LARVICIDAL AND ANTIMICROBIAL PROPERTIES OF SELECTED PLANTS FROM UPPER KUTTANAD; AN ECOLOGICALLY FRAGILE AREA OF SOUTH INDIA

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FINAL REPORT

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Submitted by

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DECLARATION

I do hereby declare that the UGC-Minor Research Project **(1889-MRP/14-15/KLMG019/UGC-SWRO, dated 04-Feb-2015)** entitled '**Larvicidal and Antimicrobial Properties of Selected Plants from Upper Kuttanad; an Ecologically Fragile Area of South India' is** an authentic record of the project work carried out by me in the Department of Zoology, St. Aloysius College, Edathua, during the year 2015- 2017. Further certify that the work presented in the report is original and carried out according to the plan in the proposal and guidelines of the University Grants Commission.

Edathua,

April 2018**.** **Dr. Shibu George**

(Principal Investigator)

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Dr. Shibu George

Principal Investigator

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LARVICIDAL ACTIVITY

Mosquitoes are well known arthropod vectors of medical importance as they may transmit many dreadful diseases among human population which not only affect the health of the people but also the economy of the Nation. To certain extent, such vectors are controlled by synthetic chemicals available in the market. But synthetic chemicals may cause serious problems such as environmental pollutions, bio magnification, insecticide resistance, adverse effect on non-target populations etc. Therefore, there is an urgent need to find a natural remedy for controlling mosquitoes in an environmentally safe manner. Previous studies have shown that plant based chemicals are the only natural remedy to overcome the above said adverse effects of synthetic larvicides. Hence, in this investigation, an attempt was made to evaluate the larvicidal activities of twenty five plants, collected from the Upper Kuttanad of Alappuzha District of Kerala state. The selected plants and their parts were screened for detecting their larvicidal property against 3^{rd} and 4^{th} instar larvae of *Aedes* mosquito using standard procedures. The whole plants or their parts were extracted in different solvents such as petroleum ether, acetone, methanol and water to assess their larvicidal activity.

The results of the larvicidal study showed that the methanol extracts of the tested plants had significant larvicidal activity than other extracts. Out of the twenty five plants studied, about twelve of them showed slight or moderate activity. However, three plants; flowers of *Ipomoea cairica*, whole plant of *Sida acuta* and whole plant of *Asystasia gangetica* showed highest and

promising larvicidal activity. Among them, the flowers of *Ipomoea cairica* were active against the tested 3rd and 4th instar larvae of *Aedes* mosquito even at smaller concentrations; 0.2 mg/ml (95% larvicidal activity) and 0.1 mg/ml (83% larvicidal activity). The extract of *Sida acuta* with a concentration of 0.2 mg/ml was very effective to kill 93% of the tested larvae. However, 0.2 mg/ml of the extract of *Asystasia gangetica* was effective to kill only 86.5% of tested larvae which is also a promising result. Therefore, the present study suggests that these three plants; *Ipomoea cairica*, *Sida acuta* and *Asystasia gangetica* possess effective larvicidal activity against the larvae of *Aedes* mosquito. Hence, they can be considered for isolating their active larvicidal principles for selecting them as larvicidal agents.

ANTIBACTERIAL ACTIVITY

The above twenty five plants were also tested for determining their antibacterial activity against standard MTCC bacterial strains. The methodology adopted for screening the antibacterial activity was disc diffusion method. The activity was also compared with standard antibiotics using antibiotic susceptibility test. Petroleum ether, acetone, methanol and aqueous extracts of whole plant or their parts were taken for the study. The results of the study revealed that the only methanol extracts of eleven of them showed considerable activity than other extracts. Among the active methanol extracts, the whole plant extract of *Ludwigia parviflora* with a crude concentration of 10 mg/disc was very active against all the tested strains. Its activity was higher than the activity of Tetracycline 30 mcg against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Escherichia coli*. The extract also showed slightly higher activity than the Gentamicin 10 mcg against the *Pseudomonas aeruginosa*. Therefore, the present study can be concluded that the crude methanol extract of whole plant of *Ludwigia parviflora* with a concentration of 10 mg/disc is equivalent to Gentamicin and is more

powerful than Tetracycline. Hence, the methanol extract of *Ludwigia parviflora* can be taken as a natural alternative to Tetracycline and Gentamicin that are commonly used against the infection caused by *Staphylococcus aureus, Pseudomonas aeruginosa, Klebsiella pneumoniae* and *Escherichia coli*. Its MIC study revealed that a concentration of 3mg/ml was the MIC against *Staphylococcus aureus*. The MIC result is also a promising one in the field of antibacterial studies. Hence, the present study suggests that the methanol extract of whole plant of *Ludwigia parviflora* can be used for isolating the active principles to develop a new broad spectrum antibiotic against *Staphylococcus aureus, Pseudomonas aeruginosa, Klebsiella pneumoniae* and *Escherichia coli*.

The other plant extracts were active against one or two tested bacterial strains only. Among them, the methanol extracts of *Pouzolzia zeylanica* (10 mg/disc) was active against *Staphylococcus aureus* only. Its activity was equivalent to that of Tetracycline 30 mcg. Similarly, the crude methanol extract (10 mg/disc) of leaf of *Gliricidia sepium* was also active against two strains such as *Klebsiella pneumoniae* and *Escherichia coli*. Their activity was equivalent to the activity of Tetracycline 30 mcg. The crude methanol extract of leaf of *Cerbera odollam* was also effective to inhibit the growth of *Escherichia coli*. Its activity was also equivalent to the activity of Tetracycline 30 mcg. Hence, the present study concluded that the extracts of *Pouzolzia zeylanica, Gliricidia sepium* and *Cerbera odollam* can be utilized for developing natural alternative for Tetracycline 30 mcg, a common antibiotic available in the market.

Key Words: Larvicidal activities, Mosquito, vectors, *Aedes aegypti*, *Ipomoea cairica*, *Sida acuta*, *Asystasia gangetica*, antibacterial activity, antimicrobial activity, antibiotics, Gentamicin, Tetracycline, bacterial strains, *Ludwigia parviflora*, *Pouzolzia zeylanica*, *Gliricidia sepium* and *Cerbera odollam*.

CHAPTER 1.

LARVICIDAL AND ANTIMICROBIAL PROPERTIES OF SELECTED PLANTS FROM UPPER KUTTANAD; AN ECOLOGICALLY FRAGILE AREA OF SOUTH INDIA

1.1 GENERAL INTRODUCTION

'Nature' is the mother of all kinds of living organisms in the world. Hence, nature provides everything for the better survival of all organisms. The traditional medicine is based on the fact that the remedy for a disease is also bestowed with the nature, either in the form of a plant or plant products. Therefore, the scientific world is now searching for identifying such plants/plant products with medicinal significances for the mankind. In recent years, many studies are going on in the world to identify natural remedies for preventing vector born diseases especially mosquito born diseases and also for searching antibacterial drugs from the nature.

The prevalence of mosquito born disease and microbial infections are severe in our country, especially in the backward areas. This is due to the fact that the most of the residents of such area are illiterate or less educated, have poor health consciousness or hygiene, and are socially, financially or culturally less developed.

In this study, Upper Kuttanad, an ecologically fragile area of Kerala State was selected for studying the larvicidal activity of extracts of selected plants or their parts against mosquito larvae and also for screening their activities against standard bacterial strains.

Upper Kuttanad is an ecologically fragile land, spreads in three districts of Kerala; Alappuzha, Pathanamthitta and Kottayam. It is one of the World wonders as the land is lying below the sea level. In India, this is the one and only place with lowest altitude. However, the life at Kuttanad is under challenge. During monsoon, people are confronted with flood, followed

by infectious epidemics such as Cholera, typhoid, leptospirosis, elephantiasis etc. The major reason is that during flood, the wastes especially septic tank wastes would be mixed with drinking water or other fresh water bodies. Vector borne diseases especially mosquito borne diseases are also prevalent in Kuttanad because of the peculiar geography of the land mass allows the stagnation of water and is favorable for the proliferation of such vectors.

Since, most of the people of Kuttanad are financially poor; they cannot approach for modern medicine. However, they are mainly depending on the traditional/indigenous systems of medicines. Therefore, providing some knowledge on larvicidal and antimicrobial properties of plants of Upper Kuttanad in traditional medicine is worthy.

Knowledge on larvicidal and antimicrobial activities of plants/plant parts of Kuttanad will be useful not only for the people of Kuttanad but also for the world for developing new drugs.

1.2 OBJECTIVES OF THE STUDY

Kuttanad is rich in plant diversity and are highly adapted to the varying ecological conditions of the land. Therefore, the phytochemical composition of these plants may be useful in medicine. However, the medicinal significances of most of the plants of this region are unknown to the science. The present study was focused on the screening of selected plants or their parts (leaf/fruit/flower etc) for recognizing larvicidal activity against mosquito larvae and antimicrobial effects against standard bacterial strains. The objectives of the study were;

1. Selection of plant or plant parts (leaf/fruit/flower etc) for testing their larvicidal activity against mosquito larvae and antimicrobial activity against selected standard bacterial strains.

2. Taxonomic identification of selected plants with the help of taxonomists.
3. Preparation of different plant extracts in different solvents such as petroleum ether, acetone, methanol and water.
4. Screening of selected extracts for identifying their larvicidal property against mosquito larvae.
5. Screening of selected extracts for identifying antimicrobial property against selected standard bacterial strains.
6. Determination of the MIC (minimum inhibitory concentration) of the active extracts (i.e. having promising activity) using the selected bacterial strains.
7. Comparison of the active extracts (i.e. having promising activity) with standard antibiotics for determining the efficacy of the extract.

CHAPTER II

LARVICIDAL ACTIVITIES OF SELECTED PLANTS FROM UPPER KUTTANAD: AN ECOLOGICALLY FRAGILE AREA OF SOUTH INDIA

2.1. INTRODUCTION

Vector and vector-borne diseases cause great threat to the people of tropical country like India. Seventeen States and six Union Territories of India are reported to have 553 million people with the risk of vector born infections and about 31 million people are expected to be the carriers of microfilaria and about 23 million are infected by filarial disease [1, 2]. In India, the incidence of malaria, dengue fever, yellow fever, filariasis, schistosomiasis and Japanese encephalitis is very high. This is because of the fact that these diseases are vector born and mosquitoes play a major role in the transmission of these diseases. Two to three million cases of malaria are reporting every year in our country. This tells about the need for doing research in the field of larvicidal drug discovery [3-5]. In addition to their disease spreading ability, mosquitoes also act as a source of allergic reaction that includes local skin and systemic sensitivity in many people, especially those of hypersensitive nature [6].

Many studies are going on in India in search of good, effective larvicidal drugs. Most of the studies are focused on plants because of the fact that plant based drugs are non toxic to human but are effective against vectors and pathogens. More than two hundred plants are identified as effective sources for killing the larvae of mosquitoes [7]. On the other hand, synthetic larvicides are harmful to both human and the ecosystem; as they have the ability to stay on for a very long time in the nature. Synthetic larvicides may also kill natural biological control agents which may result in the emergence of resistance. These reasons have resulted in search for

the natural larvicides active against the vector mosquitoes. Most of the studies in the world are focusing on plants for isolating plant based compounds as an alternative source for serving as insecticides or larvicides in mosquito control programmes [8, 9].

Vector borne diseases especially mosquito born diseases are prevalent in low land areas as in Kuttanad because their peculiar geography of the land mass allows the stagnation of water which is a home ground for the proliferation of vectors like mosquitoes. Kerala, especially Kuttanad has increased incidence of Dengue fever since the last few years. *Aedes* is the vector for dengue fever in Kerala. Even though, massive mosquito eradication programs have been organized by both Government and Non Governmental institutions, 100% success is not yet achieved. One of the hindrances to this aim is the lack of eco-friendly larvicides to kill the mosquito larvae at their natural habitat. If toxic chemicals are utilized for the eradications of larvae, the chances for biological magnification of such chemicals are also high. To overcome this, plant based natural larvicides are to be discovered.

Since, plant derived larvicidal chemicals are effective in controlling mosquitoes in their breeding sites, this study was focused to assess the larvicidal potentials of the extracts from the selected plants of Upper Kuttanad against the larvae of *Aedes* species. *Aedes aegypti* is one of the main mosquito species responsible for the transmission of many vector borne diseases. Hence, this species was considered for the larvicidal studies. In mosquito control programmes, it is the larvae rather than adults are the target for control operations due to their low mobility in the breeding habitats and the ease to control in their habitats [10]. Hence, *Aedes* larvae were taken as the test organism.

