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COMBINED EFFECT OF KUNGILIUM AND *AZADIRACTA* INDICA LEAF AND ITS BIOLOGICAL PERCEPTIONS, LARVICIDAL ACTIVITY AGAINST THE *CULEX QUINQUEFASCIATUS* MOSQUITOES

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ABSTRACT

Mosquitoes transmit serious human diseases like malaria, filariasis, Japanese encephalitis, dengue haemorrhagic fever and yellow fever causing millions of deaths every year. Extensive use of chemical insecticides for control of vector borne diseases has created problems related to physiological resistance to vectors, adverse environmental effects, high operational cost and community acceptance. The present study was aimed to determine the biological perspectives and larvicidal potential of the combined effect of *Azadiracta indica* (L.) leaf three solvents extracts with powdered substance of Kungiliyum activity against *C. quinquefasciatus* mosquioto of fourth and fifth instar larvae in laboratory condition. The rate of larval mortality was maximum noticed on ethyl acetate extract combined with kungiliyium powder treated mosquito larvae. Therefore the current research planned to aim the following objectives such as analyse the preliminary phytochemicals, antibacterial activity, examineed the green synthesis of silver nanoparticles Qualitative estimation of protein from the whole-body mass of treated dead mosquito larvae by SDS-PAGE and elucidation of bioactive compounds from better potential extract of leaf from *A. indica* by GCMS technique. Investigated the moratlity rate of *C. quinquefasciatus* mosquito larvae treated with combination of kungilium with three various solvent extracts of *A. indica* leaf. From the present result showed that maximum zone of inhibition against *Bacillus* sp., and *S. marcesence* noticed in ethyl acetate and methanol extract respectively. While the treated dead larvae were subjected in to SDS-PAGE analysis it was clearly showed that total number of protein bands (polypeptides) are maximum eleven bands were noticed in control larvae but other experimental treated larvae possessed minimum number of lowest molecular weighed protein band. Moreover, when treated with ethyl acetate and ethanol subjected dead larvae shows the 34kDa protein bands are thickened intensity was more than other bands. Furthermore, GCMS chromatograph showed fourteen different peak areas at retention time 5.52 to 35.56 minutes. The current study was clearly showed the fourteen different bioactive compounds were elucidated from the ethyl acetate leaf extract, amoung those compounds peak compound such as 3-Heptanol, 3,5-dimethyl- Hence, *A. indica* leaf ethyl acetate extract formulations are relative less toxic, eco-friendly and mosquitoes are unable to develop resistance and may be used as an alternative to other pesticides for control of vector- borne diseases particularly this extract is act as a mosquitocidal mediator.

KEYWORDS: *C. quinquefasciatus*, SDS-PAGE, antibacterial activity, GCMS.

INTRODUCTION

India is holy land which provides shelter to the more than 7500 species of medicinal plants. Plants and their products are safer tolls of treatment against infectious and non-infectious diseases in man and animals as compared to the synthetic's compounds. (EL-mahmood, et al., 2010). Hazard use of synthetic antimicrobial drugs results in multiple drug resistance and evidences of serious adverse effects (Bagavan and Rahuman, 2011). Therefore, natural herbs are gaining an importance in overcoming this problem as the traditional herbs are found to be more economical and having lesser side effects than synthetic drugs (Becker *et al.*, 2003), Mosquitoes are the most important pest, cause several infectious diseases e.g. dengue, chikungunya, malaria, yellow fever, diro filariasis tularemia, and many other diseases. *Culex pipens* are common house mosquito which transmits several infectious diseases from birds to human and human to humans for example west Nile fever, Sindbis fever, lymphatic filariasis, and Japanese encephalitis (Brugman *et al.*, 2018). The diseases transmitted by Cx.pipens cause tens of thousands of deaths annually. Moreover, a majority of these diseases, for instance are not vaccine-preventable in humans. Therefore, pest management remains an indispensable tool in the control and prevention of mosquito-borne infection *A. indica* is one such plant havinf tremendous potential of pharmacological properties. *A. indica* commonly known as Neem is a fast-growing tropical evergreen tree found mainly in India, Africa and America. Neem seeds contain approximately 99 biologically active compounds. Many of these derived products have antifeedancy, ovicidal activity, fecundity suppression besides insect growth regulation and repellancy against insects (Isman, 2006; Locantaoni *et al*., 2006). Neem products are less likely to induce resistance due to their multiple mode of action on insects. In addition to this, insect growth regulatory activity of neem weakens the cutile defence system of the larva. Azadirachtin a biologically active a compound has been promoted as a new insectide that is considered more eco-friendly than synthetic insecticides (Aliero, 2003). The pesticidal efficancy, environmental safety and public acceptability pf neem and its products for control of crop pests and led to its adoption into various mosquito control programmes. Mosquitoes transmit serious human diseases, causing millions of deaths every yea (Alouanir *et al*., 2009). Use of synthetic insecticides to control vector mosquitos has caused physiological resistance and adverse environmental effects in addition to high operational cost (Su and Mulla, 1998). Insecticides of botanical orgin have been reported as useful for control mosquitoes. *A.indica* (meliaceae) and its derived products have shown a variety of insecticidal properties. The present paper confers the larvicidal activity of neem-based biopesticide for the control of mosquitoes. Still no one work have done the similar works so the present study was aimed to determine the biological perspectives and larvicidal potential of the combined formulation of neem leaf three solvents extracts with powdered substance of Kungiliyum activity against *C .quinquefasciatus* mosquioto of fourth and fifth instar larvae in laboratory condition. Still, no.one work has done regarding this kind of work so the current research was framed the following objectives such as investigated the moratlity rate of *C. quinquefasciatus* mosquito larvae treated with combination of kungilium and three various solvent extracts of *A.indica* leaf. Secondly analysed the preliminary phytochemicals and detected the Antibacterial activity of *A. indica* (Lin.) leaf extracts against the pathogenic bacterial species. Examined the green synthesis of silver nanoparticles of solvent and control aspects of *A. indica* leaves Elucidation of bioactive compounds in petroleum ether extract of leaf from *A. indica* by GCMS technique. Finally qualitative estimation of protein from the whole-body mass of treated dead mosquito larvae by SDS-PAGE.

