ANTIBACTERIAL, ANTI OXIDANT, LARVICIDIL ACTIVITY OF SEAWEED EXTRACT (TURBINERIA ORNATA) AGAINST BACTERIAL ISOLATES FROM DIABETIC FOOT ULCER

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ABSTRACT

To investigate and evaluate the antibacterial activity, larvicidal activity, and antioxidant of methanol, chloroform, diethyl ether extracts of brown seaweed *Turbineria ornate* (*phaephyceae)* from Mandapam coastal region of Rameshwaram. The seaweed was extracted by two types methods namely soxhlet extraction and crude extraction. The extracts of *Turbineria ornate* were tested for their antibacterial activity against five multidrug resistant both gram positive and gram negative bacterial isolates from diabetic foot ulcers. The highest inhibition activity among the two types of extracts is obtained with the *Turbineria orneta* in soxhlet MeOH extract showed a zone of inhibition in maximum of 16 mm against *Bacillus* sp., and *E. coli* whereas a minimum of 13 mm was observed in *Pseudomonas aeroginosa* in 60 µg ml^{-1}. The larvicidal activity of methanol, chloroform, and diethyl ether extracts against early 4^{th} in star larva of *Aedes agypti* by using WHO standard values and the concentration of 200, 400,600, 800, 1000ppm. The mortality of larva was made 24hrs and 48hrs after treated with seaweed extraction. Among the seaweed extract, the methanol showed highest mortality of larvae with LC_{50} value 54.2±1.09 in 1000ppm. The functional group of *Turbineria ornate* was characterized by FT-IR, it has carboxyl bond, uranic acid, sulfate groups, and glycosidic linkages. The antioxidant activity was carried by free radical scavenging DPPH method standard with ascorbic acid, the methanol extract was 88.85±13.42. *Turbineria ornate* also has tannin, polyphenol, glycosides, saponins. It was done by phytochemical analysis. Altogether, these results confirms the seaweed have strong antibacterial, larvicidal activity .

Keywords:- *Turbineria ornate*, *Aedes agypti*, antibacterial, larvicidal activity,Antioxidant activity.

INTRODUCTION

Diabetes is a chronic disease that the insulin level increase in the blood which is known as diabetes mellitus(DM). It is otherwise termed as glucose metabolism disease. The chronic hyperglycemia which are results from insulin secretion, insulin action or both[(1)].DM was one of the oldest diseases. Before 3000 years ago it was first reported by Egyptian[(2)]. Diabetes posses a great threat to human health and governments faced a huge socioeconomic burden. The international diabetes federation (IDF) has updated the data of diabetes mellitus reaching 8.8% in 2015 and the global health expenditure was 12% due to diabetes mellitus in that same year[(3)].

Diabetes mellitus posses a big economic burden to health system costs, indirect costs arising from losses occasioned by patient disability and premature mortality, time spent by family members accompanying patients when seeking care, and intangible costs in terms of psychological pain to the family and loved ones. The one of the major health challenges to economical development was the growing burden of diabetes and other non communicable diseases[(4)]. The increasing coagulation, endothelial dysfunction, impaired fibrinolysis and hyper reactivity are the factors that enhancing the prothrombotic condition of patients with DM[(5)].

The diabetic mellitus also cause foot ulcer that is an open sore or wound which occurs in the 15% of patients with diabetes and is commonly located on the bottom of the foot. Nearly 6% of the foot ulcered patient will be hospitalized due to infection. The lack of feeling in the foot, irritation (such as friction or pressure), poor circulation, and trauma, foot deformitiesas well as duration of diabetes are the factors that causes foot ulcers. Patients who have diabetes for many years can develop neuropathy, a reduced or complete lack of ability to feel pain in the feet due to nerve damage caused by elevated blood glucose levels over time. The nerve damage often can occur without pain, and one may not even be aware of the problem. The foot ulcers also has organism which causing chronic diabetic foot ulcers were commonly multidrug-resistant. This was also observed among biofilm formers. So In this study that the biofilm organism were inhibited by *turbineria ornate* extraction which is known as seaweed.