2.2 REVIEW OF LITERATURE

Are the plants acting as effective larvicidal agents? Yes, the following literatures revealed that the plants or their products are effective larvicidal agents. *Azadirachta indica*, commonly known as Chinaberry or Persian lilac tree, is native to northwestern India. Its fruit extract is active against insects in the following ways; as antifeedant, growth retarding agent, agent for reducing fecundity, for producing moulting disorders, morphogenetic defects, and changes in behavior [11, 12]. Various extracts such as, aqueous, ethanol, methanol, acetone and chloroform of *Lantana camara aculeata* showed larvicidal activity against the fourth instar larvae of *Aedes aegypti, Anopheles stephensi* and *Culex quinquefasciatus*. Bioactive compounds of tannins, alkaloids, flavonoids, anthocyanin, quinines, triterpenoids, flavonoids, saponin and steroids were found in the leaves of above plants for contributing the above activity [13].

A Bagavan *et al* reported that the seed ethyl acetate extracts of *Abrus precatorius* and leaf extracts of *Croton bonplandianum*, flower chloroform and methanol extracts of *Musa paradisiaca* and flower bud hexane extract of *Syzygium aromaticum* showed promising larvicidal activity against *Anopheles vagus* [14]. Leaf, stem or bark extracts of *Jatropha curcas*, *Citrus grandis* and *Tinospora rumphii* exhibited larvicidal activity against the larvae of the dengue-vector, *Aedes aegypti* [15]. Jayapal Subramaniam *et al* reported that the leaf of *Aloe vera* is a potential larvicidal agent against the first to fourth instars larvae of *Aedes aegypti* [16]. The leaf extracts of *Blepharis maderaspatensis, Elaeagnus indica, Maesa indica, Phyllanthus wightianus* and *Memecylon edule* showed moderate to good larvicidal activities against the fourth-instar larvae of *Aedes aegypti* [17].

Another report showed that *Culex quinquefasciatus*, a vector responsible for serious disease, filariasis can be effectively inhibited by the use of aqueous extract of flower of *Nerium oleander* [18]. The water extract of seed of *Moringa oleifera is* also active against the larvae of *Aedes aegypti* [19]. The larvicidal activity of *Solanum lycocarpum* against *Culex quinquefasciatus* was studied by Thamer Matias Pereira *et al* and the result of their study showed that the dichloromethane and ethyl acetate extracts of green fruits of *Solanum lycocarpum* is active to inhibit the growth of third and fourth instar larvae of *Culex quinquefasciatus*; an important species involved in the transmission of lymphatic filariasis in different parts of the world [20]. The essential oils of *Ocimum americanum* and *Ocimum gratissimum* also have potential activity for the control of *Aedes aegypti* [21].

The essential oils of Camphor oil, Clove oil and Eucalyptus oil exhibited high larvicidal effect against the larvae of *Aedes aegypti*, *Anopheles stephensi* and *Culex quinquefasciatus* [22]. The petroleum ether and N-butanol extract of whole plant of *Cassia occidentalis* (Linn.) is very effective for inhibiting the growth of larvae of filarial vector, the *Culex quinquefasciatus* [23]. The mesocarp extract of fruit of *Balanites aegyptiaca* contains saponins and they exhibit significant larvicidal activity against the common dengue vector, *Aedes aegypti* [24]. Seed extract of *Apium graveolens* can be used as an effective mosquito repellent agent in the vector control programme [25]. The leaf of *Cadaba indica* can be used as a larvicidal agent against dengue vector, *Aedes aegypti* [26]. The bark of *Annona squamosa* L, the leaf of *Chrysanthemum indicum* L. and the leaf of *Tridax procumbens* L. are found to possess larvicidal activities against malaria vector, *Anopheles subpictus* Grassi and Japanese encephalitis vector, *Culex tritaeniorhynchus.* The leaf of *Annona muricata* L. and *Annona senegalensis* is also active against larvae of *Culex quinquefascintus* [27, 28].

Mangrove plant *Rhizophora mucronata* showed mosquito larvicidal property against two species of mosquitoes; *Anopheles stephensi* and *Aedes aegypti* [29]. The leaves of *Plectranthus glandulosus* possess promising larvicidal effect against *Anopheles gambiae*, *Aedes aegypti* and *Culex quinquefasciatus* [30]. Piperitenone oxide, an essential oil obtained from *Mentha longifolia* and *Mentha suaveolens* is a very effective phytochemical to fight against the larvae of *Culex quinquefasciatus* [31].

The above literatures reveal that many of the plants contain some active principles for fighting against the mosquito larvae. A great number of plants were screened for their larvicidal activities by many researchers but most of the extant species are not yet studied for their larvicidal properties. So, this study was primarily focused to screen the larvicidal activities of some selected plats from the Upper Kuttanad of Alappuzha District, Kerala State.

2.3. MATERIALS AND METHODS

2.3.1. Preparation of Plant Extracts:

The following table 1 shows the list of plants selected for the larvicidal screening studies;

Table No.1: Plants selected for the larvicidal screening studies.

Sl. No.	Scientific Name	Family	Part Used
1	*Vernonia cinerea* L.	Compositae	Whole plant
2	*Asystasia gangetica* L.	Acanthaceae	Whole plant
3	*Ixora finlaysoniana* Wall.ex G.Don.	Rubiaceae	Flower
4	*Tiliacora acuminate* (Lam.)	Menispermaceae	Leaf & Stem
5	*Gliricidia sepium* Jacq.	Fabaceae	Leaf
6	*Ludwigia parviflora* L.	Onagraceae	Whole plant
7	*Triumfetta rhomboidea* Jacq.	Malvaceae	Leaf & Stem
8	*Sida acuta* Burm.f.	Malvaceae	Whole plant
9	*Sphagneticola trilobata* (*Wedelia trilobata* L.)	Asteraceae	Leaf & Stem
10	*Waltheria indica* L.	Malvaceae	Whole plant
11	*Eryngium foetidum* L.	Apiaceae	Whole plant
12	*Senna alata* (*Cassia alata* L.)	Fabaceae	Flower and leaf
13	*Cerbera odollam* Gaertn.	Apocynaceae	Leaf
14	*Ipomoea cairica* (Linn.) Sweet.	Convolvulaceae	Flower
15	*Alternanthera bettzickiana* (Regel) G. Nicholson	Amaranthaceae	Whole plant
16	*Pseudarthria viscida* (L.) Wight & Arn	Fabaceae	Leaf & stem
17	*Aerva lanata* (L.) Juss.ex Schult.	Amaranthaceae	Whole plant
18	*Adiantum pedatum* L.	Pteridaceae	Leaf

19	*Eleutheranthera ruderalis* (Sw.) Sch. Bip.	Asteraceae	Whole plant
20	*Leea indica* (Burm.f.) Merr.	Vitaceae	Leaf & stem
21	*Pouzolzia zeylanica* (L.) Benn.	Urticaceae	Whole plant
22	*Aegle marmelos* L	Rutaceae	Leaf
23	*Pseuderanthemum reticulatum*	Acanthaceae	Leaf
24	*Ocimum basalicum var. Pilosum* (Wild.) Benth.	Lamiaceae	Leaf
25	*Euphorbia hirta* L.	Euphorbiaceae	Whole plant

The plants or plant parts were collected from different geographical areas of Upper Kuttanad, Alappuzha district of Kerala State (during different periods of September 2015 to March 2017). The taxonomic position of the selected plants was identified with the help of Sri. Bijesh P.P., Botanist, Sreedhareeyam Ayurvedic Research Centre, Koothattukulam, Ernakulam-Kerala. The collected plant sample was washed thoroughly under tap water, dried under sunlight and powdered using a mixer grinder. Serial extraction of the powder was made by a Soxhlet extractor using solvents such as petroleum ether, acetone, methanol and distilled water. The soxhlet extracts were filtered using Whatman No.1 filter paper and then concentrated. The concentrate was considered as stock and kept in refrigerator. From the stock, a concentration of 100mg/ml, 50 mg/ml and 25 mg/ml was prepared in de-chlorinated tap water and was used for the preliminary larvicidal screening.

2.3.2. Mosquito Culture:

3rd and 4th instar larvae of *Aedes aegypti* were collected from ditch water/ stagnant water areas of Kuttanad and they were identified in the Department of Zoology, St. Aloysius College, Edathua, Alappuzha District, Kerala State. Larvae were kept in plastic trays containing tap water

at 27 ± 2°C and with a humidity range of 75–85%. A mixture of Brewer's yeast, dog biscuits and algae (collected from ditchwater/pond) in a ratio of 3:1:1 were given to the larvae as food.

2.3.3. Larvicidal Bioassay:

The 3rd and 4th instar larvae of *Aedes aegypti* were used for the preliminary screening. Sample concentrations of 100mg/ml, 50 mg/ml and 25 mg/ml were used for preliminary larvicidal screening. The larvicidal activity was assessed by the procedure of WHO with slight modification as described by Kamaraj *et al* [32-34]. For the screening test, 20 larvae were taken in 249 ml of water and 1.0 ml of the plant extract with desired concentration (100mg/ml, 50 mg/ml and 25 mg/ml respectively) was added. Therefore, the final concentration of plant extract in the testing sample water was 0.4mg/ml, 0.2mg/ml and 0.1mg/ml respectively. In control experiment, 1 ml of distilled water was added without the plant extract. The numbers of dead larvae were counted after 24 h of exposure to the plant extract; the larvae were considered as dead, if they showed no sign of motility when touched with a glass rod. The percentage of mortality was recorded from the average of three replicates using the following formula,

$$\frac{n}{N} \text{ X } 100$$

where ‘n’ is the number of dead larvae and ‘N’ is the total number of larvae taken for the study.

2.4. RESULTS

The results of the preliminary screening of selected plants for determining their larvicidal activity are given in the table 2. Triplicate screening studies showed that the petroleum ether extracts and aqueous extracts of the selected plants were possessed no significant activity against the tested larvae. Hence, the effects of acetone and methanol extracts of selected plants are given in the table 2.

Table 2: Effect of acetone & methanol extracts of selected plants against the 3rd and 4th instar larvae of *Aedes aegypti*.

Sl. No.	Name of the Plant	No. of larvae died* in Acetone Extract			No. of larvae died* in Methanol Extract		
		0.4 mg/ml	0.2 mg/ml	0.1 mg/ml	0.4 mg/ml	0.2 mg/ml	0.1 mg/ml
1	*Vernonia cinerea*	1.3	-	-	5.3	1	-
2	*Asystasia gangetica*	10	5.6	1.3	20	17.3	12
3	*Ixora finlaysoniana*	2	-	-	9.6	2	-
4	*Tiliacora acuminate*	-	-	-	-	-	-
5	*Gliricidia sepium*	-	-	-	-	-	-
6	*Ludwigia parviflora*	2.6	-	-	8	2	-
7	*Triumfetta rhomboidea*	-	-	-	-	-	-
8	*Sida acuta*	13.6	10	6.3	20	18.6	15.6
9	*Sphagneticola trilobata*	8.6	5	3	18.6	15.6	9
10	*Waltheria indica*	-	-	-	9	5	1
11	*Eryngium foetidum*	-	-	-	4.3	1.3	-
12	*Senna alata*	-	-	-	-	-	-
13	*Cerbera odollam*	-	-	-	-	-	-
14	*Ipomoea cairica*	16	14.3	11.6	20	19	16.6
15	*Alternanthera bettzickiana*	-	-	-	-	-	-
16	*Pseudarthria viscida*	-	-	-	2.6	-	-
17	*Aerva lanata*	-	-	-	12	4	-
18	*Adiantum pedatum*	-	-	-	-	-	-
19	*Eleutheranthera ruderalis*	-	-	-	-	-	-
20	*Leea indica*	6.3	2	-	9	6.3	1.3
21	*Pouzolzia zeylanica*	-	-	-	-	-	-
22	*Aegle marmelos*	7.6	2	-	13	9.3	3.6
23	*Pseuderanthemum reticulatum*	9	4	2	15.6	10.3	4.3
24	*Ocimum basalicum*	-	-	-	-	-	-
25	*Euphorbia hirta*	3	-	-	9	4	1

