"BOTANICAL LARVICIDAL POTENTIAL OF CHLOROFORM AND PETROLEUM ETHER SEED EXTRACTS OF THEVETIA NERIIFOLIA AGAINST DENGUE VECTOR AEDES AEGYPTI LARVAE IN KUMARAPALAYAM, NAMAKKAL DISTRICT"

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ABSTRACT

Aedes aegypti; well-known vector associated with the transmission of dengue, chikungunya and yellow fever; has attracted substantial attention worldwide because of the alarming increase in disease statistics.The investigations clearly established the larvicidal efficiency of both the extracts, though chloroform extracts proved more efficient than the seed extracts. The bioassay resulted in LC_{50} value of 12.08 when larvae were exposed to the chloroform seed extracts of *T. nerifolia*. Similarly, the bioassay resulted in LC_{50} value of 8.05 when larvae were exposed to the petroleum ether seed extracts of *T. nerifolia*. Our results suggest the probable use of chloroform extracts of *T. nerifolia* as an efficient and eco-friendly larvicide against *A. aegypti*. Further investigations are needed to identify the bioactive constituent and ascertain its effectiveness in the field conditions. Emergence of microbial resistance is the one of the major problems nowadays; thus, there have been tremendous efforts towards finding new chemicals, specifically herbals, for the development of new antimicrobial drugs.

KEYWORDS

Mosquito, Dengue and Chikungunya, shrub *Thevetia nerifolia,* lethal concentration of 90% (LC_{90}) value, antibioticextract

INTRODUCTION

Mosquito control has become a global health priority, owing to their vectoring role of pathogens of many diseases such as malaria, dengue, yellow fever, Zika, West Nile, affecting a significant proportion of the world population. These diseases contribute significantly to the estimated 17% of the global vector-borne disease burden of all infectious diseases, accounting for >1 billion new cases and >1 million deaths annually (WHO, 2016; Valentine et al., 2017). Among, them dengue is an important human viral disease transmitted by *Aedes aegypti* (Gubler 1998). *Aedes aegypti*, the chief carrier of Dengue and Chikungunya Virus in India, has engrossed extensive interest at universal level because of rapid spread of these diseases. Since last decade, dengue has emerged as a prime health concern in tropical and sub-tropical expanseof the world; primarily in urban and semi-urban regions; causing increased deaths every year. Since past five decades as high as 30-fold increase in the occurrence of dengue has beenobserved at the global level. Currently, approximately half of the world's population has been reported at the risk of dengue fever (WHO, 2015). As per the estimates of World HealthOrganization, 390 million dengue infections occur each year leading to clinical manifestations of96 million infections. Though dengue is considered a global concern, yet almost 75% of the world population experiencing dengue resides in the Asia-Pacific region. In India, Ministry of Family and Health Welfare has reported 36486 cases of dengue

and 92 fatalities till 31stDecember, 2014 with highest figures of 7410 cases reported in the state of Maharashtra (NVBDCP, 2015). In addition, *Aedes aegypti transmits* the overwhelming diseases such as dengue, yellow fever, chikungunya and causes burden to the country and worldwide too (Lame et al., 2014). The yellow fever mosquito, *A. aegypti* is a mosquito that can spread the dengue fever, yellow fever viruses, and other diseases (Evenhuis and Samuel, 2007). The Dengue feveris transmitted by this vector and causes various symptoms viz., vomiting, fever, headache, muscle, joint pains, nausea, rashes etc. These symptoms are responsible for making it as a syndrome causing an increased vascular permeability and shock through the patient's life. The DEN 1, 2, 3 and 4 viruses are responsible for causing the Dengue fever and Dengue hemorrhage fever by closely related *A. aegypti* (NVBDCP, Delhi 2005-2012). The mosquito can be recognized by white markings on legs and a marking in the form of a lyre on the thorax. The mosquito originated in Africa (Mousson et al., 2005) but is now found in tropical and subtropicalregions throughout the world.