Mosquitoes are blood sucking insects, which cause numerous diseases to millions of people throughout the world[(6)]. Today mosquito plays an important role for the transmission of dengue, yellow fever, malaria, filariasis and other several disease which are today among the greatest health problems in the world[(7)]. The mosquitoes act as a vector which transmits the parasites and skin allergy as well as diseases to human through their bites. There are 350–500 million clinical cases of malaria per year with about one million deaths. Especially in India, every year two million malaria cases are being reported[(8)].

The four serotypes of dengue arbovirus causes the dengue fever which are clinically can happen in classic dengue fever, asymptomatic forms, hemorrhagic dengue fever and other more severe forms. Worldwide, 2.5 billion of people are in risk to acquire the disease and 50 million are infected every year, characterizing a pandemia[(9)]. Mosquito can causes chikungunya, a severe viral disease which has been recently considered to be an important public health problem in India and in other countries like Senegal and West Africa[(10)].

There are more than 3,500 species of mosquitoes exists.*Anopheles*, *Aedes* and *Culex* are the members of three genera of mosquito which are the leading causers of mortality and morbidity in humans[(11)]. In WHO guidelines it is addressed that over one million people worldwide die due to mosquito-borne disease.Dengue fever is prevalent throughout the tropics and subtropics. Dengue is the most significant viral disease spread by mosquitoes and a major international public health concern. The present resurgence of these diseases is due to the higher number of breeding places in today's throwaway society. Further, the indiscriminate use of synthetic insecticides is creating multifarious problems such as environmental pollution, insecticide resistance and toxic hazardous to humanbeings[(12)].

Organochlorine, organophosphourous, carbamates, pyrethrins and pyrethroids are the commonly used Synthetic insecticides which are basically for the controlling of ever increasing population of vectors. These chemical insecticides cannot be overused as it is not safer due to environment hazard and the non target organisms has resulted in resistant development[(13)]. The emergence of insecticide resistance in targeted vectors[(14)], as well as their damaging on non-target organisms and environment, increase an urgent search of new and better mosquito control methods that are economical and efficient as well as safe for non-target organisms and the environment[(15)].

II. MATERIALS AND METHODS

EXTRACT PREPARATION

The 500 g of pulverized moisture free/dried seaweed was extracted in 1 L with the same volume of different organic solvents (v/v) with increasing polarity like di-ethyl ether (DEE), chloroform ($CHCl_3$) and methanol (MeOH) were added to obtained the natural concentration (soaking extractor for 15 days) of seaweeds and the other method in seaweeds were extracted by soxhlet extractor for 24 hours at 60°C.The total extract was filtered and the obtained filtrate (crude extract) was concentrated in a rotary evaporator at 40°C for dry. And the dried extract was dissolved in 100% of 20 mL (v/v) of the same solvent and stored in vials for further studies.

ROTATORY EVAPORATED METHOD

The filtration of seaweed extract was concentrated by rotatory evaporation method to evaporate the solvent. The concentrated extracts were dissolved in 1ml of same solvent and tested for further studies. The concentrated extracts were characterized by the technique of FT-IR.

SOXHLET METHOD

The 20g of crushed seaweed was taken into the soxhlet flask of soxhlet apparatus. 100ml of respective solvent was taken intio the round bottom flask. The running tap water is allowed into the cooling jacket of the soxhlet apparatus. The respective temperature was ginven to the soxhlet apparatus. the extract was extracted untill 6 hours.

ISOLATION OF DIABETIC WOUND ORGANISM

The diabetic foot wound infected were scrapped using sterile cotton swabs and immediately transferred to culture tubes .From the mixture (diabetic foot wound forming bacteria isolates + sterile double distilled water), bacterial strains were isolated and enumerated by pure culture techniques (spread plating method - 0.1 ml) on selective medium plates. Nutrient medium was prepared with sterile distilled water at 121^0C. Bacterial parameters were characterized and listed in Table 1. The average bacterial counts of the replicates were noted and the mean values were recorded. Morphologically dissimilar, distinct colonies were randomly selected and inoculated into rapid microbial limit test kits (Hi-media Laboratories Limited, India) for identification of bacterial strains.