MATERIALS AND METHOD

Collection of Samples

The medicinal plant Neem leaves *A. indica* (A. Juss.) are collected in a plastic bag and cleaned with distilled water. The Mosquito larvaes are collected from stagnated water. Neem is a member of the Mahongy family, Meliaceae.

Preparation of Plant extract

500mg Neem leaves were dried in the shade at the environmental temperature (28-30°C daytime) and grounded in a mechanical mill. The ground mixture was macerate with ethanol, Methanol, Ethyl acetate solvents also mixed with 200mg of Kungiliyum powder was gentle magnetic stirring with during seven days at 25°C. Extract obtained was filtered and concentrated under reduced pressure at 45°C and the residues obtained until for completion of the project.

Phytochemical Analysis

The preliminary phytochemical evaluation of experimental extract revealed that carbohydrate, protein, phenols, tannins, flavonoids, alkaloids, quinones, glycosides, terpenoids, steroids, coumarin, saponin were analysed with some modifications also done in the Ethanolic, methanolic, ethyl acetate and aqueous extracts (Harborne, 1973).

Antibacterial Activity

Pathogenic gram positive and negative bacteria were obtained from MCC BT lab. The bacterial culture was maintained in nutrient broth as turbid growth and kept in refrigerator 4C for further analysis. Antibacterial activity of the extract was studied by the disc diffusion method. Lawns of each organism were prepared on nutrient agar plates. The concentrated plant extract was dissolved in DMSO and were added to sterile filter disc (size 5mm) and allowed the solvent to evaporate after each addition. The discs were placed on air dried surface of the medium. The plates were then intubated at 24hrs at 37C. After incubation the degree of sensitivity is determined the measuring the zone of inhibition if growth around the discs.

Green synthesis by silver nanoparticles

Silver nitrate (Merck India) GR was used to prepare 100ml of 1mM solution of silver nitrate. Then 1ml of silver nitrate is added to 0.5ml of plant extracts. This set up was incubated in dark chamber to minimize photo-activation of silver nitrate at room temperature. The colour change from colourless to brown in colour confirms the reduction of silver ions. The antimicrobial activity of the AgNPs was determined on a Gram-positive bacterium and Gram-negative bacteria by disc diffusion method. Lawns of each organism were prepared on nutrient agar pates. The plant extract which was treated to AgNPs was dissolved in DMSO and were added to sterile filter disc (size 5mm) and allowed the solvent to evaporate after each addition. The discs were placed on air dried surface of the medium. The plates were then incubated at 24hrs at 37°C. After incubation the degree of sensitivity is determined by measuring the zone of inhibition of growth around the discs.

