennai, India. Inoculums of each bacterial strain was suspended in 5 mL of nutrient broth and incubated for 24 h at 37^{0}C. A loopful of bacteria was taken from the stock cultures and dissolved in 0.1 mL of saline. Plant extracts were screened for antimicrobial activity using the Disc Diffusion Assay [27]. The petri plates (9 cm dia.) were pre-seeded with 10 mL of Muller Hinton Agar and stock culture was streaked thoroughly to ensure uniform distribution of the micro-organisms. Sterile paper discs (5 mm diameter) containing 100µg/mL of hexane, diethyl ether, dichloromethane, ethyl acetate and methanol crude extracts were screened for antibacterial activity. Simultaneously paper discs dipped with pure respective organic solvents were used as positive controls. The petri plates were then pre-incubated for 3 h at 5^{0}C to permit maximum diffusion of the extracts into the media. Cefalexin and Gentamycine (10µg/mL) was used as negative control against gram positive and gram negative bacteria respectively [28].

2.4 Determination of MIC and MBC

A modified agar microdilution method of Lorian [29] was used to determine the MIC of hexane, diethyl ether, dichloromethane, ethyl acetate, and methanol extracts. The test tubes contained serially diluted from 1500µg/mL to 1.46µg/L were inoculated with the selected bacterial strain suspension and the test tubes were incubated at 35^{0}C for 18 h. Observations were performed in triplicates and results were expressed as the lowest concentration of plant extracts that produced a complete suppression of colony growth.

2.5 Mosquito rearing

Eggs of *Aedes aegypti*, *Anopheles stephensi* and *Culex quinquefasciatus* were collected within the college campus by placing water-filled plastic trays (23×15×6.5 cm) with a lining of partially immersed filter paper. The egg rafts of *Cx. quinquefasciatus* and the larvae of *An. stephensi* were collected from Cooum River near Kotturpuram, Chennai, and transferred to plastic bowls containing dechlorinated tap water and then brought to the laboratory. The eggs were placed in plastic trays (30×24×10 cm) each containing 2 liter of tap water and kept at room temperature (27±2^{0}C) with a photoperiod of 12:12 h (L: D) for larval hatching. The larvae of each mosquito species were maintained in separate trays under the same laboratory conditions and fed with yeast powder. The trays with pupae of each mosquito species were maintained in separate mosquito cages at 27 ± 2°C and

relative humidity of 75±5% for adult emergence. Cotton soaked in 10% aqueous sucrose solution in a petri dish to feed adult mosquitoes was also placed in each mosquito cage. An immobilized young chick was placed for 3 h inside the cage in order to provide a blood meal especially for female mosquitoes. A plastic tray (11× 10×4 cm) filled with tap water with a lining of partially immersed filter paper was then placed inside each cage to enable the female mosquitoes to lay their eggs. The eggs obtained from the laboratory-reared mosquitoes were immediately used for toxicity assays or allowed to hatch out under the controlled laboratory conditions described above. Only the newly hatched specific instars of larvae or the pupae of different mosquito species were used in all bioassays.

2.6 Larvicidal activity

The larvicidal activity of the extract was determined by following the standard procedure prescribed by WHO [30]. Mosquito larvae were exposed to test concentrations (50, 100, 150 and 200µg/l concentrations were used to determine the lethal concentration of 50% (LC50) and the lethal concentration of 90% (LC90) values. DMSO (emulsifier) in water served as a control. The larvae of these mosquito species (25 nos.) were introduced in 500-mL plastic cups containing 250 mL of aqueous medium (249 mL of dechlorinated water + 1mL of emulsifier) and the required amount of plant extract was added. Five replicates were kept for each test concentration as stated earlier and in each replicate 25 larvae were used with five replicate of control. The experiment was performed under laboratory conditions at 27 ± 2^0C. If the control mortality is between 5% and 20%, the mortalities of treated groups should be corrected according to Abbott [31] formula. The LC50, LC90, 95% confidence limit of Lower Confidence Limit (LCL) and Upper Confidence Limit (UCL), Chi- square values and the degrees of freedom were calculated by using Probit analysis with Statistical Package for Social Sciences (SPSS) 17.0 Version in MS-Excel, 2007.

2.7 Biosynthesis of silver nanoparticles

Silver nitrate, used in this study was obtained from Himedia Laboratories Pvt. Ltd., Mumbai, India. 1.5 g of the leaves (methanol extract) from *B. montana* and *L. chinensis* were boiled in 100 mL of de-ionized water. 2.5 mL of ammonium solution was added to 5 mL of 1 mM $AgNO_3$ (solution, followed by addition of plants extract 1^{-10} mL and the final volume was adjusted to 50 mL by adding the appropriate amount of de-ionized water. For silver nanoparticles, the solution turned from yellowish to bright yellow and to dark brown. The Erlenmeyer flasks were incubated at 37° C under agitation (200 rpm) for 24–48 h [32].

2.8 Preliminary studies on phytochemical screening

Qualitative analyses of the phytochemicals present in the leaf extracts were determined using the method described by Harborne [33] and Evans [34].

2.9 Determination of lethal concentrations

Lethal concentration (LC50) represents the concentration of the test material that caused 50% mortality of the test organisms within the specified period of exposure. Based on the mortality of the test organisms recorded in these bioassays, LC50 and LC90 was calculated along with their fiducial limits at 95% confidence level by probit analysis using SPSS software package (version 17.0).

3. Results

3.1 Antibacterial activity – zone of inhibition of *B. montana*

The data pertaining to the present experiments clearly reveals that the *B. montana* different extracts (hexane, diethyl ether, dichloromethane, ethyl acetate and methanol) produced varying bacterial growth inhibitory activity against the selected bacteria (gram positive bacteria; *S. mutans, B. subtilis,S. aureus, M. luteus* and gram negative bacteria; *E. coli, K. pneumonia, P. vulgaris* and *S. flexneri*) and the values obtained are shown in table 1. Among the different extracts tested the methanol extract showed significant antibacterial activity than the other four extracts. It was found that 20.83, 26.68, 24.62, 25.22 and 20.24, 19.48, 19.22 and 20.33mm zone of inhibition against gram positive and gram negative bacteria were *S. mutans, B. subtilis, S. aureus, M. luteus* and *E. coli, K. pneumoniae, P. vulgaris* and *S. flexneri* respectively. Furthermore hexane, diethyl ether, dichloromethane and ethyl acetate extracts showed remarkable antibacterial activity.