MATERIAL AND METHODS

Figure 1. Photography of *Aedes aegypti* mosquito

Taxonomical hierarchy

Kingdom: Animalia
Phylum: Arthropoda
Class : Insecta
Order : Diptera
Family :Culicidae
Genus : *Aedes*
Species: *aegypti*

Development of vaccine against these diseases is still at an early stage and therefore the only method available for reducing incidence of the disease is the control of its mosquito vector (Saranya et al., 2013). Controlling of these vectors has been attempted for long time using synthetic chemicals. Nevertheless, the chemicals in turn are causing environmental pollution and developing resistance against the existing chemicals. Hence, these glitches have emphasized the prerequisite for the development of novel, effective, affordable, biodegradable, and selective mosquito control agents (Ramar et al., 2014). Therefore, an alternative way for pest control

strategies especially the effective and low-cost method is urgently needed. In the recent past, botanical pesticides can provide effective and ecofriendly tools against mosquitoes. Green pesticides are reported to be biodegradable (Sukumar et al., 1991; Senthilnathan *et al*., 2006), economical, non-toxic to non-target pests and have high specific activities towards thetarget pests. Plant extracts and essential oils have shown good larvicidal activity against the mosquitoes, which are biodegradable, economical and more sustainable option as compared to synthetic pesticides (Ghosh et al., 2012). For example, the leaf extracts of *C. maxima* exhibit larvicidal, ovicidal and repellent activities against the *C.quinquefaciatus* (Mullai and Jebanesan, 2007). The *Cassia fistula* plant methanolic extract provided the larvicidal activity against *A. stephensi* and *C. quinquefasiactus* (Govindrajan *et al.,* 2008). The plant *Azadirachta indica* (Meliaceae), commonly referred to as neem, is a classic botanical whose insecticidal activity of the crude or derived products have been evaluated against many insects including vectors of medical importance (Mulla and Activity, 1999; Mordue and Nisbet, 2000; Dua et al., 2009).The larvicidal activity of leaf extracts against the filarial vector *Culex quinquefasciatus .Murraya koenigii, Cleistanthus collinus* and *Spheranthus indicus* (Kovenden et al*.,* 2012). *Carica papaya, Murraya paniculata* and *Cleistanthus collinus* (Rawani *et al.,* 2009), *Strychonos nuxvomica* (Arivoli and Samuel Tennyson 2012). Murugan *et al.,* 2012 had investigated a methodology to explore the mosquito control using orange peels extract of *C. sinensis* for the control of *A. stephensi, A. aegypti* and *C. quinquefasciatus* vectors respectively for malaria, dengue fever and filarial diseases. In addition, various plant-derived compounds for controlling medically and veterinary important insect vectors have been highlighted (George et al., 2014; Muema et al., 2017). The shrub *Thevetia nerifolia*, the yellow oleander, is commonly cultivated throughout Asia and India as an ornamental plant. But it is only in South Asia, there is still belief that the ingestion of oleander seeds has become a popular means of self-harm. Each year, there are thousands of yellow oleander poisoning cases in South Asia and probably hundreds of deaths, giving the mortality rate of 4-10%. It is well known that the fruits contain a highly poisonous glucoside which finds considerable use as a cattle poison (Darren *et al*., 2006). The fruit of *T. nerifolia* is globular, slightly fleshy, and green, between 1.5 and 2 inches in diameter. It contains a hard nut which is light brown in colour and triangular in shape with a deep groove corresponding to the base of its triangle. Each nut contains two pale yellow seed kernels. The average weight of a nut is 5 grams. The seed oil contains the glycerides of palmitic, stearic, oleic, linolic and arachidic acids. Many cardenolides have been identified in yellow oleander, predominantly thevetin A and B, but also peruvoside, neriifolin, thevetoxin, ruvoside, and theveridoside. These cardenolides are structurally similar to the Digitalis cardenolides.

Figure 2. Photography of *Thevetia nerifolia* plant

Taxonomy

Kingdom : Plantae
Phylum:Tracheophyta
Subphylam: Euphyllophytia
Subclass : Asteridae
Order : Gentianales
Family : Apocynaceae
Genus : *Thevetia*