Gas Chromatography-Mass spectrometry (GC-MS) analysis
The GC-MS analysis was carried out using a Clarus 500 Perkin- Elmer (Auto System XL) Gas Chromatograph equipped and coupled to a mass detector Turbo mass gold – Perking Elmer Turbomas 5.2 spectrometer with an Elite-1 (100% Dimethyl ply siloxane), 300 m x 0.25 mm x 1 µm df capillary column. The instrument was set to an initial temperature of 110°C, and maintained at this temperature for 2 min. At the end of this period, the oven temperature was raised up to 280°C, at the rate of an increase of 5°C/min, and maintained for 9 min. Injection port temperature was ensured as 250°C and Helium flow rate as 1 ml/min. The ionization voltage was 70 eV. The samples were injected in split mode as 10:1. Mass Spectral scan range was set at 45-450 (mhz). The chemical constituents were identified by GC-MS. The fragmentation patterns of mass spectra were compared with those stored in the spectrometer database using National Institute of Standards and Technology Mass Spectral database (NIST-MS). The percentage of each component was calculated from relative peak area of each component in the chromatogram. Interpretation of mass spectrum of GC-MS was conducted using the database of National Institute Standard and Technology (NIST) having more than 62,000 patterns. The spectrum of the known component was compared with the spectrum of the known components stored in the NIST.

Sodium dodecyl sulfate–polyacrylamide gel electrophoresis
After treated dead 30 number of larvae whole body content was homogenized with 3 volumes of either 1% SDS (w/v) 1328 or 8 M urea solutions for 1 min using a Polytron. The 1% SDS mixture were boiled at 100•Ž for 2 min, homogenized again for 30 s and subsequently maintained at room temperature for 30 min. The 8 M urea mixture were kept at room temperature for 30 min before centrifuging. And then, all extracts centrifuged at 10,000g for 15 min precipitate was similarly homogenized and centrifuged again. Both supernatants were separately combined and used as 1% SDS or 8 M urea extract 4> The protein concentration was determined by Lowry method. 12) The protein extracts were adjusted with sample buffers to 0.3 mg/ml for Coomassie blue/silver staining. Protein profiling was performed using SDS-PAGE on 8% resolving gel, following the procedure of (Laemmli, 1970). Fifteen microlitres of the prepared sample was separated by electrophoresis at current of 50 mA and 120 V. The broad range protein molecular weight marker (29–205 kDa) (PMWH from Bangalore Genei, India) was used to determine the protein weights. Proteins were fixed by immersing the gels in an aqueous of 10% (v ⁄ v) trichloroacetic acid solution for 1 h and stained overnight in Coomassie Blue stain [0Æ25% (w⁄ v) Coomassie Blue R-250, 50% (v ⁄ v) methanol and 10% (v ⁄ v) acetic acid]. The excess stain was washed out by destaining the gel with a solution of 25% (v ⁄ v) methanol and 10%(v/v) acetic acid. The gel was documented using a Bio Vi s geldoc equipped with a kodak camera. Protein profiles were analyzed using BioVis Gel 1D (Expert Vision Pvt, Ltd, Mumbai, India). The protein molecular weights and RF (relative front) values of different protein bands were noted (Costas, 1992). The results were presented as mean SD and to perform statistical analyses, MS office excel 2016was used. DMRT was performed using the statistical analysis method to detect major variations between treatments and control.

Statistical analysis
The results were presented as mean ± SD, and to perform statistical analyses, MS office Excel 2016 was used. DMRT was performed using the Statistic Analysis Method to detect major variations between treatments and controls.

RESULT
Larvidal activity of treated with kungilium powder combination and three different solvent extract of leaf from *A .indica*
The present study mainly aimed to conduct phytochemicals, antibacterial activity, antioxidant, and green synthesis of nanoparticles of neem leaves (*A. indica*). The obtained were discussed below. The rate of larval mortality was maximum noticed in treated with ethyl acetate extract combined with kungiliyium powder. It was highest range of death rate noticed on both fourth and fifth instar larvae, when compared with other two solvents combined formulations of the experimental extract result represented in Figure-1.