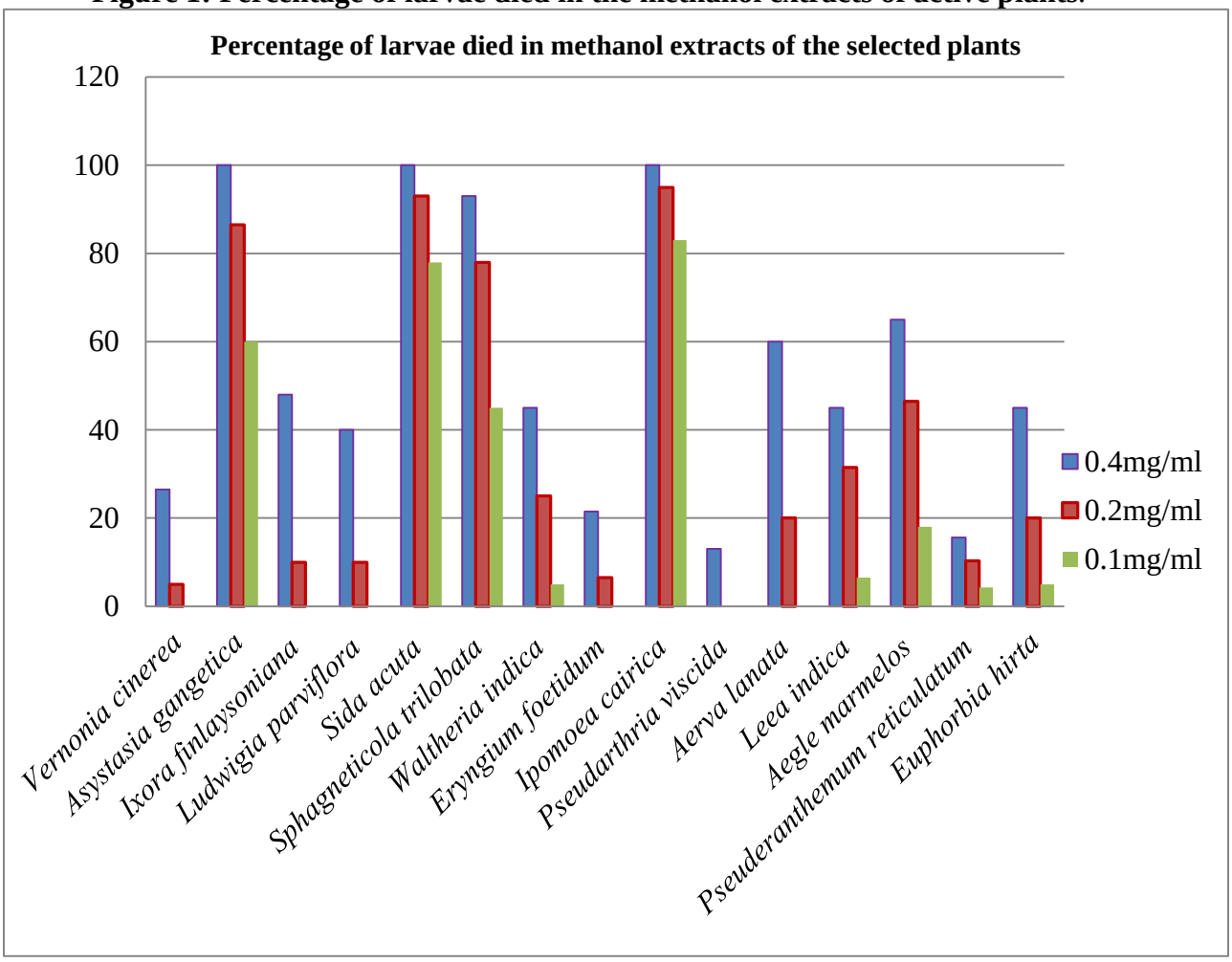
***Mean value of the triplicate experiments is represented.**

Table 3: Percentage of larvae died in acetone & methanol extracts of selected plants.

Sl. No.	Name of the Plant	Percentage of larvae died in Acetone Extract			Percentage of larvae died in Methanol Extract		
		0.4 mg/ml	0.2 mg/ml	0.1 mg/ml	0.4 mg/ml	0.2 mg/ml	0.1 mg/ml
1	*Vernonia cinerea*	6.5	0	0	26.5	5	0
2	*Asystasia gangetica*	50	28	6.5	100	86.5	60
3	*Ixora finlaysoniana*	10	0	0	48	10	0
4	*Tiliacora acuminate*	-	-	-	-	-	-
5	*Gliricidia sepium*	-	-	-	-	-	-
6	*Ludwigia parviflora*	13	0	0	40	10	0
7	*Triumfetta rhomboidea*	-	-	-	-	-	-
8	*Sida acuta*	68	50	31.5	100	93	78
9	*Sphagneticola trilobata*	43	25	15	93	78	45
10	*Waltheria indica*	-	-	-	45	25	5
11	*Eryngium foetidum*	-	-	-	21.5	6.5	0
12	*Senna alata*	-	-	-	-	-	-
13	*Cerbera odollam*	-	-	-	-	-	-
14	*Ipomoea cairica*	80	71.5	58	100	95	83
15	*Alternanthera bettzickiana*	-	-	-	-	-	-
16	*Pseudarthria viscida*	-	-	-	13	0	0
17	*Aerva lanata*	-	-	-	60	20	0
18	*Adiantum pedatum*	-	-	-	-	-	-
19	*Eleutheranthera ruderalis*	-	-	-	-	-	-
20	*Leea indica*	31.5	10	0	45	31.5	6.5
21	*Pouzolzia zeylanica*	-	-	-	-	-	-
22	*Aegle marmelos*	38	10	0	65	46.5	18
23	*Pseuderanthemum reticulatum*	45	20	10	15.6	10.3	4.3
24	*Ocimum basalicum*	-	-	-	-	-	-
25	*Euphorbia hirta*	15	0	0	45	20	5

Among the active extracts of acetone and methanol, only the methanol extracts showed significant larvicidal activities against the tested larvae. This may be due to the fact that the methanol is an effective polar solvent than acetone, so that more active phytochemicals may dissolve in the methanol than acetone.

Figure 1: Percentage of larvae died in the methanol extracts of active plants.



X axis: methanol extracts of active plants, Y axis: percentage of larvae died.

Analysis of the results on the effect of methanol extracts against 3rd and 4th instar larvae of *Aedes aegypti* showed that only 3 plants; *Asystasia gangetica*, *Sida acuta* and *Ipomoea cairica* were powerful to inhibit the growth of all the tested larvae (100%) at a concentration of 0.4 mg/ml. The 0.4 mg/ml of methanol extracts of *Sphagneticola trilobata* also showed significant activity by inhibiting the growth of 93% of tested larvae. But, 0.4mg/ml of methanol extracts of *Aegle marmelos* and *Aerva lanata* showed moderate activity (65% and 60% of larval

death respectively). All other plants extract showed 50% or less than 50% larvicidal activity in 0.4mg/ml concentration which cannot be considered as a significant activity in larvicidal studies.

On analyzing the effect of 0.2 mg/ml concentration of methanol samples, it was noticed that the three plants extracts; *Ipomoea cairica*, *Sida acuta* and *Asystasia gangetica* retained their larvicidal activity by inhibiting the growth of tested larvae with a percentage of above 85 (95%, 93% and 86.5% respectively) where as *Sphagneticola trilobata* showed a moderate activity (78% death). All other plants did not show significant results in 0.2 mg/ml concentration.

However, testing with 0.1mg/ml methanol samples of various plant extracts revealed that the extract of *Ipomoea cairica* retained its activity to inhibit the growth of 83% of tested larvae, where as *Sida acuta* was effective to inhibit 78% of tested larvae. All other extracts in 1mg/ml did not show any promising effect.

Therefore, it can be concluded that, among the tested plant extracts, the methanol extracts of flower of *Ipomoea cairica* with a concentration of 0.2 mg/ml has highest larvicidal activity (95%). Its 0.1 mg/ml has also significant activity (83%) against the 3^{rd} and 4^{th} instar larvae of *Aedes aegypti*. The second plant with effective activity was *Sida acuta* (93% larvicidal effect).

2.5. DISCUSSION

Mosquito born diseases are increasing day by day, resulting in the death of millions of people every year. This becomes a major challenge in the socio-economic realms of the developing countries. To control mosquito, chemical insecticides are not safe as they may increase the chance of insecticide resistance as well as create some environmental issues. As an alternative, plant based drugs are in focus for developing insecticide/larvicide [35]. Plants contain a variety of chemicals called as secondary metabolites and are useful to mankind as bio-control agents against microorganisms, mosquito larvae, nematodes etc. The secondary metabolites of plants are mainly produced for their protection against microorganisms or from predators. However, most of such chemicals have many biological properties and are utilized as medicine by us [37].

Since the last few decades, the indigenous plants of India have got more attention for finding their larvicidal activities as well as mosquito repellent or anti-juvenile hormone activities against the vector mosquitoes [36].

In this study, importance was given for studying the larvicidal properties of selected plants against the *Aedes* mosquito larvae. Knowledge on such plants will be beneficial to the people for applying them as larvicidal agents at a cheaper cost. The following plants were found to possess significant larvicidal activities against the larvae of *Aedes* mosquito and are discussed below.

Ipomoea cairica (Linn.) Sweet. is commonly known as railroad creeper. It is an evergreen, herbaceous, perennial climbing plant, belonging to the family Convolvulaceae. It is considered as a weed of waste area. Tropical Africa or Asia may be the place of its origin [38].

Even though it is a weed, evaluations of its medicinal properties revealed that it is an excellent herb in medicine. Its leaves possess antioxidant activity, antibacterial activity against *E.coli*, *Klebsiella pneumoniae*, *Bacillus subtilus*, *Salmonella typhi* etc., antifungal activity against *Aspergillus nigar*, *Candida albicans*, *Penicillium* sp. etc. [39]. Its leaves have effective anti-inflammatory activity as they contain tremendous anti-inflammatory chemicals like Saponins [40]. Its main bioactive compound is tannin; a powerful anticancer agent. This indicates that this plant has a good future in anticancer therapy [41]. Biochemical analysis showed that the flavonoids present in the leaves of *Ipomoea cairica* are responsible for its medicinal properties like antimicrobial, antioxidant and anti-allergic etc.[42]. Previous studies also showed that the various extracts, especially leaves of this plant possess effective larvicidal activity [43]. In the present investigation, its flowers were tested for detecting the larvicidal activities and the study results showed that its flower methanol extract was very effective against the 3^{rd} and 4^{th} instar larvae of *Aedes* mosquito. The extract was active at a smaller concentrations; 0.2 mg/ml (95% larvicidal activity) and 0.1 mg/ml (83% larvicidal activity) against the larvae. Hence, this study suggests that the flower of this plant can be considered for studying its active larvicidal principles for selecting this plant in anti-mosquito treatment.

Sida, a largest genus with cosmopolitan distribution has 17 reported species in India. *Sida acuta* Brum.f. is a small perennial shrub and is commonly known as wirewed. It comes under the family Malvaceae. Various literatures show that this plant is an excellent source of medicine; it possesses anticancer property due to its phytochemicals such as quindolinone and cryptolepinone [44]. Its antioxidant and analgesic effects were established by many researchers [45]. In traditional system of medicine like Ayurveda, it has been used to treat neurological disorders, headache, leucorrhoea, diabetics, fever, uterine disorders, dysentery and rheumatic

disorders. Its roots are used as stomachic, diaphoretic and antipyretic. It has cooling and astringent effect. Traditional systems have been using this plant for dealing the problems of blood, bile and liver. Reports also indicate that this plant has been using in the treatment of gonorrhea, elephantiasis, malaria and also in ulcers [46-48]. Report also indicates that it is effective to treat snake bite. It has anti-fertility effect and can also be used as a sedative. Antibacterial and antifungal studies using the leaf of *Sida acuta* showed that the leaves are potent source for antimicrobial agents [49]. The crude leaf extract of *Sida acuta* was found to possess larvicidal and repellant activity against the three important vector species; *Culex quinquefasciatus*, *Aedes aegypti* and *Anopheles stephensi* [50]. In the present investigation, the whole plant methanol extracts of *Sida acuta* were found to possess efficient larvicidal activity against the 3^{rd} and 4^{th} instar larvae of *Aedes* mosquito. The methanol extract of *Sida acuta* with a concentration of 0.2 mg/ml was very effective to kill 93% of the tested 3^{rd} and 4^{th} instar larvae of *Aedes aegypti*. Therefore, this study suggests that the whole plant of *Sida acuta* can be considered for isolation of larvicidal principles to use them as a prototype for large scale preparation of larvicidal drugs.

Asystasia gangetica L. is a herb, belonging to the family Acanthaceae. It is commonly known as Chinese violet. This plant is an excellent source of phytochemicals such as tannin, saponin, flavonoids, terpenoids etc. and has been used in the traditional medicines for curing various ailments [51]. Its roots are used for treating skin allergies. This plant can be used in treating rheumatism, gonorrhea and ear diseases. Its leaves have anti-helminthic properties. Its leaves posses antibacterial activity against gram positive and gram negative bacteria [52]. In south India, this plant is used in the treatment of diabetes mellitus. They are used in the treatment of asthma in Nigeria. The methanolic extract of this plant shows effective anti-inflammatory and

analgesic activities [53]. Another study revealed that the methanolic extracts of leaves of this plant possess effective hypoglycemic and hypolipidemic properties [54]. It is also evident that this plant possesses antioxidant activities [55]. In the present investigation, the whole plant methanol extract of *Asystasia gangetica* with a concentration of 0.2 mg/ml was found to possess a significant larvicidal activity. 86.5% of tested 3rd and 4th instar larvae of *Aedes* mosquito were died in that concentration. Therefore, the present investigation suggests that this plant can be taken into consideration for detailed larvicidal studies. There is no much literature evidence on its larvicidal activity. Hence, detailed pharmacological and toxicological studies are suggesting for recommending this plant in larvicidal treatment.