Table 1: Antibacterial activity (zone of inhibition) of *Atalantia monophylla* against the selected human pathogenic bacteria

Antibacterial activity - Zone of inhibition (mm)					
Gram positive bacteria					
Solvents tested	**Control ***	***S. mutans***	***B. subtilis***	***S. aureus***	***M. luteus***
Hexane	26.44	-	-	14.62	-
Diethyl ether		10.23	9.46	18.44	14.58
Dichloromethane		10.84	14.22	21.82	16.33
Ethyl acetate		11.61	16.00	22.80	18.92
Methanol		20.83	26.68	24.62	25.22
Gram negative bacteria					
Solvents tested	Control **	*E. coli*	*K. pneumoniae*	*P. vulgaris*	*S. flexneri*
Hexane	29.82	-	9.26	8.33	9.58
Diethyl ether		-	10.44	10.88	13.40
Dichloromethane		12.28	11.86	12.40	15.22
Ethyl acetate		17.84	13.00	14.82	17.86
Methanol		20.24	19.48	19.22	20.33

C* = Positive Control (Cefalexin); C ** = Negitive Control (Gentamycin)

3.2 Antibacterial activity – zone of inhibition of *L. chinensis*

Antibacterial activity of *L. chinensis* leaf different extracts produced varying bacterial growth inhibitory activity against the selected bacteria and the values obtained are shown in table 2. Among the different extracts tested the methanol extract showed highest antibacterial activity than the other four extracts. It was found that 20.48, 21.84, 18.64, 24.66 and 27.80, 18.84, 14.60 and 20.22mm zone of inhibition against gram positive and gram negative bacteria were *S. mutans*, *B. subtilis*, *S. aureus*, *M. luteus* and *E. coli*, *K. pneumoniae*, *P. vulgaris* and *S. flexneri* respectively. Furthermore hexane, diethyl ether, dichloromethane and ethyl acetate extracts showed remarkable antibacterial activity.

Table 2: Antibacterial activity (zone of inhibition) of *Hugonia mystax* against the selected human pathogenic bacteria.

Antibacterial activity - Zone of inhibition (mm)					
Gram positive bacteria					
Solvents tested	**Control ***	***S. mutans***	***B. subtilis***	***S. aureus***	***M. luteus***
Hexane	26.24	-	7.88	4.64	-
Diethyl ether		10.82	8.35	9.39	10.58
Dichloromethane		12.26	16.58	14.84	16.22
Ethyl acetate		14.00	18.22	13.82	14.48
Methanol		20.48	21.84	18.64	24.66
Gram negative bacteria					
Solvents tested	Control **	*E. coli*	*K. pneumoniae*	*P. vulgaris*	*S. flexneri*
Hexane	29.00	8.38	-	-	-
Diethyl ether		16.82	12.64	9.22	7.44
Dichloromethane		11.46	10.20	11.48	9.82
Ethyl acetate		14.22	13.84	10.83	14.68
Methanol		27.80	18.84	14.60	20.22

C* = Positive Control (Cefalexin); C ** = Negitive Control (Gentamycin)

3.3 Antibacterial activity – zone of inhibition of silver nanoparticles

Antibacterial activity of SNPs synthesized from the methanol leaf extract of *B. montana* and *L. chinensis* against the selected gram positive and gram negative human pathogenic bacteria are shown in table 3. SNPs synthesized from *B. montana* showed zone of inhibition in the following order: *M. luteus* > *S. flexnari* > *B. subtilis* > *S. aureus* > *E. coli* > *K. pneumonia* > *S. mutans* with 31.22 > 29.44 > 23.82 > 20.68 > 16.00 > 12.62 > 11.46mm zone of inhibition. SNPs synthesized from *L. chinensis* showed zone of inhibition in the following order: *S. flexnari* > *B. subtilis* > *E. coli* > *M. luteus* > *S. mutans* > *K. pneumonia* > *S. aureus* with 29.68 > 28.84 > 24.48 > 18.42 > 16.62> 16.22 > 14.68mm zone of inhibition. SNPs of *B. montana* and *L. chinensis* methanol extract did not control the growth of *P. vulgaris*.

Table 3: Antibacterial activity (zone of inhibition) silver nanoparticles (SNPs) against the selected human pathogenic bacteria

Tested groups	**Pathogens tested**	**Silver nanoparticles synthesized plants**	
		Zone of inhibition (mm)	
		Atalantia monophyla	*Hugonia mystax*
Gram positive	*Streptococcus mutans*	11.46	16.62
	Bacillus subtilis	23.82	28.84
	Staphyllococcusaureus	20.68	14.68
	Micrococcus luteus	31.22	18.42
Gram negative	*Escherichia coli*	16.00	24.48
	Klebsiell apneumoniae	12.62	16.22
	Proteus vulgaria	NA	NA
	Schizellaflexnari	29.44	29.68

Values represent in mm.

3.4 Antibacterial activity –minimum inhibitory concentration of *B. montana*

Hexane, diethyl ether, dichloromethane, ethyl acetate and methanol extract of *B. montana* leaf was tested for its antibacterial activity (MIC) against the selected gram positive and gram negative bacteria. The data pertaining to the present experiments clearly reveals that the different extracts produced varying bacterial growth (MIC) activity and the values obtained are shown in table 4. Among the different extracts tested the methanol extract showed significant antibacterial (MIC) activity than the other four extracts. It was found that 3.90625, 31.25, 7.81215, 62.5 and 7.8125, 3.90625, 62.5 and 31.25 µg/mL of MIC inhibition was observed against *S. mutans*, *B. subtilis*, *S. aureus*, *M. luteus* and *E. coli*, *K. pneumonia*, *P. vulgaris* and *S. flexneri* respectively.