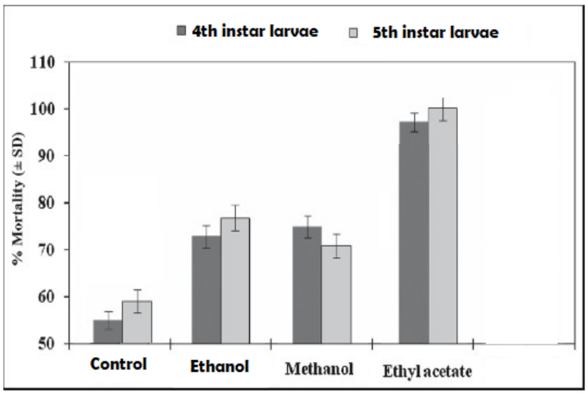

Fig:-1- Larvicidal activity of *A. indica* leaf crude extracts against the fourth and fifth instar larvae of *C. quinquefasciatus* at 100 mg/l. Values are mean ± SD of 5 replicates

Phytochemical Analysis
The preliminary phytochemical screening of twelve different compounds like carbohydrate, protein, phenols, tannins, flavonoids, alkaloids, quinones, glycosides, terpenoids, steroids, coumarin, saponin were tested on

three different solvent extracts of *Azadirachta indica* namely Ethanol, Methanol, Ethyl acetate.

Table 1: Phytochemical analysis of combination with kungilium powder and three different solvent extract of leaf from *A .indica.*

SI.NO	TEST	ETHANOL	METHANOL	ETHYL ACETATE	CONTROL
1.	Carbohydrates	++	--	--	--
2.	Protein	--	--	--	--
3.	Phenol	--	--	--	++
4.	Tannins	++	--	--	++
5.	Flavonoid's	--	++	--	++
6.	Alkaloids	--	--	--	++
7.	Quinone's	--	--	--	--
8.	Glycosides	--	++	--	--
9.	Terpenoids	++	++	--	--
10.	Steroids	++	--	++	++
11.	Coumarin	--	++	--	++
12.	Saponin	--	--	--	--

(++) presence (--) Absence

Table. 1 shows the phytochemical screening of *A. indica* extracts. Five extracts such as Ethanol, Methanol, Ethyl acetate and Aqueous of neem leaves were used for the present study. The phenols are present only in Ethyl acetate extract while steroids are present in three extracts aqueous. The flavonoids are present both aqueous and Ethyl acetate. Coumarin present in control and ethyl acetate. Terpenoids are absent in Methanol and Ethyl acetate extracts. Glycosides are only present in control (aqueous). Saponins, Protein and quinone's are absent in every extracts. Carbohydrate only present in Ethanol extract. In Ethanol extract tannins are present and in Ethyl acetate extract Alkaloids are present. In total, Ethanol extract has carbohydrates, tannins, terpenoids, steroids. The Methanol extract has only steroids and the Ethyl Acetate has Phenols, Tannins, Flavonoids, Alkaloids, Steroids, Coumarin Fig-2.

Control

Ethanol extract

Figure 2: Preliminary phytochemical analysis of combination with kungilium powder and Ethanol, Methanol, Ethyl acetate extracts from the leaf in *A. indica* (L.).

Ethyl Acetate extract

When the three combined experimental extracts namely kungilium mixed Methanol, Ethanol and Ethyl Acetate of *A. indica* are exposed to pathogenic bacterial organisms like, *E. coli*, *Klebsiella sp.*, *Serratia marcescens*, *Salmonella sp.*, *Pseudomonas sp.*, *Streptococcus sp.*, *Bacillus sp.*, and the zone of inhibition represented on Fig-3. From the present result was clearly showed ethyl extract showed that maximum zone of inhibition against *Bacillus* sp., it was significantly higher activity when compared with other experimental extracts such as Methanol and Ethanol. Correspondingly, methanol extract showed that second most antibacterial activity against *S. marcescens*, *Klebsiella sp.* and

Bacillus sp., followed by more or similar zone of inhibition noticed on *Pseudomonas sp*…and *Salmonella sp*...Furthermore, minimum activity has been observed in ethanol extract on two organisms named as *Salmonella sp* and *Pseudomonas sp* Though, least zone of inhibition has been observed in ethanol and ethyl acetate extracts against *Klebsiella sp*., 0.26cm zone of inhibition seen in methanol extract against *Klebsiella sp.* Despite, no zone of inhibition seen in any of the three extracts against *Streptococcus sp.*