2.6. CONCLUSION

Twenty five plants were collected from different regions of Upper Kuttanad, Alappuzha District of Kerala state during September 2015 to March 2017. They were screened for detecting their larvicidal property against 3rd and 4th instar larvae of *Aedes* mosquito using standard procedure (as described by the WHO, but with a slight modification). The whole plants or their parts were extracted in different solvents such as petroleum ether, acetone, methanol and water. Triplicate experiments showed that the petroleum ether and aqueous extracts were ineffective to kill the tested larvae where as the acetone and methanol extracts showed positive results. Among the active extracts, methanol extracts had significant activity than acetone extract. Therefore, methanol extract was taken for the study.

Out of the twenty five plants studied, about twelve of them showed slight or moderate activity, which cannot be considered for future studies. However, only three plants; flower extracts of *Ipomoea cairica*, whole plant extract of *Sida acuta* and whole plant extract of *Asystasia gangetica* showed highest and promising activity. Among them, the flower extracts of *Ipomoea cairica* were active against the tested 3rd and 4th instar larvae of *Aedes* mosquito even at smaller concentrations; 0.2 mg/ml (95% larvicidal activity) and 0.1 mg/ml (83% larvicidal activity). The methanol extract of whole plant of *Sida acuta* with a concentration of 0.2 mg/ml was very effective to kill 93% of the tested larvae. But, 0.2 mg/ml of the whole plant methanol extract of *Asystasia gangetica* was effective to kill only 86.5% of tested larvae. However, this result is also satisfactory for larvicidal studies. Therefore, the present study can be concluded that these three plants; *Ipomoea cairica*, *Sida acuta* and *Asystasia gangetica* possess larvicidal activities against the larvae of *Aedes* mosquito, hence, they can be considered for isolating the larvicidal principles for selecting them as a larvicidal agent.

2.7. REFERENCES

1. M. Govindarajan, A. Jebanesan, and D. Reetha, "Larvicidal effect of extracellular secondary metabolites of different fungi against the mosquito, *Culex quinquefasciatus* Say," *Tropical Biomedicine*. 2005; 22 (1):1–3.
2. Kumar A, Valecha N, Jain T and Aditya P. Dash. Burden of malaria in India: retrospective and prospective view. *Am J Trop Med Hyg*. 2007; 77: 69-78.
3. Rahuman AA, Venkatesan P, Gopalakrishnan G. Mosquito larvicidal activity of oleic and linoleic acids isolated from *Citrullus colocynthis* (Linn.) Schrad. *Parasitology Research*. 2008; 103:1383–90.
4. WHO. Sixth meeting of the technical advisory group on the global elimination of lymphatic filariasis, Geneva, Switzerland. Wkly. *Epidemiol. Rec*. 2005, 80: 401-408.
5. Das MK, Ansari MA. Evaluation of repellent action of *Cymbopoganmartinii* stapfvar sofia oil against *Anopheles sundiacus* in tribal villages of Car Nicobar Island, Andaman & Nicobar Islands, India. *J Vect Borne Dis*. 2003; 40:101–4.
6. Cheng S.S., Chang H.T., Chang S.T., Tsai K.H., Chen W.J. Bioactivity of selected plant essential oils against the yellow fever mosquito *Aedes aegypti* larvae. *Bioresource Technol*. 2003; 89: 99–102.
7. Govindarajan M, Jebanesan A and Pushpanathan T. Larvicidal and ovicidal activity of *Cassia fistula* Linn. leaf extract against filarial and malarial vector mosquitoes. *Parasitology Research*. 2008; 102(2): 289-292.
8. Tiwary M., Naik S.N., Tewary D.K., Mittal P.K. and Yadav S. Chemical Composition and larvicidal activities of the essential oil of *Zanthoxylum armatum* DC (Rutaceae) against three mosquito vectors. *J. Vector Borne Dis.* 2007; 44,198-204.

9. Prophiro JS, Rossi JCN, Pedroso MF, Kanis LA, Silva OS. Leaf extracts of *Melia azedarach* Linnaeus (Sapindales: Meliaceae) act as larvicide against *Aedes aegypti* (Linnaeus, 1762) (Diptera: Culicidae). *Rev Soc Bras Med Trop*. 2008; 41:560-564.
10. Howard AFB, Zhou G, Omlin FX. Malaria mosquito control using edible fish in Western Kenya: preliminary findings of a controlled study. *BMC Public Health*. 2007; 7:199-204.
11. Gajmer T, Singh R, Saini RK, Kalidhar S.B. Effect of methanolic extracts of neem (*Azadirachta indica* A. Juss) and bakain (*Melia azedarach* L.) seeds on oviposition and egg hatching of *Earias vittella* (Fab.) (Lepidoptera: Noctuidae). *J. Appl. Entomol*. 2002; 126: 238–243.
12. Wandscheer CB, Duque JE, da Silva MAN, Fukuyama Y, Wohlke JL, Adelmann J, Fontana JD. Larvicidal action of ethanolic extracts from fruit endocarps of *Melia azedarach* and *Azadirachta indica* against the dengue mosquito *Aedes aegypti*. *Toxicon*. 2004; 44: 829–835.
13. Periaswamy Hemalatha, Devan Elumalai, Arumugam Janaki, Muthu Babu, Kuppan Velu, Kanayairam Velayutham, Patheri kunyil Kaleena. Larvicidal activity of *Lantana camara aculeate* against three important mosquito species. *Journal of Entomology and Zoology Studies*. 2015; 3 (1): 174-181.
14. A Bagavan, A Abdul Rahuman. Evaluation of larvicidal activity of medicinal plant extracts against three mosquito vectors. *Asian Pacific Journal of Tropical Medicine*. 2011; 29-34.
15. Pedro M. Gutierrez, Jr., Aubrey N. Antepuesto, Bryle Adrian L. Eugenio, Maria Fleurellei L. Santos. Larvicidal activity of selected plant extracts against the dengue

vector *Aedes aegypti* mosquito. *International Research Journal of Biological Sciences*. 2014; 3(4): 23-32.

16. Jayapal Subramaniam, Kalimuthu Kovendan, Palanisamy Mahesh Kumar, Kadarkarai Murugan and William Walton. Mosquito larvicidal activity of *Aloe vera* (Family: Liliaceae) leaf extract and *Bacillus sphaericus*, against Chikungunya vector, *Aedes aegypti*. *Saudi Journal of Biological Sciences*. 2012; 19: 503–509.
17. M. S. Shivakumar, R. Srinivasan and D. Natarajan. Larvicidal potential of some Indian medicinal plant extracts against *Aedes aegypti* (L.). *Asian J Pharm Clin Res*. 2013; 6 (3): 77-80.
18. R. Raveen, K.T. Kamakshi, M. Deepa, S. Arivoli and Samuel Tennyson. Larvicidal activity of *Nerium oleander* L. (Apocynaceae) flower extracts against *Culex quinquefasciatus* Say (Diptera: Culicidae). *International Journal of Mosquito Research*. 2014; 1 (1): 38-42.
19. Paulo M.P. Ferreira, Ana F.U. Carvalho, Davi F. Farias, Nara G. Cariolano,V.Nia M.M. Melo, Maria G.R. Queiroz, Alice M.C. Martins and Joaquim G. Machado-Neto. Larvicidal activity of the water extract of *Moringa oleifera* seeds against *Aedes aegypti* and its toxicity upon laboratory animals. *Anais da Academia Brasileira de Ciencias*. 2009; 81 (2): 207-216.
20. Thamer Matias Pereira, Viviane de Cassia Bicalho Silva, Jose Antonio Ribeiro Neto,Stenio Nunes Alves and Luciana Alves Rodrigues dos Santos Lima. Larvicidal activity of the methanol extract and fractions of the green fruits of *Solanum lycocarpum* (Solanaceae) against the vector *Culex quinquefasciatus* (Diptera: Culicidae). *Rev Soc Bras Med Trop*. 2014; 47 (5): 646-648.

21. Eveline Solon Barreira Cavalcanti, Selene Maia de Morais, Michele Ashley A Lima, and Eddie William Pinho Santana. Larvicidal activity of essential oils from Brazilian plants against *Aedes aegypti* L. *Mem Inst Oswaldo Cruz, Rio de Janeiro*. 2004; 99 (5): 541-544.
22. S.R. Pugazhvendan and K. Elumali. Larvicidal activity of selected plant essential oil against important vector mosquitoes: dengue vector, *Aedes aegypti* (L.), malarial vector, *Anopheles stephensi* (Liston) and filarial vector, *Culex quinquefasciatus* (Say) (Diptera: Culicidae). *Middle-East Journal of Scientific Research*. 2013; 18 (1): 91-95.
23. Deepak Kumar, Rakesh Chawla, P. Dhamodaram, and N. Balakrishnan. Larvicidal activity of *Cassia occidentalis* (Linn.) against the larvae of Bancroftian filariasis vector mosquito *Culex quinquefasciatus*. *Journal of Parasitology Research*. 2014; 1-5.
24. Bishnu P. Chapagain and Zeev Wiesman. Larvicidal activity of the fruit mesocarp extracts of *Balanites aegyptiaca* and its saponin fractions against *Aedes aegypti*. *Dengue Bulletin*. 2005; 29: 203-205.
25. Wej Choochote, Benjawan Tuetun, Duangta Kanjanapothi, Eumporn Rattanachanpichai, Udom Chaithong, Prasong Chaiwong, Atchariya Jitpakdi, Pongsri Tippawangkosol, Doungrat Riyong, and Benjawan Pitasawat. Potential of crude seed extract of celery, *Apium graveolens* L., against the mosquito *Aedes aegypti* (L.) (Diptera: Culicidae). *Journal of Vector Ecology*. 2004; 29 (2): 340-346.
26. Kandasamy Kalimuthu, Kadarkarai Murugan, Chellasamy Panneerselvam and Jiang-Shiou Hwang. Mosquito larvicidal activity of *Cadaba indica* lam leaf extracts against the dengue vector, *Aedes aegypti*. *Asian Journal of Plant Science and Research*. 2012; 2 (5): 633-637.

27. C. Kamaraj, A. Bagavan, G. Elango, A. Abduz Zahir, G. Rajakumar, S. Marimuthu, T. Santhoshkumar and A. Abdul Rahuman. Larvicidal activity of medicinal plant extracts against *Anopheles subpictus* and *Culex tritaeniorhynchus*. *Indian J Med Res*. 2011; 134: 101-106.
28. Joseph J. Magadula, Ester Innocent and Joseph N. Otieno. Mosquito larvicidal and cytotoxic activities of 3 *Annona* species and isolation of active principles. *Journal of Medicinal Plants Research*. 2009; 3(9): 674-680.
29. S.V. Meenakshi and K. Jayaprakash. Mosquito larvicidal efficacy of leaf extract from mangrove plant *Rhizophora mucronata* (Family: Rhizophoraceae) against *Anopheles* and *Aedes* species. *Journal of Pharmacognosy and Phytochemistry*. 2014; 3 (1): 78-83.
30. Y.S.P. Danga, E.N. Nukenine, L. Younoussa and C.O. Esimone. Phytochemicals and larvicidal activity of *Plectranthus glandulosus* (lamiaceae) leaf extracts against *Anopheles gambiae, Aedes aegypti* and *Culex quinquefasciatus* (diptera: culicidae). *International Journal of Pure and Applied Zoology*. 2014; 2(2): 160-170.
31. Roman Pavela, Katarína Kaffkova and Michal Kumsta. Chemical composition and larvicidal activity of essential oils from different *Mentha L*. and *Pulegium* species against *Culex quinquefasciatus* Say (Diptera: Culicidae). *Plant Protect. Sci*.2014; 50(1): 36–42.
32. Kamaraj C, Bagavan A, Elango G, Abduz Zahir A, Rajakumar G, Marimuthu S, Santhoshkumar T, and Abdul Rahuman A. Larvicidal activity of medicinal plant extracts against *Anopheles subpictus* & *Culex tritaeniorhynchus. Indian J Med Res*. 2011; 134 (1): 101–106.
33. World Health Organization. Guideliness for laboratory and field testing of mosquito larvicdes. *WHO, Geneva* 2005.