Species : *nerifolia*

The *T. nerifolia* is one of the unexplored plant species for insecticidal or insect growth regulatory activity, though other biological activities were studied (Oji and Okafor, 2000; Gata Gongalves et al., 2003; Ambang et al., 2007; Ray et al., 2009). Satpathi and Ghatak (1990) found that methanolic extracts of seeds of *T. nerifolia* Merr, at 1.0 per cent resulted in 100 per cent mortality of fourth instar larvae of *P. xylostella*, 12-24 h after treatment when applied topically.Pesticidal property of Thevetia sp. was reported against diamond back moth and other agriculturally important insect pests (Lingappa et al., 2004). IGR and larvicidal activity of Thevetia sp. was evaluated against two species of mosquito and found good larvicidal but very little IGR activity (Lapcharoen et al., 2005). *Thevetia neriifolia* leaf extract was evaluated against *Tribolium confusum* adults and it was found that acetone extract was found to be the most effective toxicant followed by ethyl acetate, petroleum ether and methanol extracts (Khanam et al., 1995). Therefore, this study was designed to search for the larvicidal activity of *T. nerifolia* on *Aedes aegypti* mosquito larvae as an alternative control measure for dengue fever. A wide variety of antibiotics are commonly used for the treatment of infections caused bybacteria (Kandasamy et al., 2016). Treatment with antibiotics is not only expensive but the risk of bacterial resistance to antimicrobial agents and side effects such as acidity, burning sensation and damage to natural fauna of intestine are also involved. The situation is further worsening as the resistance to pathogens against antibiotics is developing much faster than ever. In recent years, multiple drug resistance, a threat to mankind has caused an urgent need for the search of innovative ways to control bacterial pathogens. Hence, natural antibiotics are in process of being discovered as alternative to synthetic products (Dua et al., 2014). Over the past 2 decades, there has been a lot of interest in the investigation of natural materials as sources of new antibacterial agents (Bonjar et al., 2003; Tepe et al., 2004). According to the World Health Organization (WHO), medicinal plants would be the best source to obtain a variety of drugs and active compounds. Therefore, such plants should be investigated to better understand their properties, safety and efficiency (Ellof, 1998). Plant materials such as Citrus spp. peel (Johann et al., 2007) and grape (*Vitis vinifera* L.) seeds (Baydar et al., 2006) are some natural products that display antimicrobial activity that has been applied in foods. Seeds of plants have been reported to produce a number of peptides and proteins with antimicrobial activities (Wang et al., 2009). Many types of molecules with antibacterial activity have been isolated from plants (Boonnak et al., 2009; Mahabusarakam et al., 2008). This study was therefore undertaken to determine the presence and level of antimicrobial activity (if any) associated with seed extracts, chloroform and petroleum ether seedextracts against *E. coli* (gram negative) and *S. epidermidis* (gram positive) organisms.

2.1. SEEDS COLLECTION

Thevetia nerifolia seeds were collected in and around Yercaud hills (11.7753° N, 78.2093° E), Salem district, Tamil Nadu, India (Fig. 3). The mature and disease-free seeds were collected and washed thoroughly with tap water to remove the dust particles on the seed surface. Finally, seeds were air-dried in shade and after drying sample was ground to a fine powder using an electric blender (Fig. 3).

2.2. PREPARATION OF EXTRACTS

The experimental seed powders extract with two different solvents such chloroform and petroleum ether in a successive manner, in order to produce crude extracts containing a wide range of active compounds. For the preparation of sample extracts, the method reported by Hanafy and Hatem (1991) was used. For this purpose, 100 ml of solvents (chloroform and petroleum ether) was added into 40 g of seed powder and the mixture was left for 8 hours. The mixture was periodically agitated during this period (15 min). Afterwards, it was filtered and the ether was vaporized in an evaporator (60°C). The dark colored oily extract obtained at the end of these processes was used in a non-diluted form for the analysis. The crude extract was placed in desiccators for the complete evaporation of solvents finally dried in air at room temperature on blotting paper in plastic tray antimicrobial activity tests were started on the same day. The sample extracts were kept in the refrigerator (4°C) until the analysis was accomplished.

Fig. 3. A) Map showing the Salem District & Yercaud taluk, (B) *Thevetia nerifolia* plant, (C) *Thevetia nerifolia* seeds with leaves, (D) *Thevetia nerifolia* seed, (E) Dried *Thevetia nerifolia* seeds and leaves.

2.3. COLLECTION OF MOSQUITO LARVAE

The selected vector mosquitoes' culture and establishment were followed as per the method prescribed by Mashhadani *et al.* (1980). *Aedes aegypti* colony was collected from womens SIDCO, Karuppur, Salem-11, Tamil Nadu (Fig. 4) and maintained at insectary at 27°C and 80 % Relative humidity with a photoperiod of 12 hours light and dark cycles. The collected larvae were maintained in Petri dishes with dechlorinated tap water. Larvae were fed with a diet of yeast and dog biscuits.