ANTI-BACTERIAL SCREENING

In-vitro anti bacterial sensitivity assays were carried out using the well diffusion method to the test samples (three different solvents and two different extraction methods) against certain isolated diabetic foot wound forming bacteria plated on a Muller Hinton Agar (MHA) medium. A sterile cotton swab was used to inoculate the standardized bacterial suspensions (test culture suspensions prepared in sterile 0.85% saline, matching an optical density of 0.5 McFarland standards corresponding to 10^8 CFUs ml^{-1}) on the surface of agar plates for homogeneous growth. Then 20, 40, and 60 μg ml^{-1} were loaded in each well. The plates were then incubated at 37 ± 2 ^{0}C for 24–48 hrs. After incubation, the zone of inhibition was measured with a ruler/Hi Antibiotic Zone Scale-C. The assays were performed in triplicate and the average values were presented. All media, standard disks and Hi Antibiotic Zone Scale-C were purchased from Hi-Media (Mumbai, India).

SCREENING OF LARVICIDAL ACTIVITY

The 4th in star larvae were used in the study. The larvicidal activity was observed as per the standard procedures recommended. The seaweeds extracts were dissolved in 2 ml of methanol solvent and prepared into different concentrations viz., 200, 400, 600, 800 and 1000 ppm were prepared with distilled water. Ten larvae (in a 100 ml beaker) of early fourth instar stage were used for larvicidal assay and five replicates were maintained for each concentrations. Then it was incubated at 37^0C for 24 hours. The larval mortality was calculated after 24 and 48 hours of the exposure period. All moribund mosquito larvae were considered as dead.

ANTIOXIDANT ACTIVITY

Free radical scavenging activity of different extracts of seaweed *Turbineria ornate*. The seaweed extract were measured by 1, 1diphenyl-2picryl hydrazyl (DPPH). In brief, 0.1mm solution of DPPH in methanol was prepared. This solution (1ml) was assayed to 3ml of different extracts in methanol at different concentrations (100, 200, 300, 400, 500μg/ml). Here, only those extract were used which are solubilize in methanol and their various concentrations were prepared by dilution method. The mixture was shaken vigorously and allowed to stand at room temperature for 30 minutes then, absorbance was measured at 517nm by using spectrophotometer (UV-VIS Shimadzu).

Reference standard compound being used was ascorbic acid and experiment was done in triplicate. The IC50 value of the sample, which is the concentration of sample required to inhibit 50% of the DPPH free radical, was calculated using Log dose inhibition curve. Lower absorbance of the reaction mixture indicated higher free radical activity. The percentage of DPPH scavenging effect was calculated by using following equation, DPPH scavenging effect(%) or Percentage of inhibition = $A_0 - A_1 / A_0 \times 100$ where A0 was the absorbance of control reaction and A1 was the absorbance in presence of test ore standard sample.

III. RESULTS

ISOLATION AND IDENTIFICATION OF BACTERIA

Fifteen colonies were isolated from diabetic foot wound patient; five morphologically distinct strains were separated and cultured for biochemical analysis. Nutrient and selective media plates were prepared with the addition of sterilized double distilled water. Bacterial parameters were characterized and listed. Average bacterial counts of the replicates were noted and the mean values were recorded. Characterization and identification of bacteria were done based on the results of morphologically dissimilar and distinct colonies. These were randomly selected and inoculated into rapid microbial limit test kits (Hi-media Laboratories Limited, India) for the identification of the bacterial strains in Table 1.