Table 4: Antibacterial activity (Minimum Inhibitory Concentration) of *Atalantia monophylla*against the selected human pathogenic bacteria

Antibacterial activity - Zone of inhibition (µg/mL)				
Gram positive bacteria				
Solvents tested	*S. mutans*	*B. subtilis*	*S. aureus*	*M. luteus*
Hexane	62.5	7.8125	62.5	15.625
Diethyl ether	31.25	250	31.25	7.8125
Dichloromethane	15.625	125	62.5	7.81215
Ethyl acetate	62.5	250	15.625	125
Methanol	3.90625	31.25	7.81215	62.5
Gram negative bacteria				
Solvents tested	*E. coli*	*K. pneumoniae*	*P. vulgaris*	*S. flexneri*
Hexane	62.5	125	500	62.5
Diethyl ether	250	500	250	125
Dichloromethane	250	125	250	62.5
Ethyl acetate	125	250	125	250
Methanol	7.8125	3.90625	62.5	31.25

Values represent in µg/mL.

3.5 Antibacterial activity – minimum inhibitory concentration of *L. chinensis*

The different extract of *L. chinensis* leaf was tested for its antibacterial activity (MIC) against the selected gram positive and gram negative bacteria. The data pertaining to the present experiments clearly reveals that the different extracts produced varying bacterial growth (MIC) activity and the values obtained are shown in table 5. Among the different extracts tested the methanol extract showed significant antibacterial (MIC) activity than the other four extracts. It was found that 3.90625, 7.81215, 7.8125, 7.81215 and 31.25, 62.5, 31.25 and 62.5 µg/mL of MIC inhibition was observed against *S. mutans*, *B. subtilis*, *S. aureus*, *M. luteus* and *E. coli*, *K. pneumonia*, *P. vulgaris* and *S. flexneri* respectively.

Table 5: Antibacterial activity (Minimum Inhibitory Concentration) of *Hugonia mystax* against the selected human pathogenic bacteria

	Antibacterial activity - Zone of inhibition (µg/mL)			
Gram positive bacteria				
Solvents tested	*S. mutans*	*B. subtilis*	*S. aureus*	*M. luteus*
Hexane	62.5	62.5	125	31.25
Diethyl ether	31.25	31.25	62.5	62.5
Dichloromethane	31.25	62.5	62.5	15.625
Ethyl acetate	7.81215	31.25	15.625	62.5
Methanol	3.90625	7.81215	7.8125	7.81215
Gram negative bacteria				
Solvents tested	*E. coli*	*K. pneumoniae*	*P. vulgaris*	*S. flexneri*
Hexane	250	125	500	250
Diethyl ether	250	125	250	125
Dichloromethane	250	62.5	62.5	125
Ethyl acetate	15.625	250	125	62.5
Methanol	31.25	62.5	31.25	62.5

Values represent in µg/mL.

3.6 Antibacterial activity – minimum inhibitory concentration of silver nanoparticles

Antibacterial activity-MIC of SNPs synthesized from the methanol leaf extract of *B. montana* and *L. chinensis* against the selected human pathogenic bacteria are shown in table 6. It was observed that SNPs of *B. montana* showed zone of MIC in the following order: *S. flexnari* > *K. pneumonia* > *P. vulgaris* > *E. coli* > *B. subtilis* > *M. luteus* > *S. mutaces* > *S. coccusaureus* with 250.00, 250.00, 125.00, 125.00 and 62.50, 62.50, 15.65 and 15.65 µg/mL diameter zone of MIC inhibition. It was observed that SNPs of *L. chinensis* showed zone of MIC in the following order: *S. coccusaureus* > *S. mutaces* > *M. luteus* > *E. coli* > *K. pneumonia* > *S. flexnari* > *B. subtilis* > *P. vulgaris* with 250.00, 125.00, 125.00, 125.00, 62.5.00, 62.50, 15.65 and 15.625 µg/mL diameter zone of MIC inhibition.

Table 6: Antibacterial activity (Minimum Inhibitory Concentration) of silver nanoparticles against the selected human pathogenic bacteria

Tested groups	Pathogens tested	Silver nanoparticles synthesized plants	
		Atalantia monophylla	***Hugoniamystax***
Gram positive	*Streptococcus mutaces*	15.65	125.00
	Bacillus subtilis	62.50	15.65
	Staphyllo coccusaureus	15.65	250.00
	Micrococcus luteus	62.50	125.00
Gram negative	*Escherichia coli*	125.00	125.00

	Klebsiella pneumoniae	250.00	62.5.00
	Proteus vulgaris	125.00	15.625
	Schizella flexnari	250.00	62.50

Values represent in µg/mL.

3.7 Lethal concentrations of *B. montana*

Results of the present study reflected spectrum of larvicidal activity with five different extracts of *B. montana* against the selected mosquitoes fourth instar larvae (table 7). The lethal concentrations of *B. montana* hexane, diethyl ether, dichloromethane, ethyl acetate and methanol extract against *Ae. aegypti* larvae with the LC_{50} values were 104.82, 79.54, 103.44, 96.18 and 88.32 µg/mL, respectively. The lethal concentrations of *B. montana* hexane, diethyl ether, dichloromethane, ethyl acetate and methanol extract of against *An. stephensi* larvae with the LC_{50} values were 113.40, 85.93, 107.38, 104.47 and 95.53µg/mL, respectively. The lethal concentrations *B. montana* hexane, diethyl ether, dichloromethane, ethyl acetate and methanol extract against *Cx. quinquefasciatus* larvae with the LC_{50} values were 119.82, 109.68, 112.84, 105.46 and 98.22µg/mL, respectively. Among the three vector mosquitoes larvae were tested with the five different extracts of *B. montana*, the larvae were found to be more susceptible in methanol extract than the other four extracts.

Table 7: Lethal concentrations of *Atalantia monophylla* extracts against fourth instar larvae of three vectors mosquitoes