34. WHO. Instructions for determining the susceptibility or resistance of mosquito larvae to insecticides. *WHO/VBC/81.807 Geneva: World Health Organization*. 1981: 7.
35. R. Pavela, Larvicidal activities of some Euro-Asiatic plants against *Culex quinquefasciatus* Say (Diptera: Culicidae). *Journal of Biopesticides*. 2008; 1: 81–85.
36. Singh K.V. and Bansal S.K. Larvicidal properties of a perennial herb *Solanum xanthocarpum* against vectors of malaria and dengue/DHF. *Curr. Sci.* 2003; 84: 749–751.
37. Sun R, Sacalis JN, Chin CK, Still CC. Bioactive aromatic compounds from leaves and stems of Vanilla fragrans. *Journal of Agricultural and Food Chemistry*. 2006; 49: 51-61.
38. D.F Austin and Z Huaman. A synopsis of *Ipomoea* (Convolvulaceae) in the Americas. *Taxon*. 1996; 45:5.
39. Arora S, Kumar D and Shiba. Phytochemical, antimicrobial and antioxidant activities of methanol extracts of leaves and flowers of *Ipomoea cairica*. *Int. J. Pharm Pharm Sci*. 2013; 5(1):198-202.
40. Deepa Srivastava and Shukla K. *Ipomoea cairica*: a medicinal weed with promising health benefits. *International Journal of Information Research and Review*. 2015; 2 (5): 687-694.
41. Li D and Wang P. Antifungal activity of Paraguayan plant used in traditional medicine. *J. Ethanopharmacol*. 2003; 76: 93-98.
42. Vanlalhruaii Ralte. Evaluation of phytochemical contents of *Ipomoea cairica* (L.) Sweet – a qualitative approach. *Science Vision*. 2014; 14 (3): 145-151.
43. AhbiRami R, Zuharah WF, Thiagaletchumi M, Subramaniam S and Sundarasekar J. Larvicidal efficacy of different plant parts of railway creeper, *Ipomoea cairica* extract

against dengue vector mosquitoes, *Aedes albopictus* (Diptera:Culcidae) and *Aedes aegypti* (Diptera:Culcidae). *J Insect Sci*. 2014; 14: 180.

44. Jang DS, Park EJ, Kang YH, Su BN, Hawthorne ME, Vigo JS, Graham JG, Cabieses F, Fong HH, Mehta RG, Pezzuto JM and Kinghorn AD. Compounds obtained from *Sida acuta* with the potential to induce quinone reductase and to inhibit 7, 12-dimethylbenz[a]anthracene-induced preneoplastic lesions in a mouse mammary organ culture model. *Arch. Pharmacol. Res*. 2003; 26: 585-590.
45. Simplice DK, Wendyam MC N, Densie P I, Djeneba O, Messanvi G, Comlan De S and Jacques S. *Sida acuta* Brum.f.: a medicinal plant with numerous potencies. *African Journal of Biotechnology*. 2007; 6 (25):2953-2959.
46. Pradhan Dusmanta Kumar, PandaAshok Kumar, Behera Rajani Kanta, Jha Shivesh, Mishra Manas Rajan, Mishra Ashutosh and Choudhary Sanjay. Ethno medicinal and therapeutic potential of *Sida acuta* Brum.f. *International Research Journal of Pharmacy*. 2013; 4 (1): 88-92.
47. Khare M, Srivastava SK and Singh AK. Chemistry and pharmacology of genus *Sida* (Malvaceace) – a review. *Journal of Medicinal and Aromatic Plant Science*. 2002; 24: 430-440.
48. Banzouzi JT, Prado R, Menan H, Valentin A, Roumestan C., Mallie M *et al*. Studies on medicinal plants of Ivory Coast: investigation of *Sida acuta* for *in vitro* antiplasmodial activities and identification of an active constituent. *Phytomedicine*. 2004; 11: 338-341.
49. Akilandeswari S, R Senthamarai, Prema S and R Valarmathi. Antimicrobial activity of leaf extracts of *Sida acuta* Brum. *International Journal of Pharma Sciences and Research*. 2010; 1 (5): 248-250.

50. Marimuthu Govindarajan. Larvicidal and repellent activities of *Sida acuta* Brum.f. (family: Malvaceae) against three important vector mosquitoes. *Asian Pacific Journal of Tropical Medicine*. 2010; 691-695.
51. Hamid AA, Aiyelaagbe OO, Ahmed RN, Usman LA, Adebayo SA. Preliminary phytochemistry, antibacterial and antifungal properties of extracts of *Asystasia gangetica* Linn T. Anderson grown in Nigeria. *Adv Appl Sci Res*. 2011; 2: 219-226.
52. Alen Godfrey R Jose, Abhirami T, Kavitha V, Sellakilli R and Karthikeyan J. Green synthesis of silver nanoparticles using *Asystasia gangetica* leaf extract and its antibacterial activity against gram positive and gram negative bacteria. *Journal of Pharmacognosy and Phytochemistry*. 2018; 7 (1): 2453-2457.
53. Tilloo SK, Pande VB, Rasala TM and Kale VV. *Asystasia gangetica*: Review on multipotential application. *International Research Journal of Pharmacy*. 2012; 3 (4): 18-20.
54. Pradeep Kumar R, Sujatha D, Mohammed Saleem TS, Madhusudhanan Chetty C, Ranganayakulu D. Potential hypoglycemic and hypolipidemic effect of *Morus indica* and *Asystasia gangetica* in alloxan induced diabetes mellitus. *Int. J. Res. Pharm. Sci*. 2010; 1: 51-56.
55. S.S Somanathan, D Ranganayakulu and K.N. Jayaveera. *In vitro* antioxidant activities of *Asystasia gangetica* leaf extract. *World Journal of Pharmacy and Pharmaceutical Sciences*. 2015; 4 (2): 1228-1239.

CHAPTER III

ANTIBACTERIAL ACTIVITIES OF SELECTED PLANTS FROM UPPER KUTTANAD: AN ECOLOGICALLY FRAGILE AREA OF SOUTH INDIA

3.1. INTRODUCTION

The dramatic increase of human population has not only resulted in the transformation of natural habitat to vector or pathogen prone area but also the emergence of less dominant microorganisms to a dominant or pathogenic organism. Evolution of non-virulent microorganisms to a virulent form also significantly contributes for the tremendous increase in the number of pathogenic forms in the society. All these changes have necessitated in the search for more and more effective antibiotics by the researchers. Till now, more than 8000 drugs are identified as antibiotics. However, the number of effective antibiotics available for the antimicrobial therapy is limited to two or three dozens of antibiotics. This is because of the misuse of antibiotics by the common people, who are not ready to take the full course antibiotics as advised by the physician or due to the overuse of antibiotics without determining the root cause of the disease. All these activities resulted in the emergence of dangerous strains of pathogens which can resist the action of available antibiotics [1].

Medicinal plants have been used as an effective agent for antimicrobial therapy both by traditional systems of medicine and modern medicine since the last few decades. In modern medicine, they are not used as such; instead their active principles are isolated through chromatography techniques and characterizing by spectroscopic analysis. Then the identified compounds are used as a prototype for large scale synthesis of such compounds as drugs [2]. The secondary metabolites of the plants are the sources of biologically active, medicinal drugs in

plants. They may be present at the whole plant or in flower or stem or leaf or root of the plant. In plant, they have their own roles like attracting the insects for pollination, to protect the plant from microbial attack etc. [3]. One of the advantages of such compounds is that they are non toxic to animal cells or their toxicity is too less than synthetic drugs. Hence, they are considered as a natural remedy to treat human and other animal diseases [4-5]. In this study all the twenty five plants selected for larvicidal studies were also taken for identifying their antimicrobial activity to suggest their use in the traditional systems of medicine and also to modern medicine.

3.2. REVIEW OF LITERATURE

As already mentioned in the introduction part, microorganisms are increasing their capacity to develop resistance against standard antibiotics that are available in the market. Moreover, the high cost of antibiotics becomes a challenge in the life of poor people of the developing country. Many other reasons are also necessitated the discovery of more and more effective antibiotics from the nature. So, many research activities are going on in the world in search of efficient antibiotics from the plants. Some of the previous studies are listed below.

S. Paudel *et al* reported that the fruit methanolic extracts of wild olive, *Olea cuspidata* possess antibacterial activity against human bacterial pathogens, *Pseudomonas aeruginosa* and *E.coli.*[6]. The phytochemicals such as tannin, phenol, anthraquinon and saponin of *Rhus coriaria* were found to possess antibacterial activity against multi-drug resistant *Staphylococcus aureus* [7]. Another report showed that the extracts of *Syzygium cumini* and *Lawsonia inermis* were effective against antibiotic resistant *Staphylococcus aureus* strains [8]. The extracts of *Caryophyllus aromaticus* and *Syzygyum joabolanum* possessed highest antimicrobial activity against *Pseudomonas aeruginosa* [9]. The leaf, stem and flowers of *Acacia aroma* contain antibacterial activity against gram-positive bacteria and gram-negative bacteria [10].

Thymol, one of the secondary metabolites of *Ocinum gratissimum* and *Eugenia uniflora* has effective antimicrobial activity against *Staphylococcus* sp., *Escherichia coli* and *Shigella* sp. Report also showed that the methanolic extract of *Phyllanthus niruri* possesses highest antibacterial activity against *Staphylococcus* sp. [11]. Abhishek Mathur *et al* reported that the ethanol extracts of three plants; *Bixa orellana, Justica secunda* and *Piper pulchrum* have effective antibacterial activity against *Staphylococcus aureus, Streptococcus hemolytic, Bacillus cereus, Pseudomonas aeruginosa* and *Escherichia coli*. They also reported that the MIC of these

three plants was lower than that of standard antibiotics [12]. The leaf extracts of *Catharanthus roseus* of the Saudi Arabia showed antibacterial activity against some human pathogenic microorganisms such as *Staphylococcus aureus* and *Escherichia coli* [13].

The leaf extracts of *Moringa oleifera* have powerful antibacterial activity against Gram negative bacteria such as *Shigella shinga, Pseudomonas aeruginosa, Shigella sonnei* and *Pseudomonas* spp. and also against Gram positive bacteria such as *Staphylococcus aureus, Bacillus cereus, Streptococcus-B- haemolytica, Bacillus subtilis, Sarcina lutea* and *Bacillus megaterium* [14]. The flower buds of *Caryophyllus aromaticus* were found to possess highest antibacterial activity against *Staphylococcus aureus* [15]. Sarah M. Wigmore *et al* reported that the extracts of Eucalyptus, Melaleuca, Prostanthera and Westringia from Australia showed an MIC of 0.25 mg/ml against *Pseudomonas aeruginosa* and *Staphylococcus aureus* [16]. Another report showed that the leaves of guava (*Psidium guajava*), green tea (*Camellia sinensis*), neem (*Azadirachta indica*) and marigold (*Calendula officinalis*) have effective antimicrobial activity against *Pseudomonas aeruginosa, Klebsiella* spp. and *Staphylococcus aureus* [17]. Leaf extracts of *Melia azedarach* possess antibacterial activity against *Bacillus cereus, Staphylococcus aureus, Escherichia coli* and *Pseudomonas aeruginosa* [18]. The fruit skin of pomegranate (*Punica granatum*) shows powerful antibacterial activity against both Gram positive *Staphylococcus aureus* and Gram negative *Pseudomonas aeruginosa* [19]. Adegoke AA *et al* reported that the ethanolic extracts of *Phyllanthus amarus* possess strong inhibition on the growth of multidrug resistant pathogens such as *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa* and *Klebsiella Spp.* They reported that the antibacterial activity of *Phyllanthus amarus* may be due to the presence of alkaloids, tannins or flavonoids present in them [20].

The crude acetone and ethanol extracts of *Corriander sativum*, *Abutilon indicum*, *Boerhavia diffusa andrographis paniculata*, *Plantago ovata*, *Bacopa monnieri*, *Bauhinia variegata*, *Flacouratia ramontchi*, *Embelia tfgerium*, *Euphorbia ligularia*, *Zinziber officinale*, *Terminalia chebula*, *Azadirachta indica*, *Ocimum sanctum* and *Cinnamomum cassia* showed antibacterial activity against urinary tract pathogens such as *Proteus mirabilis*, *Escherichia coli*, *Proteus vulgaris*, *Klebsiella pneumoniae*, *Enterobacter cloacae*, *Providencia pseudomallei*, *Pseudomonas aeruginosa* and *Klebsiella oxytoca* [21]. The fruit extracts of *Flacourtia inermis* possess promising antibacterial activity against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Escherichia coli*. The 2, 3 dihydroxybenzoic acids were the active principles, rendering the fruits of *Flacourtia inermis* its antibacterial activity [22, 23].