The biochemical analysis is known as preliminary test for the identification of the organism. In this present study that few biochemical analysis were carried out for the morphologically selected organism such as Indole, MR, VP, citrate, oxidase Catalase and the results were noted in Table 1

Table 1: Morphological characteristics of organisms

Test	Isolate 4	Isolate 5	Isolate 6	Isolate 1	Isolate 4a
Gram staining	+	-	+	-	-
Shape	Rod	Rod	Rod	Rod	Rod
Medium	NA	PIA	BLA	EMB	XLD
Colony colony	White	Green	Pink	Metallic green	Black
Indole test	-	-	-	+	-
Methyl red	-	-	-	+	+
VP test	-	-	-	-	+
Citrate test	-	+	-	-	-
TSI	K/A K/K	A/K K/A	K/A K/K	K/A K/K	K/A K/K
Oxidase	-	+	-	-	-
Catalase	+	+	-	+	-

ANTI-BACTERIAL ACTIVITY BY SOAKED EXTRACT

The Bioassay-guided extract was used in order to isolate antibacterial compounds from brown seaweed *Turbineria orneta*. The fractions that were collected in every stage of purification were subjected to anti-bacterial study. Antibacterial efficacy of soaked DEE extract of *Turbineria orneta* showed a zone of inhibition is maximum of 13 mm against *Escherichia coli*, and a minimum of 10 mm was observed in *Pseudomonas aeroginosa* in 60 µg ml^{-1}. (table 2) The Effective antibacterial activate solvents against diabetic foot wound forming bacteria were observed in the following order: MeOH >$CHCl_3$ = DEE

ANTI-BACTERIAL ACTIVITY BY SOXHLET METHOD

Antimicrobial efficacy of *Turbineria orneta*in soxhlet MeOH extract showed a zone of inhibition in maximum of 16 mm against *Bacillus* sp., and *E. coli*whereas a minimum of 13 mm was observed in *Pseudomonas aeroginosa* in 60 µg ml^{-1}. Maximum antibacterial activities against wound forming bacteria were in the following order: MeOH > CHCl3 > DEE in Tables 3. The content of bioactive compounds in the *Turbineria ornate* extract might account for the different results found in their anti-bacterial activity. The antibacterial screening of phenolic compounds extracted from soxhlet MeOH extract of *Turbineria ornata*are known to bind with thiol groups of DNA and RNA and affect the protein biosynthesis of bacteria. Studies have demonstrated that bioactive compounds interact with sulfhydryl (SH) groups of proteins as well as the bases of DNA leading either to respiratory inhibition or the unwinding of DNA.

LARVICIDAL ACTIVITY:

The larvicidal activity of different solvents viz., methanol, chloroform, diethyl ether against *A. aegypti* and the results were noted. (Table 4 which shows that in case of 4th instar of *A. aegypti* 1000 ppm concentration exhibits mortality at 24 and 48 hours for 200, 400, 600, 800 and 1000 ppm concentration). The larvicidal mortality rate which increase with time of exposure (24 h>48 h). Table reveals significant difference in larvicidal mortality of log probit analysis at 95% confidence lc50 and lc90.(Table 4). The high mortality of larve from methanolic extract in 54.2±1.09.

ANTIOXIDANT ACTIVITY

The total antioxidant capacity of *T. ornata* was measured by DPPH free radical scavenging method. The antioxidant activities increase with increasing concentration of the sample. At the concentration of 1 000mg/mL, the crude extract of *T. ornata* exhibited higher antioxidant activity [(88.85± 13.42) %] as compared with the standard, ascorbic acid.(Table 5).