Species tested	LC_{50} (µg/L)	95% Fiducial limit(µg/L)		LC_{90} (µg/L)	95% Fiducial limit (µg/L)		Slope	Regression	χ^2 value
		LCL	UCL)		LCL	UCL)			
Aedes aegypti									
hexane	104.82	96.72	114.48	180.56	168.49	198.23	4.145	0.934	0.564
diethyl ether	79.54	65.66	89.88	176.29	161.86	200.90	3.266	0.978	3.715
dichloromethane	103.44	37.68	162.78	197.59	145.73	219.00	3.201	0.811	7.833
ethyl acetate	96.18	87.44	104.22	172.88	158.44	187.44	4.543	0.956	0.153
methanol	88.32	77.22	98.64	169.26	156.28	188.28	4.221	0.875	3.170
Anopheles stephensi									
hexane	113.40	104.38	120.84	186.73	174.67	204.82	4.200	0.944	0.424
diethyl ether	85.93	70.28	96.28	182.22	166.82	205.56	3.433	0.927	2.331
dichloromethane	107.38	98.20	159.00	203.28	154.22	570.64	3.521	0.844	4.772
ethyl acetate	104.47	94.86	112.46	180.48	167.84	201.88	4.299	0.932	0.177
methanol	95.53	69.22	142.68	182.36	136.35	210.22	3.912	0.768	4.243
Culex quinquefasciatus									
hexane	119.82	109.93	128.74	197.64	184.72	216.68	3.943	0.921	2.400
diethyl ether	109.68	98.34	119.44	208.65	189.34	231.59	3.000	0.812	4.132
dichloromethane	112.84	98.72	122.78	211.44	192.80	232.92	3.591	0.820	4.550
ethyl acetate	105.46	96.48	114.66	184.92	162.94	202.95	3.884	0.933	3.057
methanol	98.22	62.87	158.40	199.46	147.76	190.46	3.462	0.874	2.432

LC_{50}=Lethal Concentration brings out 50% mortality and LC_{90} = Lethal Concentration brings out 90% mortality. LCL = Lower Confidence Limit; UCL = Upper Confidence Limit; Values in a column with a different superscript alphabet are significantly different at P <0.05 level DMRT Test.

3.8 Lethal concentrations of *L. chinensis*

The lethal concentrations of *L. chinensis* hexane, diethyl ether, dichloromethane, ethyl acetate and methanol extract tested against fourth instar larvae of *Ae. aegypti* human vector mosquito with the LC_{50} values were 124.76, 102.37, 116.43, 107.54 and 90.95µg/mL, respectively (table 8). The lethal concentrations of *L. chinensis* hexane, diethyl ether, dichloromethane, ethyl acetate and methanol extract against *An. stephensi* larvae with the LC_{50} values were 117.80, 98.92, 108.85, 105.93 and 96.94µg/mL, respectively. The lethal concentrations of *L. chinensis* hexane, diethyl ether, dichloromethane, ethyl acetate and methanol extract against *Cx. quinquefasciatus* larvae with the LC_{50} values were 128.37, 110.90, 119.24, 112.86 and 107.22µg/mL, respectively. Among the three vector mosquitoes larvae were tested with the five different extracts of *L. chinensis*, the larvae were found to be more susceptible in methanol extract than the other four extracts.

Table 8: Lethal concentrations of *Hugonia mystax* extracts against fourth instar larvae of three vectors mosquitoes

Species tested	LC_{50} (µg/L)	95% Fiducial limit (µg/L)		LC_{90} (µg/L)	95% Fiducial limit (µg/L)		Slope	Regression	χ^2 value
		LCL	UCL)		LCL	UCL)			
Aedes aegypti									
hexane	124.76	114.75	132.73	209.22	193.62	228.20	3.565	0.828	4.900
diethyl ether	102.37	89.24	112.32	202.65	185.76	221.44	5.700	3.045	3.568
dichloromethane	116.43	105.12	124.36	206.40	390.54	227.37	3.456	0.943	3.613
ethyl acetate	107.54	97.56	116.38	185.47	168.54	198.42	4.214	0.687	3.812
methanol	90.95	80.29	102.29	172.72	156.39	189.55	3.992	0.900	5.335

Anopheles stephensi									
hexane	117.80	108.14	126.42	193.33	178.98	211.54	4.144	0.821	4.962
diethyl ether	98.92	85.35	110.53	208.26	187.43	234.63	3.289	0.884	5.900
dichloromethane	108.85	99.13	118.00	192.58	178.22	213.28	3.092	0.955	5.363
ethyl acetate	105.93	94.67	115.24	193.89	179.54	212.55	3.616	0.983	2.645
methanol	96.94	84.52	137.18	179.94	135.22	293.27	3.819	0.990	6.423
Culex quinquefasciatus									
hexane	128.37	119.29	138.82	214.45	197.53	233.30	3.378	0.821	5.000
diethyl ether	110.90	99.35	121.64	213.94	194.37	236.64	3.711	0.843	3.269
dichloromethane	119.24	109.67	129.57	210.87	395.19	235.96	3.426	0.822	2.922
ethyl acetate	112.86	103.13	120.96	196.13	178.33	216.55	3.592	0.937	2.659
methanol	107.22	96.87	116.38	193.82	179.37	213.89	3.648	0.944	2.327

LC_{50}=Lethal Concentration brings out 50% mortality and LC_{90} = Lethal Concentration brings out 90% mortality. LCL = Lower Confidence Limit; UCL = Upper Confidence Limit; Values in a column with a different superscript alphabet are significantly different at P <0.05 level DMRT Test.

3.9 Lethal concentrations of silver nanoparticles (SNPs)

The larvicidal activity of SNPs from *B. montana* against the fourth instar larva of three vector mosquitoes such as *Ae. aegypti*, *An. stephensi* and *Cx. quinquefasciatus* were tested with different concentration, such as 10, 15, 20 and 25µg/mL are shown in table 9. The data clearly revealed that 35.33%, 54.45%, 85.17and 97.26% of larval mortalities were observed against *A. aegypti* when exposed to 10, 15, 20 and 25µg/mL concentrations respectively. *An. stephensi* when treated with SNPs of *B. montana* showed 33.54%, 49.63%, 77.44% and 97.86% of larval mortalities at 10, 15, 20 and 25µg/mL concentrations respectively. The larval mortalities of *Cx. quinquefasciatus* were found to be 31.58%, 47.37%, 73.54% and 97.63%, when exposed to 10, 15, 20 and 25µg/mL concentrations respectively. Besides, maximum larval mortality was observed against *An. stephensi* (97.86%).

SNPs of *L. chinensis* against the fourth instar larva of *Ae. aegypti*, *An. stephensi* and *Cx. quinquefasciatus* were tested with different concentration, such as 10, 15, 20 and 25µg/mL are shown in table 9. The data clearly revealed that 31.34%, 54.22%, 82.56% and 96.66% of larval mortalities were observed against *Ae. aegypti* when exposed to 10, 15, 20 and 25µg/mL concentrations respectively. *An. stephensi* when treated with SNPs showed 29.23%, 49.56%, 75.33% and 95.30% of larval mortalities at 10, 15, 20 and 25µg/mL concentrations respectively. The larval mortalities of *Cx. quinquefasciatus* were found to be 25.55%, 45.58%, 72.46% and 94.46%, when exposed to 10, 15, 20 and 25µg/mL concentrations respectively. Besides, maximum larval mortality was observed against *An. stephensi* (97.86%).