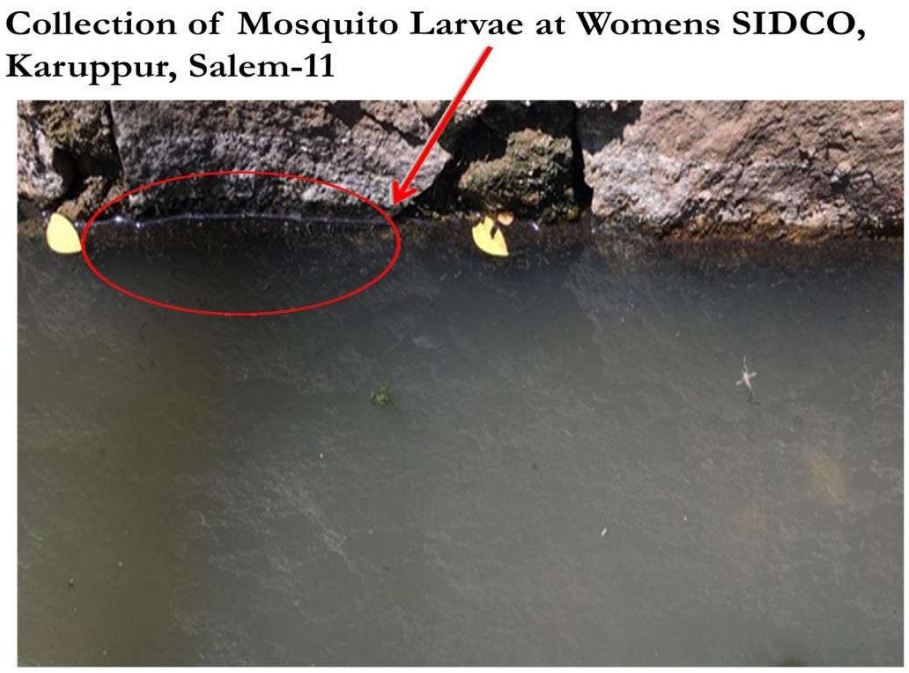


Fig. 4. Collection of Mosquito Larvae at Womens SIDCO, Karuppur, Salem, Tamil Nadu

2.4. LARVICIDAL ACTIVITY

Based on preliminary screening results, the aqueous seeds extract of *T. nerifolia* were subjected to dose response assay for larvicidal activity against the larvae of *A. aegypti*. Larvicidal activity of the extract was determined by following the standard procedure (WHO, 2005). Initially, mosquito larvae were exposed to a wide range of test concentrations and a control to find out the activity range of the aqueous extract of seed under test. After determining the mortality of larvae in this wide range of concentrations, a narrow w range of 05, 10, 20, 40 and50 mg/L concentrations were used to determine the lethal concentration of 50% (LC_{50}) and the lethal concentration of 90% (LC_{90}) 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 and the required amount of seed extract was added. Five replicates were setup for each test concentration. In each replicate 25 larvae were used, with five

replicates of control. The number of dead larvae was counted after 24 h. The larval mortality was calculated by using the formula of Abbott (1925) and LC_{50} value was calculated after 24 h by probit analysis (Finney, 1971).

2.5. ANTIBACTERIAL ACTIVITY OF *T. nerifolia* SEEDS EXTRACT

The antibacterial activity of *T. nerifolia* seeds extract was evaluated against *E. coli* and *Staphylococcus epidermidis*. The antibacterial activity was determined using the well diffusion method. The wells were prepared on plates with Muller-Hinton agar (MHA) medium. Then, the plates were seeded with different bacterial strains using sterile swab. Four wells were prepared using gel puncture in each plate. Each well was loaded with 50 µL of *T. nerifolia* aqueous seeds extract. Then, the plates were incubated at 35 °C for 24 h and zone of inhibition was observed.

2.6. DATA ANALYSIS

The average larval mortality data were subjected to analysis for calculating LC_{50} and other statistics at 50% lower confidence limits and chi-square values were calculated using Graph Pad In state and Prism version 5.00 for Windows, Graph Pad Software, San Diego, CA, USA.