FT-IR ANALYSIS

The extraction of *T.ornata* by three different solvents are analysed by FT-IR. The series of absorption peaks from 600 to 4000 cm^{-1} can be found. The methanolic extract of *T.ornata* showed at bands 3688.60, 2825.48, 1868.18, 1486.01, 1190.10, 904.50, and 715.35 cm^{-1}(Figure 1). The DEE extracts of *T.ornata* showed bands at 3672.40, 2881.05, 1736.90, 1425.48, 1369.95, 1231.07, 1092.76, 979.23, and 849.49 cm^{-1} (Figure 2) the chloroform extracts of *T.ornata* showed bands at 3674.36, 2987.14, 1729.93, 1491.31, 1181.01, 1025.86, 978.53 and 754.75cm^{-1}(Figure 3). The FT-IR spectra of *T.ornata* showed bands characteristic of glycosidic linkages, sulfates and uronic acids. The band between 1230-1290cm^{-1} is represents S=O stretching vibration of the sulfate group[16]. The peaks between 3674.76-1425.48cm^{-1} represents the O-H, C-H, groups. In addition, the signals close to 1609 and 1420 cm1 were due to the asymmetric and symmetric stretch vibration of COO of uronic acid[17][18]. Based on the FT-IR spectrum that the *T. ornate* is acidic in nature.(Table 6)

Table 2 Anti-bacterial Activity by using soaking method of seaweed extraction with different solvents

Organism	Seaweed Extraction 60 µg ml^{-1}		
	Methanol (MeOH)	chloroform (CHCl3)	di-ethyl ether (DEE)
Bacillus sp.	8	7	12
Pseudomonas aeroginosa	8	6	10
Corynebacterium sp.	10	11	12
Escherichia coli	10	12	13
Salmonella sp.	-	-	11

Table 3 antibacterial activity (soxhlet apparatus extraction)

Organism	Seaweed Extraction 60 µg ml^{-1}		
	Methanol (MeOH)	chloroform (CHCl3)	di-ethyl ether (DEE)
Bacillus sp.	16	7	7
Pseudomonas sp.	13	6	7
Corynebacterium sp.	14	11	9
Escherichia coli	16	12	8
Salmonella sp.	14	7	6

Table 4: larvicidal activity of methanol extract of seaweed

Seaweeds	seaweeds prepared with different solvent	% Mortality (Concentration of ppm)				
		200	400	600	800	1000
Turbineria sornate	DEE	5.2±1.09	9.2±1.78	17.4±0.57	32.8±1.48	41.2±1.09
	Chloroform	7.8±0.83	13.2±0.4	24.2±1.09	36.4±0.57	49.6±0.54
	Methanols	11.0±1.22	19.4±1.64	34.6±0.83	46.0±0.89	54.2±1.09