Table 9: Larvicidal activity of silver nanoparticles (SNPs) against the fourth instar larvae of three vectors mosquitoes

Test organisms	DEET	10µl/500mL	15µl/500mL	20µl/500mL	25µl/500mL
Atalantia monophylla					
Aedes aegypti	98.00±0.82^{e} (81.87)	35.33±1.48^{a} (34.72)	54.45±1.22^{b} (47.18)	85.17±0.78^{c} (65.50)	97.26±1.84^{d} (82.87)
Anopheles stephensi	98.00±0.82^{e} (81.87)	33.54±1.62^{a} (35.75)	49.63±253^{b} (45.17)	77.44±0.51^{c} (62.54)	97.86±2.42^{d} (82.87)
Culex quinquefasciatus	98.00±0.82^{e} (81.87)	31.58±1.27^{a} (34.51)	47.37±2.65^{b} (42.85)	73.54±0.49^{c} (56.53)	97.63±0.53^{d} (82.86)
Hugonia mystax					
Aedes aegypti	98.00±0.82^{e} (81.87)	31.34±1.24^{a} (34.48)	54.22±1.94^{b} (47.72)	82.56±1.22^{c} (65.17)	96.66±1.67^{d} (76.62)
Anopheles stephensi	98.00±0.82^{e} (81.87)	29.23±1.26^{a}(33.37)	49.56±0.67^{b}(45.44)	75.33±0.37^{c}(62.95)	95.30±1.53^{d} (76.19)
Culex quinquefasciatus	98.00±0.82^{e} (81.87)	25.55±1.23^{a}(31.93)	45.58±1.43^{b}(42.84)	72.46±0.26^{c} (58.34)	94.46±1.94^{d} (75.55)

Control (positive): DEET. Value represents mean ± S.D. of five replications. *Mortality of the larvae observed after 24h of exposure period. LC_{50}=Lethal Concentration brings out 50% mortality and LC_{90} = Lethal Concentration brings out 90% mortality. LCL = Lower Confidence Limit; UCL = Upper Confidence Limit; Values in a column with a different superscript alphabet are significantly different at P <0.05 level DMRT Test. Parenthesis hold angular transformed values.

3.10 Qualitative analysis of phytochemical screening

Preliminary phytochemical screening of *B. montana* and *L. chinensis* were assessed and the results are shown in table 10. It was observed that the maximum groups of phytochemicals were present in high polar solvents, especially in methanol extracts of both the plants. Alkaloids, flavonoids, saponins, tannins, triterpenes, courmarins, anthraquinones and phenolics were observed from the methanol extract of *B. montana*. Similarly, in *L. chinensis* the presence of flavonoids, saponins, tannins, triterpenes and phenolics in methanol extract.

Table 10: Qualitative analysis of phytochemical screening from *Atalantia monophylla* and *Hugoniamystax*in different leaf extracts

Sl. NO	Phytochemical	Name of the medicinal plants and different plant extracts									
		A. monophylla					*H. mystax*				
		HEX	DEE	DCM	EA	MO	HEX	DEE	DCM	EA	MO
1	Carbohydrates	-	-	-	-	-	-	-	-	-	-
2	Alkaloids	+	-	-	+	+	-	-	+	+	+
3	Flavonoids	+	-	+	-	+	+	+	+	+	+
4	Saponins	-	+	-	+	+	-	+	-	-	+

5	Tannins	+	+	+	+	+	+	+	+	+	+
6	Triterpenes	-	+	+	+	+	+	+	+	+	+
7	Resins	-	-	+	-	-	+	+	-	-	-
8	Coumarins	+	-	+	+	+	+	+	+	-	+
9	Anthroquinnones	+	-	-	+	+	+	+	+	+	+
10	Phenolics	+	+	+	+	+	+	-	+	+	+

HEX = Hexane; DEE = Diethyl ether; DCM = Dichloromethane; EA = Ethyl acetate; MO = Methanol
+ = indicate presence of phytochemical group.
- = indicate absence of phytochemical group.

4. Discussion

Medicinal plants are traditionally used as medicine of various diseases and pesticidal activities worldwide. These plants are having different collection of bioactive compounds which are cordial alternative remedies for synthetic chemical. It is estimated that about 35,000 to 70,000 plants species are having medicinal properties out of 422127 reported worldwide plant species. In India more than 80% of the population belong to the rural areas and only depends on the traditional medicines. Today, the environments are occupied by plenty of synthetic chemical which causing high mortality and more side affect to non-target organism. Recently, plant materials have been used for control of pathogenic microorganism and pesticidal activity. Therefore attempts must be directed towards the development of effective natural, non-toxic drug for treatment. The present work was a pioneer to explore the antibacterial and mosquitocidal potentials of Indian medicinal plants and finding of the present study is comparable with earlier reports. They reported that the Indian medicinal plants extracts of methanol, ethanol and aqueous were assessed against medically important bacteria such as *Staphylococcus* sp., *E. coli*, *Klebsiella* sp., *Pseudomonas* sp. Least antibacterial activities were recorded in ethanolic and aqueous extracts when compared to methanolic extracts and maximum activities were observed in methanolic extract of *P. niruri* against *Staphylococcus* sp which compares well with present findings [35]. The medicinal plants of *B. ciliata, J. officinale* and *S. album* extracts tested against pathogenic bacteria *S. aureus, B. subtilis, P. vulgaris, P. aeruginosa* and *E.coli*. Among the three medicinal plants extracts tested *B. ciliata* demonstrated significant activity against bacterial pathogens and *B. ciliate* cold water extract showed the maximum activity against *B. subtilis* [36]. The antibacterial potential of *Z. multiflora* hydroalcoholic extract provided MIC and MBC 1.25 and 2.5 mg/mL were observed against *K. pneumonia* and *P. aeruginosa* and the aqueous extracts of *M. longifolia* MIC and MBC 12.5 and 5 mg/mL were observed against *K. pneumonia* and *S. marcescens* [37].