RESULT

3.1. larvicidal activity of aqueous seed extracts of *T. nerifolia* against *A. aegypti*

In the present study, the larvicidal activity of aqueous seed extracts of *T. nerifolia* along with chloroform and petroleum ether seed extracts were studied. The parasite larvae of *A. aegypti* at fourth instars were exposed to varying concentrations (5, 10, 20, 40, 50 mg/L) of *T. nerifolia* aqueous seed extracts and chloroform & petroleum ether seed extracts. The maximum mortality of the crude seed extracts of *T. nerifolia* against *A. aegypti* noticed was 41.68±6.11 % at 50 mg/L concentration (Table 1). The chloroform seeds extract showed 100 % mortality rate against larvae of *A. aegypti* at fourth instars stage (Table 2). The chloroform (negative control) was showed 100 % mortality within 6 hours. The petroleum ether seeds extract showed 98.66±2.30 % mortality rate against larvae of *A. aegypti* at fourth instars stage (Table 3). The petroleum ether (negative control) was showed 97.24±2.30 % mortality within 6 hours while no mortality was observed with distilled water (positive control). The LC_{50} values for fourth instars after 24hrs of aqueous seed extracts of *T. nerifolia* and chloroform mediated *T. nerifolia* seed extract exposure were 68.20, 11.05 mg/L against against *A. aegypti*. Similarly, TheLC_{50} values for fourth instars after 24hrs of aqueous seed extracts of *T. nerifolia* and petroleum ether mediated *T. nerifolia* seed extract exposure were 68.20, 8.05 mg/L against *A. aegypti*.

3.2. Antibacterial activity of aqueous seed extracts of *T. nerifolia*

The *T. nerifolia* seed extract showed bactericidal activity against *E. coli* (gram negative) and *S. epidermidis* (gram positive) organisms. The zone of inhibition showed by the chloroform mediated *T. nerifolia* seed extract against the *S. epidermidis* ranged 11 mm, similarly petroleum ether mediated *T. nerifolia* seed extract showed zone inhibition is 9 mm and antibiotic gentamicin showed zone inhibition is 8 mm. However, no zone inhibition was observed by the *T.nerifolia* seed extract against *S. epidermidis* (Fig. 5 and 6).

Table. 1. Aqueous seeds extract of *T. nerifolia* against *A. aegypti* vector.

Treatment	Conc. mg/L	Mortality after 24 hrs	LC_{50} mg/L	LCL-UCL	R^2	Slope
Aqueous seeds extract	05	30.00±4.61	68.2	7.80-10.84	0.906	0.318
	10	36.34±2.30				
	20	39.60±2.30				
	40	39.66±2.30				
	50	41.68±6.11				

Table. 2. Chloroform mediated *T. nerifolia* seed extract and chloroform (negative control) against *A. aegypti* vector.

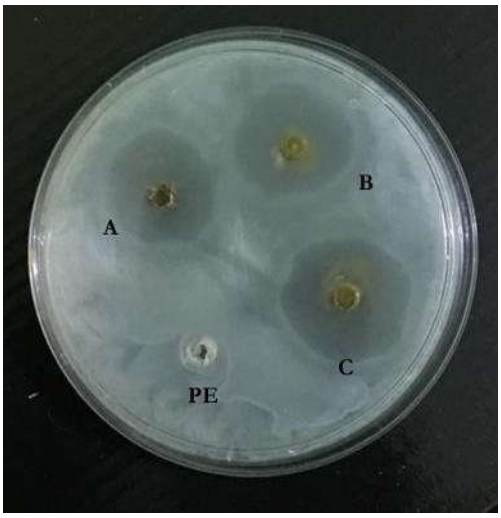
Treatments	Conc. mg/L	Mortality after 24 hrs	LC_{50} mg/L	LCL-UCL	R^2	Slope
Chloroform (negative control)	05	06.02±2.06	12.05	2.14-30.85	0.974	16.29
	10	15.00±4.89				
	20	97.00±2.19				
	40	97.16±2.06				
	50	100.00±0.00				
Chloroform seeds extract	05	16.66±4.84	12.08	0.597-31.0	0.996	14.02
	10	18.00±5.65				
	20	100.03±1.63				
	40	100.00±0.00				
	50	100.00±0.00				

Table. 3. Petroleum ether mediated *T. nerifolia* seed extract and petroleum ether (negative control) against *A. aegypti* vector.

Treatments	Conc. mg/L	Mortality after 24 hrs	LC_{50} mg/L	LCL-UCL	R^2	Slope
petroleum ether (negative control)	05	26.00±4.00	9.02	8.10-25.61	0.958	2.13
	10	47.66±4.61				
	20	72.66±4.61				
	40	83.13±6.11				
	50	97.24±2.30				
petroleum ether seed extract	05	34.63±0.57	8.05	9.35-27.17	0.945	2.31
	10	59.56±9.23				
	20	87.23±4.61				