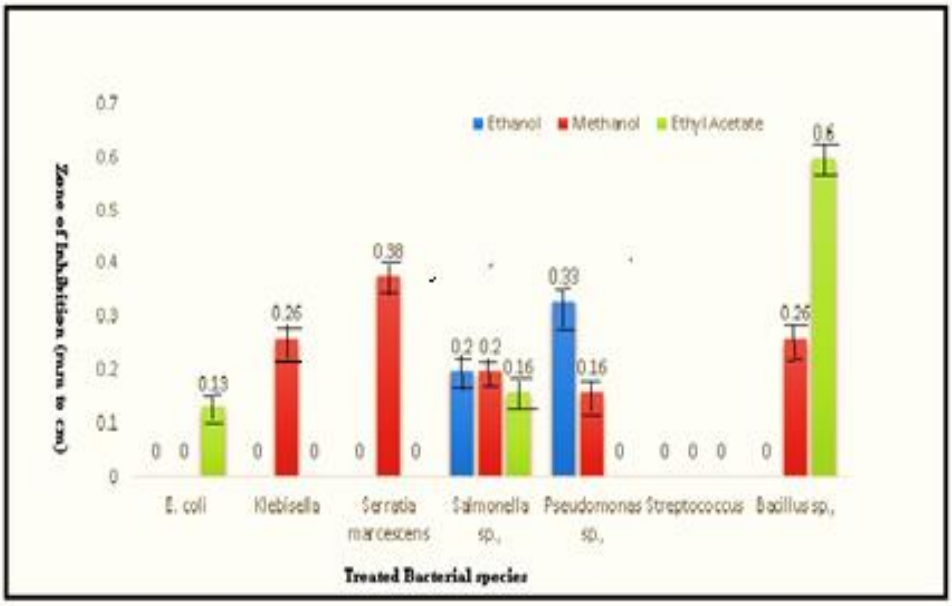

Figure 3: Antimicrobial activity of combination of kungilium with Ethanol, Methanol and Ethyl Acetate extracts of *A. indica* against pathogenic bacteria.

Green Synthesis Of Nanoparticles

The Methanol, Ethanol and Ethyl acetate extract is treated with AgNP'S and checked antimicrobial activity using eight pathogenic microbes.

Figure 4: Production of AgNP'S using with combined experimental extracts.

Antibacterial activity of AgNP'S treated leaf extract of *A. indica* leaves against microbes

In the ethanol extract of *A.indica* AgNP'S treated showed 1.96, 3.4cm of zone of inhibition, an ethanol extract of *A.indica* d in Methanol extract and in ethyl acetate 0.76cm of zone of inhibition is formed against *Klebsiella sp*., 3.73cm zone of inhibition has formed in the extract of Ethanol, 3.6cm in methanol, 0.93 cm in ethyl acetate extract against *Pseudomonas*. In the treatment of extract against *E. coli*, we can see 1.9cm zone of inhibition in ethanol extract, 1cm in methanol, and no formation of zone of inhibition in ethyl acetate extract. And also, zone of inhibition 1.73cm formed in control against *E. coli*. 2.6cm zone of inhibition is seen in ethanol extract, 3.26cm in methanol, 2.1cm in control and no formation in ethyl acetate against *streptococcus sp*., In the experiment against *proteus sp*., we can see 1.2cm of zone of inhibition in ethanol extract, 2.3cm in methanol and 0.76 in ethyl acetate extract. In the ethanol extract it showed 1.4cm zone of inhibition, 2.3cm in methanol extract, 1.3cm in ethyl acetate against 1.3cm zone of inhibition showed in ethanol extract, 1.16cm in methanol, 0.53cm in ethyl acetate and 2.16cm in control against *S. marcescens*. In the ethanol extract against *Salmonella* 1.46cm seen, 0.63cm in methanol, 1.4cm in control and no zone of inhibition formed in ethyl acetate. The tabulated zone of inhibition is presented in bar diagram (fig.-3).