The above literatures showed that the plants are effective sources for fighting against bacterial pathogens. They can be utilized by the traditional as well as modern medicine to treat against bacterial diseases. However, most of the existing plants are not yet examined to detect their antimicrobial property. Therefore, the present study was focused on the antibacterial activity of the selected plants of Upper Kuttanad against standard bacterial strains to suggest their use as antibacterial agent in traditional and modern medicines.

3.3. MATERIALS AND METHODS

3.3.1. Preparation of Plant Extracts:

The following table 4 shows the list of plants selected for the antibacterial studies (same plants; those used for larvicidal studies were taken for the antibacterial studies);

Table No.4: Plants selected for the preliminary antibacterial screening

Sl. No.	Scientific Name	Family	Part Used
1	*Vernonia cinerea* L.	Compositae	Whole plant
2	*Asystasia gangetica* L.	Acanthaceae	Whole plant
3	*Ixora finlaysoniana* Wall.ex G.Don.	Rubiaceae	Flower
4	*Tiliacora acuminate* (Lam.)	Menispermaceae	Leaf & Stem
5	*Gliricidia sepium* Jacq.	Fabaceae	Leaf
6	*Ludwigia parviflora* L.	Onagraceae	Whole plant
7	*Triumfetta rhomboidea* Jacq.	Malvaceae	Leaf & Stem
8	*Sida acuta* Burm.f.	Malvaceae	Whole plant
9	*Sphagneticola trilobata* (*Wedelia trilobata* L.)	Asteraceae	Leaf & Stem
10	*Waltheria indica* L.	Malvaceae	Whole plant
11	*Eryngium foetidum* L.	Apiaceae	Whole plant
12	*Senna alata* (*Cassia alata* L.)	Fabaceae	Flower and leaf
13	*Cerbera odollam* Gaertn.	Apocynaceae	Leaf
14	*Ipomoea cairica* (Linn.) Sweet.	Convolvulaceae	Flower
15	*Alternanthera bettzickiana* (Regel) G. Nicholson	Amaranthaceae	Whole plant
16	*Pseudarthria viscida* (L.) Wight & Arn	Fabaceae	Leaf & stem
17	*Aerva lanata* (L.) Juss.ex Schult.	Amaranthaceae	Whole plant

18	*Adiantum pedatum* L.	Pteridaceae	Leaf
19	*Eleutheranthera ruderalis* (Sw.) Sch. Bip.	Asteraceae	Whole plant
20	*Leea indica* (Burm.f.) Merr.	Vitaceae	Leaf & stem
21	*Pouzolzia zeylanica* (L.) Benn.	Urticaceae	Whole plant
22	*Aegle marmelos* L	Rutaceae	Leaf
23	*Pseuderanthemum reticulatum*	Acanthaceae	Leaf
24	*Ocimum basalicum var. Pilosum* (Wild.) Benth.	Lamiaceae	Leaf
25	*Euphorbia hirta* L.	Euphorbiaceae	Whole plant

The plants or plant parts were collected from different geographical areas of Upper Kuttanad, Alappuzha district of Kerala State (during different periods of September 2015 to March 2017). The taxonomic position of the selected plants was identified with the help of Sri. Bijesh P.P., Botanist, Sreedhareeyam Ayurvedic Research Centre, Koothattukulam, Ernakulam-Kerala. The collected plant sample was washed thoroughly under tap water, dried under sunlight and powdered using a mixer grinder. Serial extraction of the powder was made by a Soxhlet extractor using solvents such as petroleum ether, acetone, methanol and distilled water. The soxhlet extracts were filtered using Whatman No.1 filter paper and then concentrated. The concentrate was considered as stock and kept in refrigerator. From the stock, a concentration of 10mg/disc was prepared and was used for the preliminary antibacterial screening.

3.3.2. Bacterial Strains:

Standard MTCC strains purchased from Institute of Microbial Technology, Chandigarh (IMTECH), India were used for the study (Invoice No. MTCC/3/14/10497, dated 16-12-2015). The strains were;

1. *Staphylococcus aureus* (MTCC Code 3160)
2. *Escherichia coli* (MTCC Code 40)
3. *Pseudomonas aeruginosa* (MTCC Code 4673)
4. *Klebsiella pneumoniae* (MTCC Code 3040)

3.3.3. Antibiotic Discs:

Standard antibiotic discs purchased from Himedia Laboratories Pvt.Ltd, Mumbai, India were used for antibiotic sensitivity comparison. Following antibiotics were used for the study.

1. Chloramphenicol (30 mcg)
2. Gentamicin (10 mcg)
3. Penicillin G (10 u)
4. Ciprofloxacin (5 mcg)
5. Tetracycline (30 mcg)
6. Amikacin (30 mcg)

3.3.4. Culture Media:

The dehydrated Muller Hinton Agar (MHA) medium purchased from Himedia Laboratories Pvt.Ltd. Mumbai, India was used. The medium was rehydrated, sterilized in an autoclave and was poured into sterilized petri dishes and allowed to set. The plates were stored at 4 - 10 °C in refrigerator. Before inoculation, the surface of the petriplates was dried in an incubator.

3.3.5. Antibacterial Assay by Disc Diffusion Method:

The antibacterial activity and antibiotic sensitivity were tested by Disc Diffusion Method as described by Kirby *et al* in 1966 [24]. The dried plates were inoculated by the test strains

uniformly over the surface using a sterile cotton swab. A sterile 6 mm Whatmann No.1 filter paper loaded with appropriate extract was placed on the surface of the inoculum and gently pressed by a sterile forceps. Control discs (made up of each solvent like petroleum ether, acetone, methanol and water) were also placed on the surface of inoculum. The plates were incubated at 37 °C for 16 to 20 hrs. The zone of inhibition of bacterial growth around the disc was measured in millimeters. Tests were repeated three times and the mean values were calculated (mean fractions were avoided) and recorded.

Antibiotic sensitivity was compared with methanol extracts only because all other extracts (Petroleum ether, Acetone and Aqueous) were insignificant against the tested strains.

3.3.6. Determination of MIC of the Active Extracts:

The extract having promising activity was selected for studying their MIC against the most susceptible bacterial strain. A modified agar dilution method was employed for detecting the MIC [25]. (Detailed procedure is explained in the result section).

3.4. RESULTS

The results of the antibacterial activity of various extracts of selected plants against standard strains are given in Table 5 and the antibiotic susceptibility test by using standard antibiotics against the tested strains in Table 6.

Table No.5: Antibacterial activity of various plant extracts against standard strains:

Sl. No	Name of the plants	Bacterial strains tested (Zone of inhibition in mm)															
		1 (S)				2 (P)				3 (K)				4 (E)			
		Pe	A	M	Aq	Pe	A	M	Aq	Pe	A	M	Aq	Pe	A	M	Aq
1	*Vernonia cinerea*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2	*Asystasia gangetica*	-	-	7	-	-	-	-	-	-	-	-	-	-	-	-	-
3	*Ixora finlaysoniana*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
4	*Tiliacora acuminate*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5	*Gliricidia sepium*	-	6	7	-	-	6	8	-	-	8	10	-	-	7	12	-
6	*Ludwigia parviflora*	-	8	21	-	-	8	11	-	-	9	11	-	-	7	13	-
7	*Triumfetta rhomboidea*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
8	*Sida acuta*	-	-	12	-	-	-	7	-	-	-	8	-	-	-	7	-
9	*Sphagneticola trilobata*	-	-	10	-	-	-	-	-	-	-	6	-	-	-	-	-
10	*Waltheria indica*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
11	*Eryngium foetidum*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
12	*Senna alata*	-	-	12	-	-	-	-	-	-	-	-	-	-	-	-	-
13	*Cerbera odollam*	-	-	13	-	-	-	6	-	-	-	8	-	-	-	14	-
14	*Ipomoea cairica*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

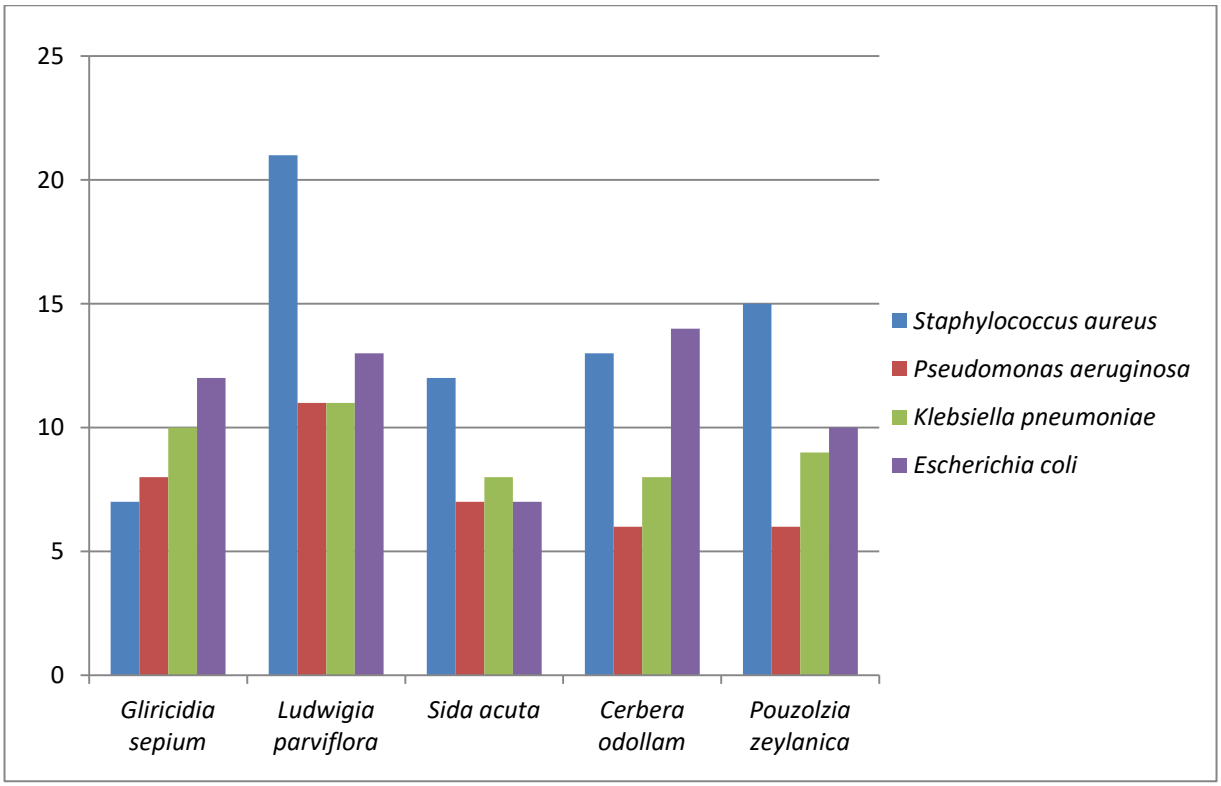
15	*Alternanthera bettzickiana*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
16	*Pseudarthria viscida*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
17	*Aerva lanata*	-	-	10	-	-	-	-	-	-	-	-	-	-	-	-	-
18	*Adiantum pedatum*	-	-	-	-	-	-	6	-	-	-	-	-	-	-	7	-
19	*Eleutheranthera ruderalis*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
20	*Leea indica*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
21	*Pouzolzia zeylanica*	-	7	15	-	-	6	6	-	-	6	9	-	-	6	10	-
22	*Aegle marmelos*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
23	*Pseuderanthemum reticulatum*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
24	*Ocimum basalicum*	-	-	11	-	-	-	-	-	-	-	-	-	-	-	-	-
25	*Euphorbia hirta*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

1(S): *Staphylococcus aureus*, 2 (P): *Pseudomonas aeruginosa*, 3(K): *Klebsiella pneumoniae*, 4 (E): *Escherichia coli.*

Table No. 6: Antibiotic susceptibility test using standard antibiotics against the tested strains:

Bacterial Strains	**Standard Antibiotics Tested (Zone of inhibition in mm)**					
	Chloramphenicol (30 mcg)	Gentamicin (10 mcg)	Penicillin G (10 u)	Ciprofloxacin (5 mcg)	Tetracycline (30 mcg)	Amikacin (30 mcg)
Staphylococcus aureus	25	27	0	35	15	34
Pseudomonas aeruginosa	11	10	0	30	0	16
Klebsiella pneumoniae	22	16	0	24	10	21
Escherichia coli	22	20	0	30	11	25

Figure 2: Antibacterial activity of the crude methanol extracts of selected plants against standard bacterial strains:



X axis: - methanol extracts of active plants; Y axis: zone of inhibition in mm.