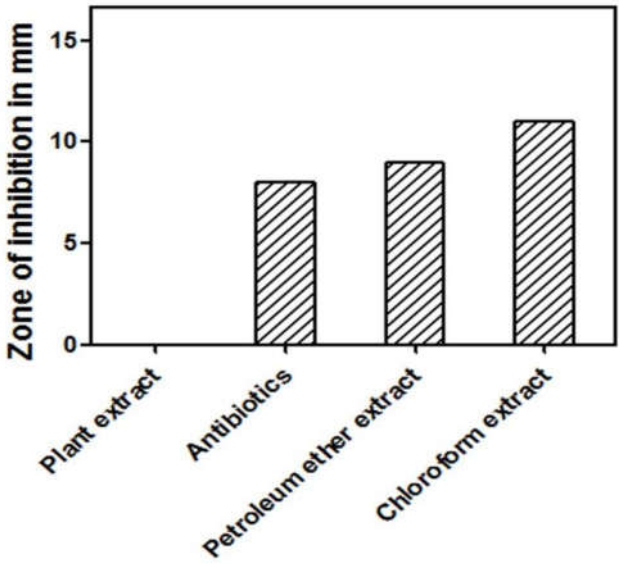
40	95.76±2.30
50	98.66±2.30

The zone of inhibition showed by the petroleum ether mediated *T. nerifolia* seed extract against the *E. coli* ranged 8 mm, similarly petroleum ether mediated *T. nerifolia* seed extract showed zone inhibition is 1 mm and antibiotic gentamicin showed zone inhibition is 6 mm.



However, no zone inhibition was observed by the *T. nerifolia* seed extract against *E. coli* (Fig. 7 and 8). *Staphylococcus epidermidis* showed the highest susceptibility with zones ranging from 8-11 mm with chloroform mediated *T. nerifolia* seed extract. In the same manner, *E. coli also showed* the highest zones inhibition ranging from 6-8 mm with chloroform mediated *T. nerifolia* seed extract. Therefore, on comparing the zones produced by the *T. nerifolia* seed extract and theroutinely used antibiotic gentamicin, chloroform and petroleum ether mediated *T. nerifolia* seed extract had proved to be considerably efficient. As observed from figures 5 & 8, out of the total 4 assessed samples, the chloroform and mediated *T. nerifolia* seed extract was found to be effective against *E. coli* and *S. epidermidis* human pathogens.

Fig. 5. Zone inhibitions of different solvent (chloroform and petroleum ether) extract of *T. nerifolia* seed against human pathogenic bacteria *Staphyloccus epidermidis*. Abbreviations:



PE – plant extract, A- Antibiotics, B – Petroleum ether extract, C - Chloroform extract.

Fig. 6. Antibacterial activity of different solvent (chloroform and petroleum ether) extract of *T. nerifolia* seed against human pathogenic bacteria *Staphyloccus epidermidis*.

Fig. 7. Zone inhibitions of different solvent (chloroform and petroleum ether) extract of *T. nerifolia* seed against human pathogenic bacteria *Escherichia coli*. Abbreviations: PE – plant extract, A- Antibiotics, B – Petroleum ether extract, C - Chloroform extract.

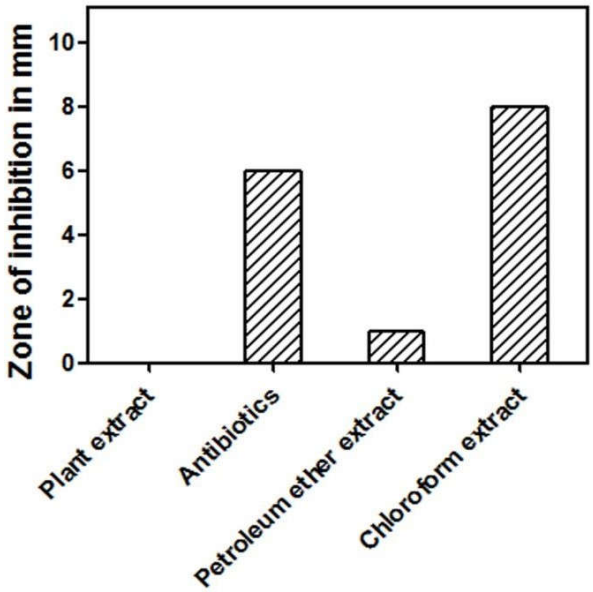


Fig. 8. Antibacterial activity of different solvent (chloroform and petroleum ether) extract of *T. nerifolia* seed against human pathogenic bacteria *Escherichia coli*

DISCUSSION

4.1. larvicidal activity of seed extracts of *T. nerifolia* against *A. aegypti* mosquito