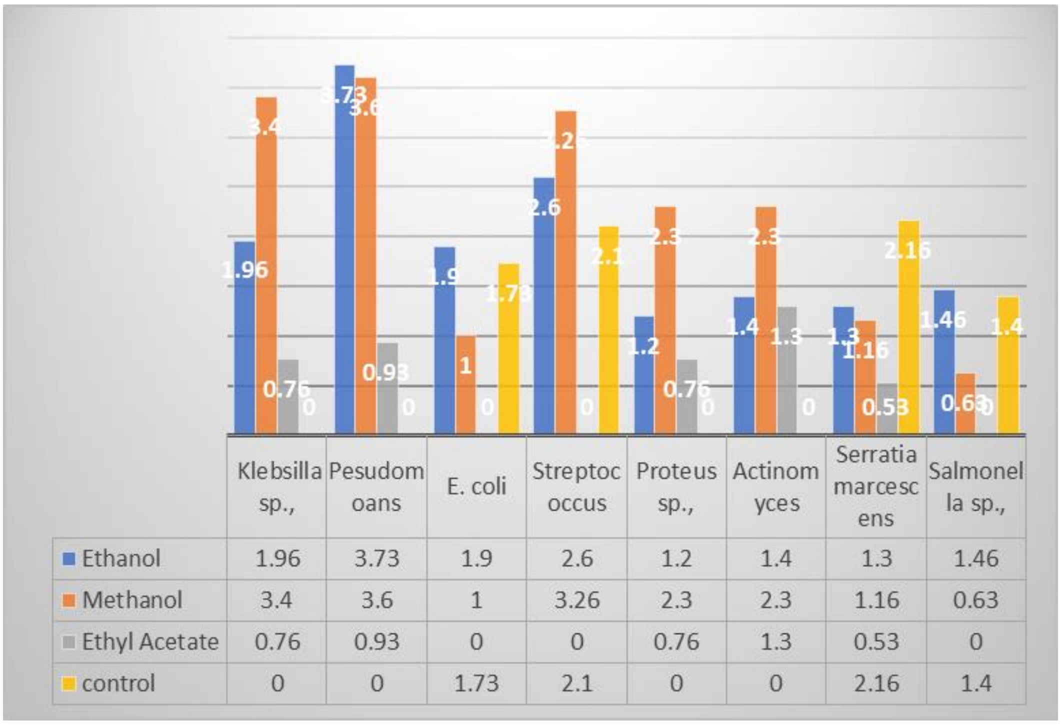

Figure 4: Antimicrobial activity of AgNPS treated extracts against pathogenic microbes.

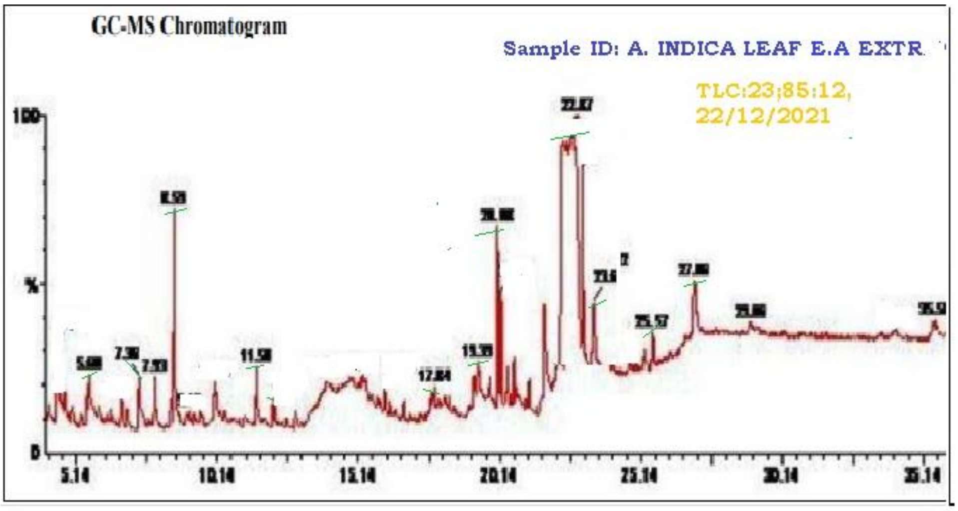

Figure 5: Elucidation of bioactive compounds in petroleum ether extract of leaf from *A. indica* by GCMS technique.

In the present study, GC-MS chromatogram shows the presence of fourteen different peaks which confirm the presence of 14 compounds along with their respective RT From the experimental extract of ethyl acetate also combined with kungilium powdered substance. The spectra of the compounds were matched with Wiley 8.0 and NIST libraries. The identified compounds, their retention time (RT), molecular weight, molecular formulae, and percentage composition (%area) are given in Table 2. The individual fragmentation of major components is illustrated in Figure-5. GC-MS Analysis The chromatograph of methanol extract of leaves of A. indica showed different area peaks at different retention time. The chromatograph showed 14 different peak areas at retention tim 5 5e 5.52 to 35.56 minutes. The current study was clearly showed the fourteen different bioactive compounds were elucidated from the ethyl acetate leaf extract through a GCMS technique, amoung those compounds peak compound such as 3-Heptanol, 3,5-dimethyl- followed by other subsequent compound named as 1,4-Benzenediol, 2,6-dimethyl along with other six different bioactive compounds are also separated those are all the compounds are better potential activity of mosquito larvicidal as well as the antibacerial efficiency more than other compound.

Table 2: Identified Bioactive constituents from kunjilium mixed with *A. indica* leaf extract (Ethyl acetate) by GC-MS.