Antimicrobial activity of *P. granatum, S. aromaticum, Z. officinales* and *T. vulgaris* ethanolic extracts were produced potential effects at 10 mg/mL concentration, *C. cyminum* extract was effective against *S. aureus* and *P. granatum* and *S. aromaticum* ethanolic extracts were observed a maximum activity which is similar to the present investigation [38]. A systematic study on the antibacterial activity of water, oil and methanol extracts of guava, green tea, neem and marigold against important pathogenic bacteria, *Pseudomonas* spp., *V. cholerae, V. parahaemolyticus, Klebsiella* spp., *E. coli, Salmonella* spp. and *S. aureus*. Guava leaf boiled water extracts showed the maximum zone of inhibition 22 mm against *V. parahaemolyticus* and neem leaf boiled water extract showed moderate zone of inhibition 10 and11 mm against *E. coli* and *Klebsiella* spp respectively [39]. Antibacterial activity of certain medicinal plants used against urinary tract infection (UTI) causing pathogens. Among the different oils tested ajwain oil provided maximm antibacterial activity and lowest activity was recorded in fennel oil [40]. Antibacteerial properties of silver nanoparticles synthesized from jamun extract against different drug resistant bacteria *S. aureus, E. coli* and *S. pyogenes*, the silver nanopartilces produced significant activities and it can be used as an alternative antibacterial agent against diseases caused drug resistant bacteria [41] and the similar trend was observed in the present findings. The antibacterial potential of *T. viride* used for the synthesis of biogenic silver nanoparticles was assessed against human pathogenic bacteria and significant inhibitory activities were observed against tested all pathogenic bacteria [42]. The potential antibacterial activity of green synthesis of silver nanoparticles of *M. officinalis* was observed significant inhibitory activity against the *S. aureus* and *E. coli* [43] which compares well with present findings.

The larvicidal activities recorded in the present study finds comparison with published literatures. A study demonstrated the mosquitocidal properties of aqueous, ethanol, methanol, acetone and chloroform extracts of *L. camara aculeata* were tested against the 4th instar larvae of *Ae. aegypti, An. stephensi* and *Cx. quinquefasciatus*, all selected extracts showed significant larvicidal activity [44] which similar to the present findings. The larvicidal activity of *C. occidentalis* extracts against the larvae of *Cx. quinquefasciatus*, 100% larval mortality observed in petroleum ether and N-butanol extract of *C. occidentalis* at 200 and 300 ppm [45]. The study reported on the extracts of *L. camara, T. procumbens* and *D. stramonium* petroleum ether extract showed 100% larval mortality after 48hrs exposure of plants extracts. The least LC_{50} values of 219 µg/mL showed in *T. procumbens* petroleum ether extract followed by Lantana and Datura had 251and 288 µg/mL respectively [46]. The different plants extracts of *A. precatorius, C. bonplandianum, C. dactylon, M. paradisiaca* and *S. aromaticum* against fourth instar larvae of *An. vagus, Ar. subalbatus* and *Cx. vishnui*. The Larvicidal potential of seed ethyl acetate extracts of *A. precatorius* and leaf extracts of *C. bonplandianum*, flower chloroform and methanol extracts of *M. paradisiacal* and flower bud hexane extract of *S. aromaticum* against *An. vagus* with LC_{50} values of 19.31, 39.96, 35.18, 79.90 and 85.90 µg/mL, respectively. Leaf ethyl acetate and methanol extracts of *C. dactylon*, flower methanol extract of *M. paradisiaca*, flower bud methanol extract of *S. aromaticum* against *Ar. subalbatus* with LC_{50} values of 21.67, 32.62, 48.90 and 78.28 µg/mL,

respectively. Seed methanol of *A. precatorius*, flower methanol extract of *M. paradisiacal* and flower bud hexane extract of *S. aromaticum* against *Cx. vishnui* with LC_{50} values of 136.84, 103.36 and 149.56 µg/mL, respectively which similar to the present investigation [47].

A systematic study on the larvicidal activities leaf and bark extracts of *A. squamosa*, *C. indicum* and *T. procumbens* hexane, chloroform, ethyl acetate, acetone, and methanol dried against the fourth instar larvae of *An. subpictus* and *Cx. tritaeniorhynchus*. All plant extracts showed moderate activity. However, the maximum toxic effect of *A. squamosa* bark methanol extract, *C. indicum* leaf ethyl acetate extract and *T. procumbens* leaf acetone extract against the larvae of *An. subpictus* LC_{50} values were 93.80, 39.98 and 51.57 mg/L respectively. *A. squamosa* bark methanol extract, *C. indicum* leaf methanol extract and *T. procumbens* leaf ethyl acetate extract against the larvae of *Cx. tritaeniorhynchus* LC_{50} values were104.94, 42.29 and 69.16 mg/L respectively [48]. The larvicidal properties of silver nanoparticles (AgNPs) synthesized from *B. kewensis* leaf extract against the fourth instar larvae of *An. stephensi* and *Ae. aegypti*, the LC_{50} and LC_{90} values of AgNPs were 78.4, 144.7 and 84.2 and 117.3 ppm, respectively [49]. The green synthesis of cadmium nanoparticles using marigold and rose flower petal extract, marigold flower petal extract shows 100 % mortality after 72 h of incubation with 10 ppm of Cd-nanoparticles. Therefore, out of two flower petal mediated nanoparticles, only marigold showed better performance towards mosquito larvicidal activity than rose petal extracts [50]. A study on synthesized silver nanoparticle from *P. tuberosa*, the maximum activities were observed at 20 ppm concentration against *Cx. vishnui* with LC_{50} and LC_{90} values of 8.25 and 17.99 ppm respectively. Moreover, the LC_{50} and LC_{90} values of 9.65, 27.18 and 7.94 and 22. 47 ppm recorded against 3rd and 4th instars larvae of *Cx. quinquefasciatus* and the similar trend was also recorded in the study [51]. Therefore, the present investigation lead the way of exploration of Indian medicinal plants *B. montana* and *L. chinensis* for eradication of selected medically important human pathogenic bacteria and vector mosquitoes and consequently gaining a real momentum to consider this medicinal plant product for intense control programme for antibacterial and mosquitocidal activity.

5. Conclusion

In this study we evaluated the antibacterial and mosquitocidal properties of two Indian medicinal plants extracts displayed the potential activity which could be a good source for the alternative of synthetic chemical. The plant extracts are traditional and folk medicine can be as effective as modern medicine to combat pathogenic microorganisms. Moreover, the plants products have no side effect, easily biodegradable, safer and potential activity against human pathogens. Further work is needed to isolate the active phyto-compounds which carry out pharmaceutical and field studies for the treatment of infectious diseases and important human vector mosquitoes.