The mosquito *Aedes aegypti* has domiciliary habits, hiding in dark and closed places, which leads to difficulties in its eradication (Gubler 1998). So, the most efficient way to control dengue resides in preventing the insect breeding through the use of larvicides (Consoli and Oliveira 1994, WHO 1999). Plant extracts exert myriads of biological activities on pests including larvicidal, repellent, ovicidal, insect growth regulator, *etc*. This can be due to various phytochemicals present in the plants that might be acting synergistically to produce such responses. Botanical pesticides are biodegradable and rarely develop resistance against the pests because of the synergistic activity of complex biomolecules thus moderating the long-term environmental effects of synthetic pesticide use (Maurya et al., 2012). *Thevetia nerifolia* is one such plant that has the potential to be used in vector control program due to the rich phytochemicals. In the current study, a significant concentration-dependent larvicidal activity of *T. nerifolia* seed extracts was demonstrated on third and early fourth instar larvae of *A. aegypti*. Among the three extracts (petroleum ether, chloroform and aqueous extract) used chloroform exhibited best results against the mosquito larva. Furthermore, the extractive value is more in chloroform (100 %), followed by petroleum ether (97.2 %) and aqueous extract (25.0 %). It shows that petroleum ether and chloroform soluble contents are more in the seeds of *T. nerifolia* than water-soluble contents. This could also be the reason for the chloroform and petroleumether extracts to act better than the aqueous extracts. Likewise, a review study by Sukumar et al. (1991) reported a great correlation between solvent polarities and nature of compounds extracted. Among different solvents, petroleum ether extract exhibited maximum larvicidal activity than aqueous extract against the mosquito larva. Larvicidal bio efficacy of *T. nerifolia* seed extracts may be due to phytochemicals stored in the plant, which exhibit biological action either independently or jointly against larvae of *A. aegypti*. Azmathullah et al. (2011) reported that alkaloids, saponins, and tannins are known to possess medicinal and pesticidal properties and protect the plants from insects, pests, and diseases. Bagavan et al. (2008) reported that saponins isolated from *Achyranthes aspera* through bioassay-guided fractionation possessed a larvicidal efficacy against *Aedes aegypti* and *Culex Quinquefasciatus*. Furthermore, Kotkar et al. (2002) reported that flavonoids isolated from water extracts of *Annona squamosa* were effective as insecticides against mosquitoes affecting 80% of *C. Chinensis*. The seed extract was found to have better larvicidal activity than the leaf extract as corroborated by various reports (vara et al., 2010; Wafa et al., 2014).Our study clearly demonstrated that water extract of *T. nerifolia* seeds have lethal action against *A. aegypti* larvae and low toxic effects on laboratorial animals, which is in agreementwith the literature (Wahedi et al., 2020) An additional benefitis that *T. nerifolia* seed is available throughout the year, especially when the mosquito population is higher. The plants grow in nature without any extra care or cost and simple technology would be necessary to separate the most suitable fractions to be exploited as a possible chemical to be employed in mosquito controlprograms.

4.2. Antibacterial activity of seed extracts of *T. nerifolia* against human pathogens

In the present scenario of emergent multi drug resistance in human pathogenic infections, it has become highly necessary to search for novel antimicrobial substances from other sources, such as plants (Abdelaziz et al., 1990). This exploration will hopefully lead to the development of a phytomedicine to act against microbes (Doughari et al., 2007). Plants have been a valuable source of natural products for maintaining human health and infections control because, microbial infections pose a health problem throughout the world, and plants are a possible source