Repeated experiments with control discs showed that they did not possess any inhibitory effect. This indicates that the solvent alone is ineffective to produce antibacterial activity. Similarly, the petroleum ether extracts and aqueous extracts were ineffective against the tested strains. This may be due to the fact that the antibacterial principles of the tested plants may be soluble in organic, polar solvents like methanol or partially polar solvent like acetone. Out of the twenty five plants tested for antibacterial activity, the acetone and methanol extracts of eleven of them showed various ranges of activity; slight activity, moderate activity or higher activity against one or more bacterial strains. An inhibition zone of 6-10 mm is considered as lesser activity, 11-15 mm as moderate activity and those of 15 mm and above is considered as higher

and powerful. An activity with 15 mm or above can only be considered for further studies including purification of the active principles.

The methanol extracts of *Asystasia gangetica, Senna alata, Aerva lanata, Ocimum basalicum* showed lesser or moderate activity against *Staphylococcus aureus* only. All other strains were not inhibited by the extracts. Methanol extracts of *Adiantum pedatum* showed lesser activity (6 mm and 7 mm) against *Pseudomonas aeruginosa* and *Escherichia coli*. The methanolic extracts of *Sphagneticola trilobata* was moderatively active against *Staphylococcus aureus* (10mm inhibition) and slightly active against *Klebsiella pneumoniae* (6mm inhibition). The acetone and methanol extracts of *Gliricidia sepium* were active against the all four tested strains. But the activity was not a promising one except the activity of methanol extracts against *Escherichia coli* (12 mm inhibition zone).

The acetone and methanol extracts of *Pouzolzia zeylanica* showed activity against all the four tested strains. Its acetone extracts were less effective (inhibition zone of 6 or 7 mm only) against all the strains. However, its methanol extracts showed prominent activity against *Staphylococcus aureus* with an inhibition zone of 15 mm and less activity was obtained against *Pseudomonas aeruginosa* (6 mm), *Klebsiella pneumoniae* (9 mm) and *Escherichia coli* (10 mm). The methanol extracts of *Cerbera odollam* showed moderate activity against *Staphylococcus aureus* (13 mm inhibition) and *Escherichia coli* (14 mm inhibition). Lesser activity was obtained against *Pseudomonas aeruginosa* (6 mm) and *Klebsiella pneumoniae* (8 mm) by the methanolic extracts. The methanol extracts of *Sida acuta* were active against all the four tested strains, but a moderate activity was found against *Staphylococcus aureus* with an inhibition zone of 12mm and all other strains were inhibited with less than 8 mm diameter (*Pseudomonas aeruginosa*; 7 mm

inhibition, *Klebsiella pneumoniae*; 8 mm inhibition and *Escherichia coli*; 7 mm inhibition) and these activities are not prominent in antibacterial studies.

The acetone extracts of *Ludwigia parviflora* were showed slight activity against all the tested strains (inhibition zone was less than 9 mm; not a prominent activity). But its methanol extract was very active against *Staphylococcus aureus* with an inhibition zone of 21 mm diameter. This activity is promising in the field of antibacterial studies. However, its methanolic extracts were moderately active against *Pseudomonas aeruginosa*; 11 mm inhibition, *Klebsiella pneumoniae*; 11 mm inhibition and *Escherichia coli*; 13 mm inhibition.

3.4.1. Determination of MIC of the methanol extracts of *Ludwigia parviflora* against *Staphylococcus aureus*:

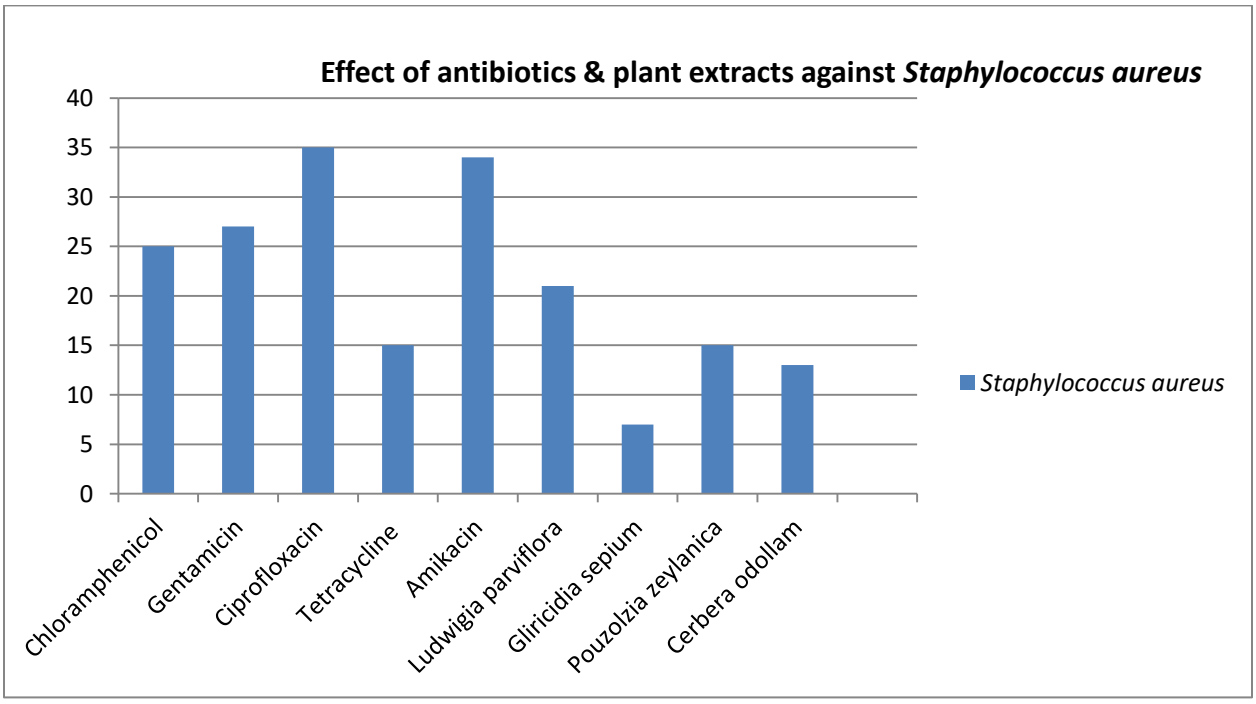
Since the methanol extract of whole plant of *Ludwigia parviflora* showed promising activity against *Staphylococcus aureus*, its MIC was studied. A modified agar dilution method was employed for detecting the MIC [25]. In this method, the methanolic extract of *Ludwigia parviflora* was incorporated in MHA so as to get various concentrations of the extract (1mg/ml, 3 mg/ml, 6 mg/ml and 10mg/ml respectively). The different tubes containing the extract in different concentrations were inoculated with 0.01 ml of the overnight incubated broth culture of *Staphylococcus aureus.* These were incubated at 37^0C for 12 –18 hrs. Tests were performed in triplicate and the lowest concentration of the extract that produced a complete suppression of colony growth was taken as MIC. The MIC study revealed that a test concentration of 3mg/ml was found as the MIC of *Ludwigia parviflora* against *Staphylococcus aureus*. This result is also a promising one in the field of antibacterial studies.

3.4.2. Comparison of the antibacterial activity of standard antibiotics with that of the active extracts:

The activity of standard antibiotics was compared with the active plant extracts by disc diffusion method [24]. Results are given in Table 6 and in Figures 3 – 6.

On analyzing the effects of standard antibiotic against the tested strains, it is evident that all the tested antibiotics such as Chloramphenicol, Gentamicin, Ciprofloxacin, Tetracycline and Amikacin were active against the four tested strains. (But none of the strains were inhibited by the Penicillin G which indicates that the Penicillin G is an outdated antibiotic in the antibacterial therapy). However, the strength of activity of effective antibiotics was different in different bacterial strains.

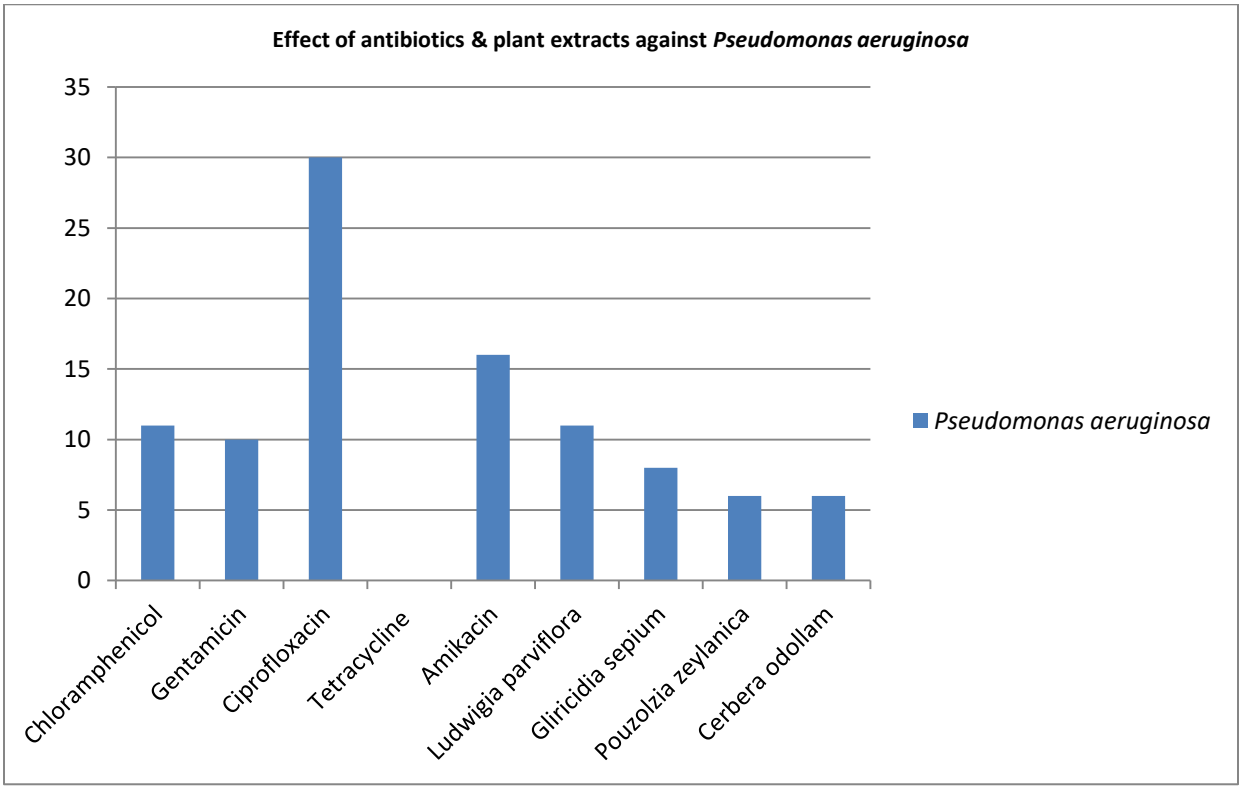
Figure 3: Comparison of the antibacterial activity of standard antibiotics with that of the active extracts against *Staphylococcus aureus*:



X axis: - Standard Antibiotics and active plant extracts; Y axis: - zone of inhibition in mm.

Maximum inhibition against *Staphylococcus aureus* was given by Ciprofloxacin 5 mcg with an inhibition zone of 35 mm diameter, and minimum inhibition by Tetracycline 30 mcg, with an inhibition zone of 15 mm. When comparing the results of the preliminary screening, it is clear that the crude methanol extract (10 mg/disc) of whole plant of *Ludwigia parviflora* (inhibition zone of 21 mm), has higher activity than Tetracycline 30 mcg and the crude methanol extract (10 mg/disc) of whole plant of *Pouzolzia zeylanica* (15 mm inhibition) is equivalent to Tetracycline 30 mcg. Therefore, the methanolic extracts of these two plants; *Ludwigia parviflora* and *Pouzolzia zeylanica* can be considered for a detailed study to suggest them as an alternative to Tetracycline 30 mcg in the treatment of disease caused by *Staphylococcus aureus*.