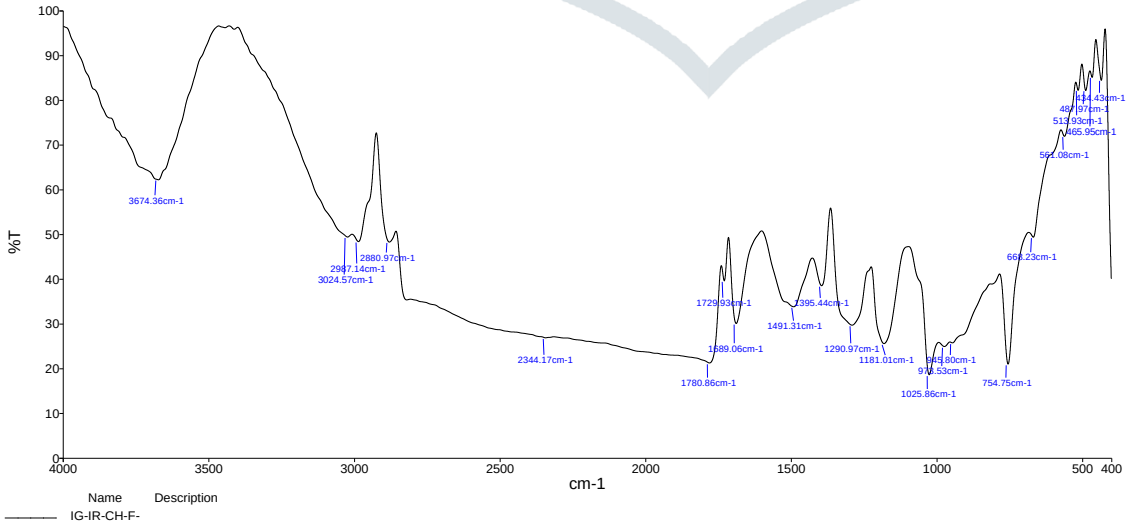

Figure 1 FT-IR analysis for chloroform

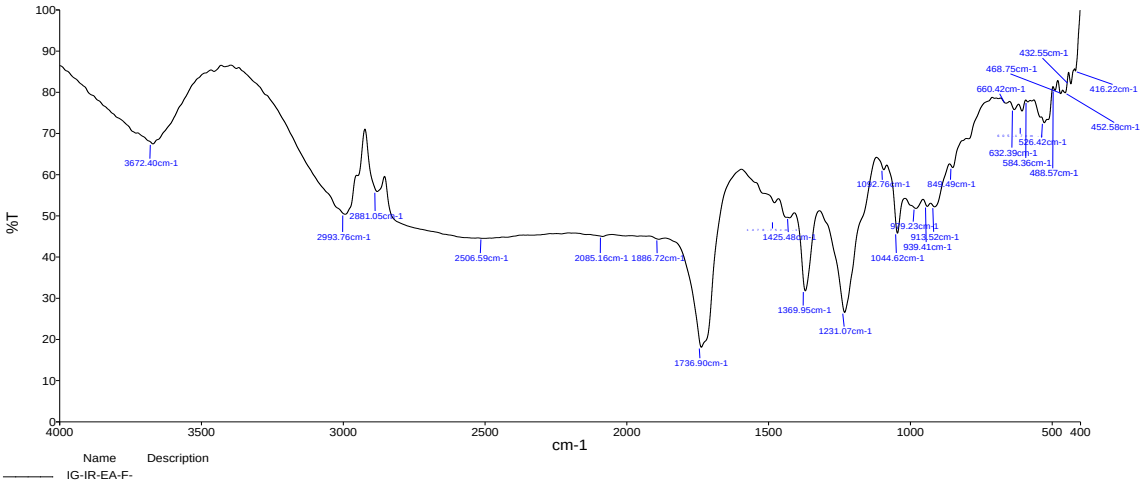

Figure : 2 FT-IR analysis for diethyl ether

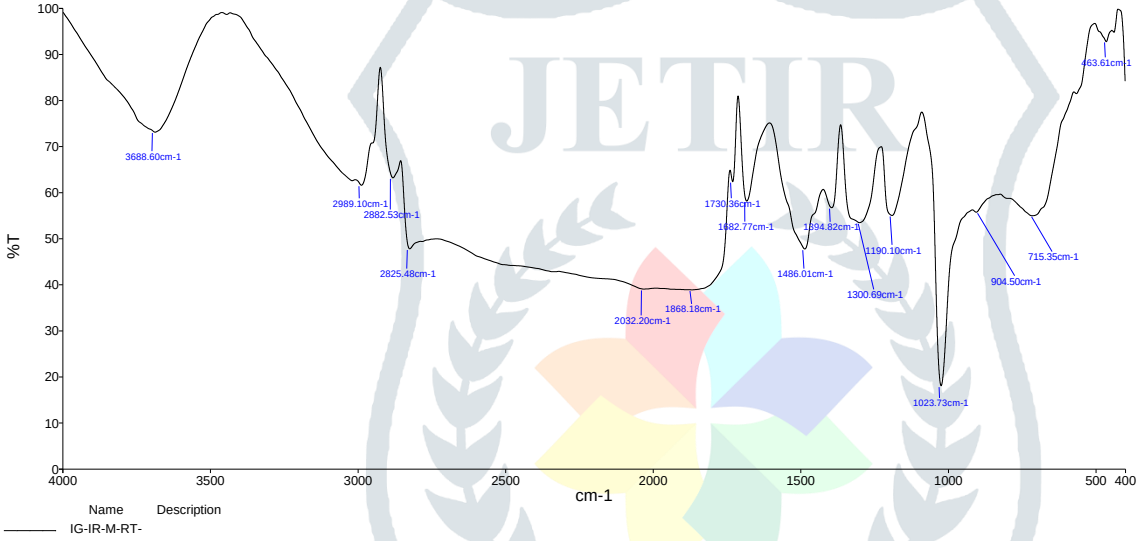

Figure : 3 FT-IR analysis for methanol

Wavelength (cm^{-1})	Characterization of functional group of *T.ornata*
3923-1640	O-H, C-H, O-C-O and C-OH bonds
1609-1433	Uranic acid
1240-1260	Stretch of sulfate groups
1139-1036	Acidic polysaccharides
899.61-910.24	Glycosidic linkage
819.75-836.20	Sulfate groups

TABL:6 FT-IR analysis of methanol extract of seaweed

IV. DISCUSSION

The marine environment representing approximately half of the global biodiversity is an enormous resource for new compounds. Seaweeds are rich sources of bioactive compounds that helps in the develpements of new pharmaceutical products[19]. Many studies were reported about the biological activities of algal extracts from different cregions around the world[20]. In the present study, the different solvents viz., methanol, chloroform, and diaethyl ether of *Turbineria orneta* possessed antibacterial activity against all the clinical and standard bacterial strains tested. The methanol extract of *Turbineria orneta* showed the highest antibacterial activity than other extracts against *B. subtilis* and followed by all bacterial strains tested. The extracts were tested against gram positive and gram negative bacteria in agar well diffusion. The seaweed *Turbineria orneta* are inhibit the growth of both gram positive and gram negative organism from wound of diabetic patient.

Though synthetic insecticides are effective they create many problems like development of insecticide resistance[21]. Therefore, usage of indigenous plant based products, could provide standardized measure for protection to the human population against various disease caused by mosquito. Many approaches that have been developed to control the mosquito menace. One such approach to prevent mosquitoborne disease is to kill at its larval stage. Many studies made use of plant extracts for mosquito control approach. The crude extracts of plants by using different solvents have potential larvicidal activity[22].