of antimicrobial agents (Ashish et al., 2013). According to the World Health Organization (WHO), medicinal plants would be the best source to obtain a variety of drugs and active compounds. Therefore, such plants should be investigated to better understand their properties, safety and efficiency (Mothana et al., 2009). Seed extract of different medicinal plants were screened for antibacterial activity. The method for the determination of antibacterial activity was agar well diffusion Assay and the zones of inhibition were measured. Zone of inhibition varied among the samples. Figure 4 - 7 shows the antibacterial activity of different extracts of *T. nerifolia* seeds against pathogenic bacteria. The antibacterial activity test results for influences of *T. nerifolia* seed extracts on pathogenic bacteria are characterized by the inhibition zone around the discs. Figure 4 & 5 shows the highest zone of inhibition (ZI) of chloroform extracts (11 mm) for petroleum ether (9 mm) was demonstrated significantly against the *S. epidermidis*.in other hand, *E. coli* showed the same zone of inhibition (ZI) (8 mm) against chloroform extracts (Fig. 6 & 7).Interestingly, chloroform and petroleum ether solvent extracts showed antimicrobial activity against selected pathogenic bacteria, which was not found in aqueous of *T. nerifolia* seeds extracts. A higher concentration of the extract could be expected to increase the antimicrobial activity, as increasing the concentration of an extract should increase the diameter of the inhibitory zone formed due to greater abundance of active components in the extract (Lis-Balchin et al., 2000). In Fig. 4 & 5, the chloroform extract of *T. nerifolia* was revealed as demonstrating the highest antibacterial activity against *S. epidermidis* (11 mm). In the same manner, the chloroformextracts inhibited *S. epidermidis* (9 mm). The lowest zone of inhibition (ZI) was found with the *T. nerifolia* seed extract against *S. epidermidis* (Fig. 4 and 5). Similarly, in Fig. 6 & 7, thepetroleum ether extracts of *T. nerifolia* were revealed as demonstrating the highest antibacterial activity against *E. coli* (8 mm). In the same manner, the petroleum ether extracts inhibited *S. epidermidis* (1 mm). The lowest zone of inhibition (ZI) was found with the water and petroleum ether extract against *S. epidermidis*. These results were consistent with those reported by other researchers (Petros, 2008), who found that *Moringa* leaf extracts were effective against *S. aureus*and *E. coli*. In addition, Talreja (2010) reported that the *Moringa oleifera* flower has antimicrobial activity against gram-positive and gram-negative bacteria. Dada et al. (2014) reported that ethanolic extract of Jatropha leaves consist of polyphenol compounds, such as flavonoids, tannin and saponins, which have antibacterial activity against *Staphylococcus aureus* and coliform bacteria. However, the lowest zone of inhibition was shown with the water extract against human pathogens. Generally, the different seed extracts revealed a concentration-dependent inhibition effect on the bacteria tested. Based on the results obtained, both chloroform and petroleum ether solvent extracts showed different rates of inhibitory effect on both gram-positive and gram-negative bacteria. It is hoped that these results will lead to the identification of compounds that could help to establish antibacterial drugs from natural products. Further purification of components can be carried out and the components may be subjected to animal studies.

CONCLUSSION

Aedes aegypti; well-known vector associated with the transmission of dengue, chikungunya and yellow fever; has attracted substantial attention worldwide because of the alarming increase in disease statistics. Laboratory investigations were carried out to evaluate the impact of extracts; prepared from the seeds of *T. nerifolia*; on the survival, behaviour and morphology of *A. aegypti* larvae. Healthy and disease-free seeds of *T. nerifolia* extracted in chloroform and petroleum ether were screened for their larvicidal activity against early fourth instars of dengue vector in accordance with the WHO protocol. The investigations clearly established the larvicidal efficiency of both the extracts, though chloroform extracts proved more efficient than the seed

extracts. The bioassay resulted in LC_{50} value of 12.08 when larvae were exposed to the chloroform seed extracts of *T. nerifolia*. Similarly, the bioassay resulted in LC_{50} value of 8.05 when larvae were exposed to the petroleum ether seed extracts of *T. nerifolia*. Our results suggest the probable use of chloroform extracts of *T. nerifolia* as an efficient and eco-friendly larvicide against *A. aegypti*. Further investigations are needed to identify the bioactive constituent and ascertain its effectiveness in the field conditions. Emergence of microbial resistance is the one of the major problems nowadays; thus, there have been tremendous efforts towards finding new chemicals, specifically herbals, for the development of new antimicrobial drugs. *T. nerifolia* is an important traditional medicinal plant, mentioned in various ancient literatures such as Ayurveda. The plant is selected to evaluate the possibility for novel pharmaceuticals having antibacterial potential. Stem-bark of the plant was extracted using various solvents. In this study the efficacy of chloroform and petroleum ether extracts of whole seed of *T. nerifolia* against common human pathogens *S. epidermidis* and *E. coli* were investigated. The disc diffusion method was used to determine antimicrobial activity of *T. nerifolia*. The result indicate that the chloroform and petroleum ether extracts inhibit the growth of bacteria in following order *S. epidermidis* > *E. coli*. Compared with standard antibioticextract was more potent than antibiotic drug. Respectively the observed antimicrobial activities were believed to be due to the presence of phytochemicals in the extract. The study promises an interesting future for designing potentially active antibacterial agents from *T. nerifolia*.

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