6. Acknowledgements

Authors are gratefully acknowledged to Professor and Head, Department of Zoology, Principal, Govt. Arts College (Autonomous) Nandanam for their support and laboratory facilities provided and Department of Science & Technology-Fast Track Young Scientist Project (DST, New Delhi) Ref. NO. SB/FT/LS-356/2012 and University Grants Commission (UGC, New Delhi; Ref. No. 42-583/2013 (SR).

7. References

1. Jastaniah SD. The antimicrobial activity of some plant extracts, commonly used by Saudi people, against multidrug resistant bacteria. Life Science Journal. 2014; 11(8):78-84.
2. Bisht R, Katiyar A, Singh R, Mittal P. Antibiotic resistance a global issue of concern. Asian Journal of Pharmaceutical and Clinical Research. 2009; 2:34-9.
3. Mill Robertson FC, Onyeka CI, Tay SCK, Walana W. In vitro antimicrobila activity of antibact, and herbal medicinal product against standard and clonical bacterila isolates. Journal of Medicinal Plants Research. 2015; 9(11): 370-8.
4. Srivastava J, Chandra H, Nautiyal AR, Kalra SJS. Antimicrobial resistance (AMR) and plant derived antimicrobials (PDAms) as an alternative drug line to control infections. 3 Biotech. 2014; 4:451-60.
5. Das K, Tiwari RKS, Shrivastava DK. Techniques for evaluation of medicinal plant products as antimicrobial agents: current methods and future trends. Journal of Medicinal Plants Research. 2010; 4:104-11.
6. Fernebro J. Fighting bacterial infections - future treatment options. Drug Resistance Updates. 2011; 14:125-39.
7. Adwan G, Mhanna M. Synergistic effects of plant extracts and antibiotics on *Staphylococcus aureus* strains isolated from clinical specimens. Journal of Science Research. 2008; 3:134-139.
8. Oyetayo VO. Microbial load and antimicrobial property of two Nigerian herbal remedies. African Journal of Traditional, Complementary and Alternative Medicines. 2008; 5(1):74-78.
9. World Health Organization. Traditional Medicine Growing Needs and Potential - WHO PolicyPerspectives on Medicines, No. 002, May, World Health Organization, Geneva, Switzerland, 2002.
10. Giamarellou H. Multidrug-resistant Gram-negative bacteria: how to treat and for how long. International Journal of Antimicrobial Agents. 2010; 36:50-54.
11. Ahmad I, Mehmood Z, Mohammad F. Screening of some Indian medicinal plants for their antimicrobial properties. Journal of Ethnopharmacology. 1998; 62:183-193.
12. Fradin MS, Day JF. Comparative efficacy of insect repellents against mosquito bites. The New England Journal of Medicine. 2002; 4(1):13-8.
13. Das MK, Ansari MA. Evaluation of repellent action of *Cymbopogan martinii* martinii Stapf var Sofia oil against *Anopheles sundiacus* in tribal villages of Car Nicobar Island, Andaman & Nicobar Islands, India. Journal of Vector Borne Diseases. 2003; 40:101-4.
14. Russell TL, Kay BH, Skilleter GA. Environmental effects of mosquito insecticides on saltmarsh invertebrate fauna. Aquatic Biology. 2009; 6:77-90.
15. Bird B, Githingi H, Macharia JW, Kasiti JM, Muriithi JI, Gacheru RM. Multiple virus lineages sharing recent