S.No.	Peak name	Retention time	Peak Area	Formula:	MW
1.	2,4-Dihydroxy-2,5-dimethyl3(2H)-furan-3-one:	5.52	0.457	$C_6H_8O_4$	144
2.	4-Decenoic acid, 3-methyl-, (E)-	4.14	0.6345	$C_{11}H_{20}O_2$	184
3.	3-Amino-2-oxazolidinone	8.47	0.453	$C_3H_6N_2O_2$	102
4.	Butanamide Formula: MW:	9.85	0.2036	C_4H_9NO	87
5.	1,5-Diazabicyclo [4.4.0] dec-5- en-2-one	13.10	0.0791	$C_8H_{12}N_2O$	152
6.	2(4H)-Benzo furanone, 5,6,7,7atetrahydro-4,4,7a-trimethylFormula	15.86	0.2709	$C_{11}H_{16}O_2$	180
7.	N-Methylpyrrole-2-carboxylic Acid	18.49	0.1048	$C_6H_7NO_2$	125
8.	D-Glucopyranoside, à-D- glucopyranosyl	26.75		$C_{12}H_{22}O_{11}$	342
9.	3-Heptanol, 3,5-dimethyl-	23.61	4.6519	$C_9H_{20}O$	144
10.	1,4-Benzenediol, 2,6-dimethyl:	25.23	5.5321	$C_8H_{10}O_2$	138
11.	of 3, 7, 11, 15- tetramethyl-2-hexadecen-1-ol	40.90	0.0945	$C_7H_{11}NO_3$	157
12.	à-D-Glucopyranoside, à-D- glucopyranosyl Formula: MW:	60.5648	2.0087	$C_{12}H_{22}O_{11}$	342
13.	Bicyclo[4.4.0]dec-2-ene-4-ol, 2-methyl-9- (prop1-en-3-ol-2-yl)- Formula:	34.16	1.0618	$C_{15}H_{24}O_2$	236
14.	5-Isopropyl-3,3-dimethyl-2- methylene-2,3-dihydrofuran	35.56	1.7680	$C_{10}H_{16}O$	152

When the fifth instar larvae have been treated with three different experimental extracts, ethyl acetate solvent extract expressed the larval mortality was maximum, so further experiment was assessed in the dead larvae whole mass for protein qualification as well a as quantification. From the current result clearly indicated that the macromolecule of protein altered the reason for mortality of the mosquito larvae. While the treated dead larvae were subjected in to SDS-PAGE analysis it was clearly showed that total number of protein bands (polypeptides) are maximum 11 bands were noticed in control larvae but other experimental treated larvae possessed minimum number of lowest molecular weighed protein band. Moreover, when treated with ethyl acetate and ethanol subjected dead larvae shows the 34 Kda protein bands are thickened intensity was more than other bands also remaining major high molecular weighed protein bands also been disappeared. From the present result clearly expressed larval mortaliy manly occurred due to protein molecular alteration effect with the bioactive compounds present in the ethyl acetate experimental extract (Figure-6).

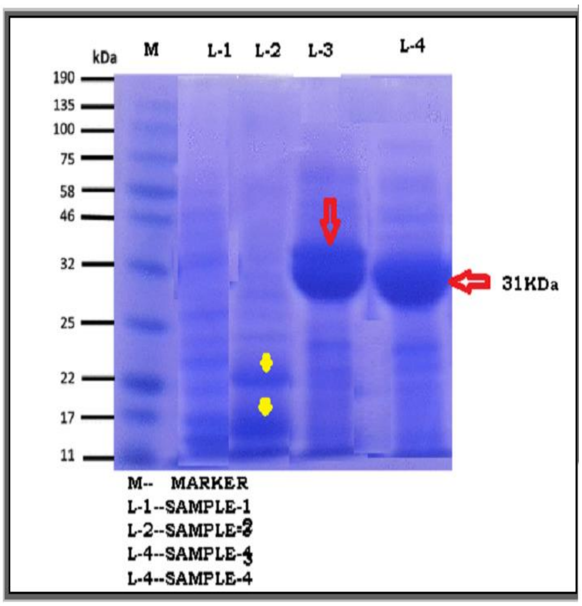

Figure 6: Qualitative Analysis of SDS Protein Profile of combined form of extract with Kungilium Treated Mosquito Larvae.