To evaluate the potential larvicidal activity of the plant preliminary screening is a good measure[23]. Insecticides of botanical origin may serve as suitable alternative biocontrol techniques in the future[24]. The larval control can be the effective appropriate way in controlling the mosquitoes in breeding habitats, which are man-made[25]. The early findings the effect of ethanol extract of Annona squamosa leaf was effective in larvicidal activity against *C. quinquefasciatus*[26].

The results of the present study also confirm similar results with methanol extracts of *Turbinariaorneta*. The methanol extracts of *T.orneta* show higher larvicidal activity against the fourth instar larvae of Aedes aegypti[27]. The extracts of *Jatropha. curcas* and *Euphorbia tirucalli* were highly effective against the larvae of *Aedes aegypti*, and the LC50 values were 35.39, 256.77, 384.19, 703.76, and 13.14 ppm against *Aedes aegypti*[28].

V. CONCLUSION

Based on the elaborative experiment and detailed observative study, the following points were derived. The wound bacterial strains isolated from diabetic foot woundpredominantly belonged to the Gram-positive compare Gram-negative. This study suggested that, among the two methods, soxhlet MeOH extract of *Turbineria orneta* possesses high antioxidant and antibacterial activity which might be helpful in preventing or slowing the progress of various oxidative stress related disorders. Moreover, anti-bacterial activity against *Bacillus* sp., *Pseudomonas aeroginosa*, *Corynebacterium* sp., *Escherichia colia* and *Salmonella* sp., wound pathogen. Therefore it can be concluded that antibacterial efficacy performed against wound pathogen a maximum inhibition growth 16 mm against *Bacillus* sp., and *E. coli*whereas a minimum of 13 mm was observed in *Pseudomonas aeroginosa* at 60 μg ml^{-1}. There are few reports on the antioxidant capacity of seaweeds were analysed at 88% and the *Turbineria ornate* extracts were responsible to larvicidal activity. Hence further research is underway to analyse and isolate the active compounds responsible for the antioxidant and antimicrobial activity from the seaweeds.

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