common ancestry were associated with a large Rift Valley fever outbreak during 2006-2007. Journal of Virology. 2008; 82:1152-11166.
16. World Health Organization. World Health Organization Media Center, yellow fever fact sheet no: 100. (http://www.who.int/mediacentre/factsheets/fs100/en) [Accessed September 2011].
17. Pancharoen C, Kulwichit W, Tantawichien T, Thisyakorn U, Thisyakorn C. Dengue infection: a global concern. Journal of The Medical Association of Thailand. 2002; 85:25-33.
18. Murugan K, Hwang JS, Kovendan K, Prasanna Kumar K, Vasugi C, *et al*. Use of plant products and copepods for control of the dengue vector, *Aedes aegypti*. Hydrobiologia. 2011; 666:331-338.
19. World Health Organisation. Malaria Factsheet No.94. Geneva: World Health Organisation. [Online] Available from http://www. who.int/mediacentre/factsheets/ fs094/en/. [Accessed July 2010].
20. World Health Organization. Pesticides and their application for the control of vectors and pests of public health importance: World Health Organization, WHO Pesticides Evaluation Scheme, 2006, 113.
21. Bernhard L, Bernhard P, Magnussen P. Management of patients with lymphoedema caused by filariasis in North-eastern Tanzania: alternative approaches. Physiotherapy. 2003; 89:743-749.
22. Hemingway J, Ranson H. Insecticide resistance in insect vectors of human disease. Annual Review of Entomology. 2000; 45:371-91.
23. Karunamoorthi K, Ilango K. Larvicidal activity of *Cymbopogon citratus* (DC) Stapf. and Croton macrostachyus Del. against Anopheles arabiensis Patton, a potent malaria vector. European Review for Medical and Pharmacological Sciences. 2010; 14(1):57-62.
24. Krishnappa K, Elumalai K. *Abutilon indicum* and *Diplocyclos palmatus* botanical extracts against ovicidal, pupicidal and repellent activities of *Aedes aegypti*, *Anopheles stephensi* and *Culex quinquefasciatus* (Diptera : Culicidae). Asian Pacific Journal Tropical Biomedicine. 2012; 1:1-7.
25. Kuppusamy Elumalai, Kaliyamoorthy Krishnappa. Mosquitocidal properties of *Oxystelma esculentum* (Asclepiadaceae) against *Ades aegypti* (Linn.) (Diptera:Culicidae). Journal of Coastal Life Medicine. 2014; 2(5):402-410.
26. Giovanni Benelli, Alice Caselli, Angelo Canale. Nanoparticles for mosquito control: challenges and constraints. Journal of King Saud University - Science. 2017; 29(4):424-435.
27. Ericsson HM, Sherris JC. Antibiotic sensitivity testing. Report of an International Collaborative Study. Acta Pathol Microbiol Scand B Microbiol Immunol. 1971; 217:1-90.
28. Hailu Tadega, Endris Mohammedb, Kaleab Asresc, Tsige Gebre Mariama. Antimicrobial activities of some selected traditional Ethiopian medicinal plants used in the treatment of skin disorders. Journal of Ethnopharmacology. 2005; 100:168-175.
29. Lorian V. Antibiotics in lobaratory medecine 4th Edu, Williams and Wilkins, Baltimore Londan, 1996.
30. World Health Organization. Guidelines for laboratory and field testing of mosquito larvicides. Geneva: World Health Organization, 2005, [Online] Available from O/CDS/WHOPES/ GCDPP/2005.13.
31. Abbott WS. A method for computing the effectiveness of an insecticide. Journal of Economic Entomology. 1925; 18:265-267.
32. Kasthuri J, Kathiravan K, Rajendiran N. Phyllanthin assisted biosynthesis of silver and gold nanoparticles: a novel biological approach. Journal of Nano Research. 2009; 11:1075-1085.
33. Harborne JB. Phytochemical methods, a guide to modern techniques of plant analysis. London: chapman and hall, 1984, 49-188.
34. Evans WC. Trease and evans pharmagnosy 15th Edu, WB saunders company Ltd, 2002, 135-150.
35. Selvamohan T, Ramadas S. Shibila Selva Kishore. Antimicrobial activity of selected medicinal plants against some selected human pathogenic bacteria. Advances in Applied Science Research. 2012; 3(5):3374-3381.
36. Usman Ali Khan, Hazir Rahman, Zeeshan Niaz, Muhammad Qasim, Jafar Khan, Tayyaba, *et al*. Antibacterial activity of some medicinal plants against selected human pathogenic bacteria. European Journal of Microbiology and Immunology. 2013; 3(4):272-274.
37. Saeide Saeidi, Kazem Hassanpour, Mehdi Ghamgosha, Mohammad Heiat, Ramezan Ali Taheri, Ali Mirhosseini, Gholamreza Farnoosh. Antibacterial activity of ethyl acetate and aqueous extracts of *Mentha longifolia* L. and hydroalcoholic extract of *Zataria multiflora* Boiss. plants against important human pathogens. Asian Pacific Journal of Tropical Medicine 7(Suppl 1). 2014; S186-S189.
38. Ashraf A, Mostafa, Abdulaziz A, Al-Askar, Khalid S, Almaary, *et al*. Antimicrobial activity of some plant extracts against bacterial strains causing food poisoning diseases. Saudi Journal of Biological Sciences. 2018; 25:361-366.
39. Atikya Farjana, Nagma Zerin, Md. Shahidul Kabir. Antimicrobial activity of medicinal plant leaf extracts against pathogenic bacteria. Asian Pacific Journal of Tropical Diseases. 2014; 4(2):S920-S923.
40. Amit Kumar, Neeraj Jhadwal, Madan Lal, Manjeet Singh. Antibacterial activity of some medicinal plants used against UTI causing pathogens. International Journal of Drug Development and Research. 2012; 4(2): 278-283.
41. Shikha Behera, Nayak PL, Nayak PL. In vitro antibacterial activity of green synthesized silver nanoparticles using jamun extract against multiple drug resistant bacteria. World Journal of Nanoscience and Technology. 2013; 2(1):62-65.
42. Abdallah Mohamed Elgorbana, Abdullah Naser Al-Rahmah, Shaban Rushdy Sayed, Abdurahman Hirad, Ashraf Abdel-Fattah Mostafa, Ali Hassan Bahkali. Antimicrobial activity and green synthesis of silver nanoparticles using *Trichoderma viride*. Biotech and Biotechnological Equipment. 2016; 30(2):299-304.
43. Alvarode Jesus Ruiz-Baltazar, Simon Yobbany Reyes-Lopez, Daniel Larranaga, Miriam Estevez, Ramiro Perez. Green synthesis of silver nanoparticles using a *Melissa*

officinalis leaf extract with antibacterial properties. Results in Physics. 2017; 7:2639-2643.
44. Periaswamy Hemalatha, Devan Elumalai, Arumugam Janaki, Muthu Babu, Kuppan Velu, Kanayairam Velayutham, Patheri Kunyil Kaleena. Larvicidal activity of *Lantana camara aculeata* against three important mosquito species. Journal of Entomology and Zoology Studies. 2015; 3(1):174-181.
45. Deepak Kumar, Rakesh Chawla, Dhamodaram P, Balakrishnan N. Larvicidal activity of *Cassia occidentalis* (Linn.) against the larvae of *Bancroftian Filariasis* vector mosquito *Culex quinquefasciatus*. Journal of Proteome Research, 2014, 1-5.
46. Anitha Rajasekaran, Geethapriya Duraikannan. Larvicidal activity of plant extracts on *Aedes aegypti* L. Asian Pacific Journal of Tropical Biomedicine, 2012, 1578-S1582.
47. Bagavan A, AbdulRahuman A. Evaluation of larvicidal activity of medicinal plant extracts against three mosquito vectors. Asian Pacific Journal of Tropical Medicine. 2011; 4(1):29-34.
48. Kamaraj C, Bagavan A, Elango G, Abduz A, Zahir, Rajakumar G, *et al*. Larvicidal activity of medicinal plant extracts against *Anopheles subpictus* & *Culex tritaeniorhynchus.* Indian Journal of Medical and Research. 2011; 134(1):101-106.
49. Rajamani Bhuvaneswari, Raju John Xavier, Manickam Arumugam. Larvicidal property of green synthesized silver nanoparticles against vector mosquitoes (*Anopheles stephensi* and *Aedes aegypti*). Journal of King Saud University - Science. 2016; 28(4): 318-323.
50. Amita Hajra, Snehali Dutta, Naba Kumar Mondal. Mosquito larvicidal activity of cadmium nanoparticles synthesized from petal extracts of marigold (*Tagetes* sp.) and rose (*Rosa* sp.) flower. Journal of Parasitic Diseases. 2016; 40(4):1519-1527.
51. Anjali Rawani. Mosquito larvicidal activity of green silver nanoparticle synthesized from extract of bud of *Polianthus tuberosa* L. International Journal of Nanotechnology and Applications. 2017; 11(1):17-28.