DISCUSSION

Neem trees are found throughout India with a myriad of uses in medicine, as well as pest control (National research Council, 1992). Neem-based pesticides are now extensively used in agriculture practices all over the world. It contains azadirachtin, which is a predominant insecticidal active ingredient, having antifeedant, ovipositional deterrence repellence Abalaka *et al*., 2012), growth disruption, sterility and larvicidal action against insects (Amer and Mehlhorn, 2006). There are various reports of control of mosquito breeding under field conditions. An emulsion of neem oil in water was found to be effective in controlling breeding of *C. quinquefasciatus* (Sukumar et al., 1991; Kumar *et al*. 2018). Where an application of neem cake powder resulted in drastic reduction in the late instar larve and pupae of culicidae mosquiotoes in paddy filed. Previously several reseachers also had been opined the various bioactive compounds elucidated through a GCMS anlaysis (Collins and Paskewitz, 1995; Batra *et al.,* 1998; Cowan, 1999; Chung *et al*., 2016). Moreover, chromatogram of the ethyl acetate extract of *A. indica* showed 14 major peaks and have been identified after comparison of the mass spectra with NIST library, indicating the presence of 3,7,11,15 – tetramethyl-phytocomponents (Abalaka et al., 2012;). From, the results, it was observed that presence of 3,7,11,15- 2-hexadeen- 1- ol (synonym: Phytol), 9,12,15 – Octadecatrienoic acid (synonym: Linolenic acid; α-Linclenic acid), 8,11,14- Eicosatrienoic acid (synonym: Homo-γ-Linolenic acid), Hexadecanoic acid (synoym: palmitic acid) and Tridecanoic acid (synonym: Tridecylic acid) were the major components in the extract. The phytochemicals that contribute to the medicinal property such as phytol is reported to have antioxidant, antiallergic (De-Fathima *et al*., 2006) and anti-inflammatory activities (Arunkumar and Muthuselvam, 2009). Phytol has also shown antimicrobial activity against Mycobacterium tuberculosis and various pathogenic bacterial organisms. Previusly similar opinion also proposed by (Amer *et al*., 2017) such as 4-benzenediol, 2,6-dimethyl is known for its potential antibacterial, antifungal. No significant different of larvicidal activity of the formulation was also observes during 18 months storage period at room temperature. Neem-based combined extracts have a wide range of effects against insect pests including repellence (Mittal and Subbarao, 2003), feeding, toxicity, sterility and growth regulator activity and are relatively safe towards non- target biota with only minimal risk of direct adverse effects on aquatic biota from contamination of water bodies (Kreutzweiser, 1997; Goektepe *et al.*, 2004; Gajmer *et al*., 2018). Allelochemicals such as azadirachtin, nimbin, nimbidin, nimbolides, nimolic acid, salannin, melianttriol, azadirachtol present in neem affect the biochemical and physiological processes of insect system and nullify the insect detoxification mechanism thereby not allowing the pest to develop resistance (Gajmer *et al*., 2002). As an emulsifiable concentrate, the neem oil formulation had greatly reduced sized particles and evenly mixed within the water column with a few suspended particles on the water surface (Gunasekaran et al., 2013). The spread of these fine particles probably increased the efficacy of formulation. These ezymes were find down regulated too in the larvae of *C. quinquefasciatus* mosquitoes exposed to temephos.

CONCLUSION

The neem leaf extract was found effective in controlling mosquito larvae in different breeding sites under natural field conditions. As neem trees are widely distributed in India, their formulations may prove to be an effective and eco-friendly larvicide, which could be used as an alternative for malaria control. The present study was clearly showed that amoung the three solvents named as ethanol, methanol and ethyl acetate extracts of *A*, *indica* leaf maximum mortality rate has been noticed in kunjilium mixed with ethyl aetate extract on both larval stages such as fourth and fifth instar larvae of *C. quinquefasciatus* mosquito. Furthermore, antibacterial activity also been observed in the same extract against the tested pathogenic organisms. Basic conclusion of the current study was clearly showed the fourteen different bioactive compounds were elucidated from the ethyl acetate leaf extract through a GCMS technique, amoung those compounds peak compound such as 3-Heptanol, 3,5-dimethyl- followed by other subsequent compound named as 1,4-Benzenediol, 2,6-dimethyl along with other six different bioactive compounds are also separated those are all the compounds are better potential activity of mosquito larvicidal as well as the antibacerial efficiency more than other compounds. Hence the present study showed that *A. indica* leaf ethyl acetate extract was probable promising mosquitocidal also antibacterial agent. Hence, combined action of kunjilium powder with *A. indica* leaf ethyl acetate extract formulations are relative less toxic, eco-friendly and mosquitoes are unable to develop resistance and may be used as an alternative to other pesticides for control the vector- borne diseases particularly this extract was act as a mosquitocidal mediator.

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