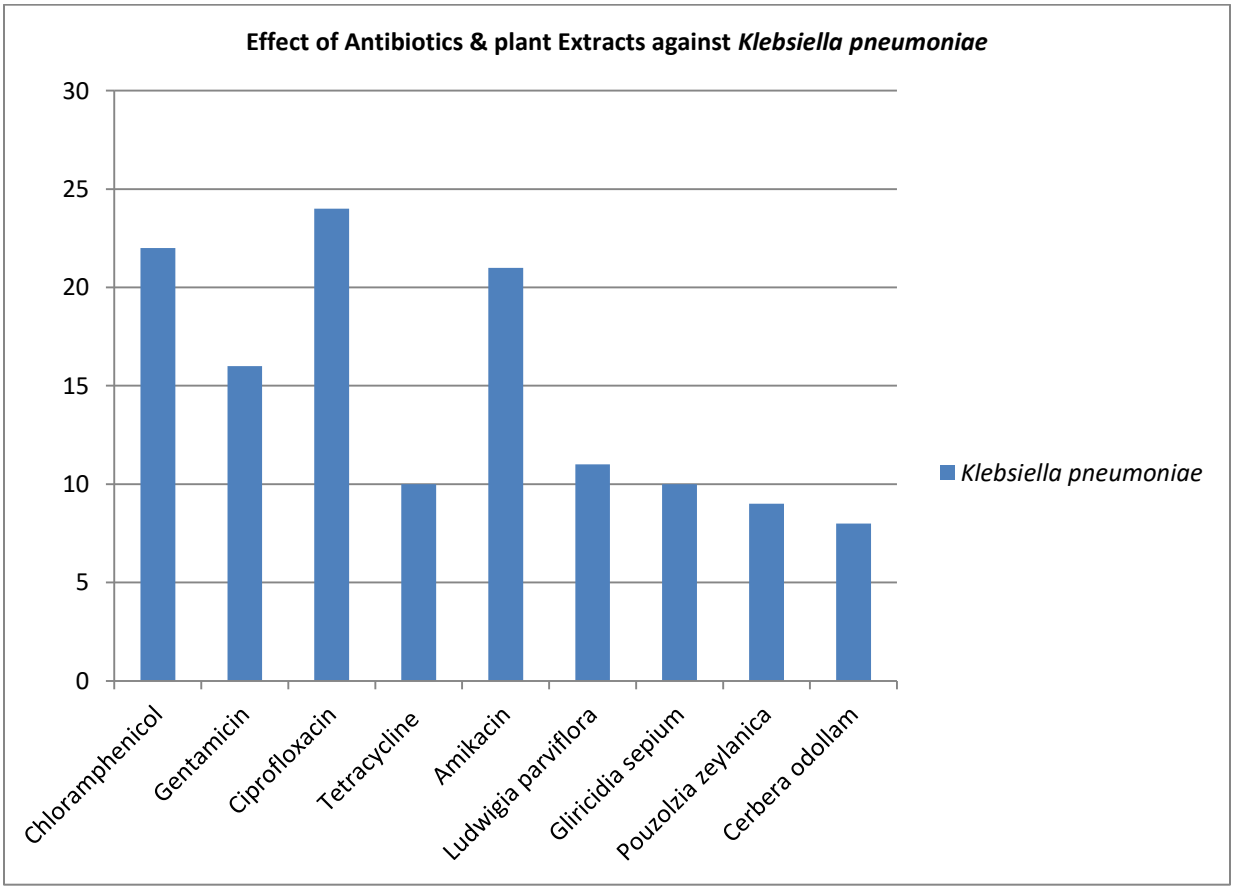
Figure 4: Comparison of the antibacterial activity of standard antibiotics with that of the active extracts against *Pseudomonas aeruginosa*:



X axis: - Standard Antibiotics and active plant extracts; Y axis: - zone of inhibition in mm.

On analyzing the effects of antibiotics against the *Pseudomonas aeruginosa*, it was seen that Ciprofloxacin 5 mcg showed highest inhibition (30 mm inhibition zone) and minimum inhibition (10 mm) is shown by Gentamicin 10 mcg. An interesting result is that the Tetracycline 30 mcg was totally resisted by the *Pseudomonas aeruginosa*. When comparing the tested samples, the crude methanol extract (whole plant) of *Ludwigia parviflora* showed 11 mm inhibition against *Pseudomonas aeruginosa*. Therefore, it can be concluded that the methanol extract of *Ludwigia parviflora* (10 mg/disc) is a perfect alternative for Gentamicin 10 mcg and Tetracycline 30 mcg against the infection caused by *Pseudomonas aeruginosa*.

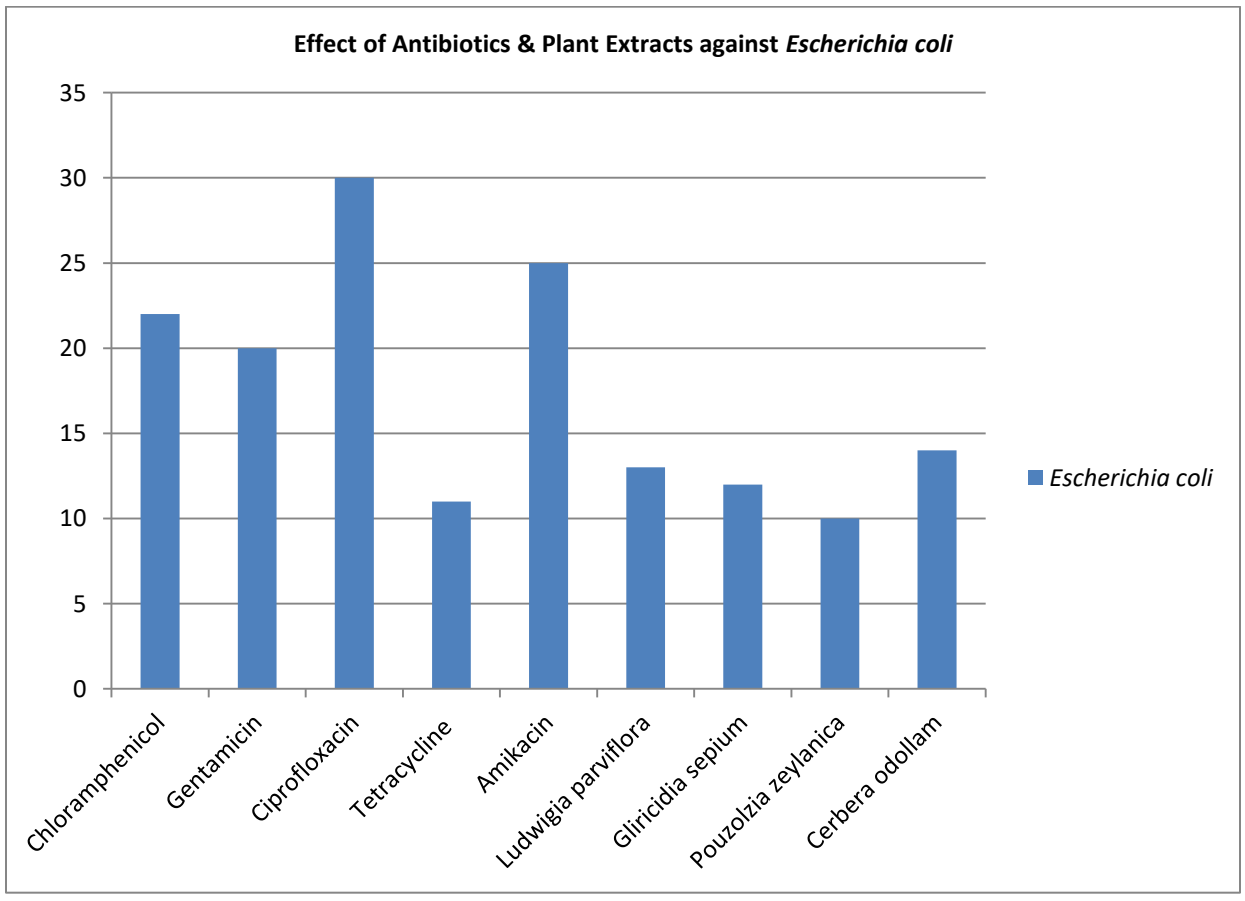
Figure 5: Comparison of the antibacterial activity of standard antibiotics with that of the active extracts against *Klebsiella pneumoniae*:



X axis: - Standard Antibiotics and active plant extracts; Y axis: - zone of inhibition in mm.

Chloramphenicol 30 mcg showed highest activity against *Klebsiella pneumoniae* (22 mm inhibition) and least inhibition by Tetracycline 30 mcg with a zone of 10 mm inhibition. Among the active extracts, the methanol extract (10 mg/disc) of leaf of *Gliricidia sepium* showed 10 mm inhibition and the methanol extract (10 mg/disc) of whole plant of *Ludwigia parviflora* showed 11 mm inhibition against the *Klebsiella pneumoniae*. Hence, it can be concluded that the crude methanol extracts of leaf of *Gliricidia sepium* and whole plant of *Ludwigia parviflora* can be considered as an alternative for Tetracycline 30 mcg; a common antibiotic used in the treatment of infections caused by *Klebsiella pneumoniae*.

Figure 6: Comparison of the antibacterial activity of standard antibiotics with that of the active extracts against *Escherichia coli*:



X axis: - Standard Antibiotics and active plant extracts; Y axis: - zone of inhibition in mm.

Highest inhibition against *Escherichia coli* was shown by Ciprofloxacin 5 mcg (30 mm inhibition) and least inhibition by Tetracycline 30 mcg (inhibition zone of 11 mm). Among the active plant extracts, the methanol extracts of three plants; leaf of *Gliricidia sepium* (10 mg/disc) showed 12 mm inhibition, whole plant of *Ludwigia parviflora* (10 mg/disc) showed 13 mm inhibition and the leaf of *Cerbera odollam* (10 mg/disc) showed 14 mm inhibition against the *Escherichia coli*. Therefore, the methanol extracts of leaf of *Gliricidia sepium*, whole plant of *Ludwigia parviflora* and leaf of *Cerbera odollam* can be considered for detailed study to suggest them as an alternative to Tetracycline 30 mcg in the treatment of diseases caused by *Escherichia coli*.

3.5. DISCUSSION

Since antiquity, plants have been used as source of medicine because of their magical power for curing diseases. They not only contain nutrients but also chemicals for providing health benefits. Anticancer, antioxidant, anti-inflammatory, anti-helminthic and antimicrobial agents are common in many plants. These chemicals are the byproducts of plant metabolism and are having specific physiological actions in our body [26, 27]. The aim of present study was to identify the antimicrobial activity of the selected plants of Upper Kuttanad. In this study, about twenty five plants were tested for their antimicrobial activity against standard bacterial strains. But the following four plants only showed various degrees of activity against one or few bacterial strains.

Ludwigia parviflora L. belongs to the family Onagraceae. It is commonly known as water primrose and is found in wet places, sandy river bed, along streams, rice field etc. It has been used in traditional medicine to relieve fever [28]. Its leaves and roots are also used in the treatment of ulcer, wound healing etc. [29]. Its decoction is useful in the treatment of dysentery [30]. One report from Selvamuthu B *et al* showed that its leaves contain some antibacterial activity [31]. The present study also suggests that the methanol extract of whole plant of *Ludwigia parviflora* possesses powerful antibacterial activity against *Staphylococcus aureus*, *Pseudomonas aeruginosa, Klebsiella pneumoniae* and *Escherichia coli*. It can be considered as a natural alternative to the common antibiotics such as Tetracycline and Gentamicin that are commonly used against the infection caused by *Staphylococcus aureus*, *Pseudomonas aeruginosa, Klebsiella pneumoniae* and *Escherichia coli*. Since the methanol extract of whole plant of *Ludwigia parviflora* showed promising activity against all the tested bacterial strains, the present study suggests that this plant should be taken into consideration for isolating the active

principles from it to develop a new broad spectrum antibiotic as an alternative to Tetracycline and Gentamicin.

Pouzolzia zeylanica (L.) Benn belongs to the family Urticaceae. The plant is distributed throughout the tropical countries. This plant has been used in the treatment of cough, urinary related problems, bacterial infections and helminthic infections by the traditional medicines in Vietnam. It is also used to control fly larvae during fish processing [32]. The brine shrimp lethality bioassay by Swati Paul and Dibyajyoti Saha suggests that this plant contains powerful anticancer activity [33]. Leaves are used to cure sores, boils and also to relieve gastric pain. Dibyajyoti Saha *et al* also reported that this plant has antibacterial activity against *Staphylococcus aureus* and *Escherichia coli* [34]. This study also indicates that the methanolic extract of whole plant of *Pouzolzia zeylanica* was active against *Staphylococcus aureus* and the activity was equivalent to that of Tetracycline 30 mcg. Therefore, this plant can be considered as a natural alternative for Tetracycline 30mcg against *Staphylococcus* infections; hence, further studies are suggesting for the isolation of narrow spectrum antibiotic from *Pouzolzia zeylanica* against *Staphylococcus aureus*.

Gliricidia sepium Jacq. belongs to the family Fabaceae. This plant has many uses; it is used as fuel wood, animal feed etc., its leaves have protein content, hence it serves as an effective cattle feed. The leaf extracts of *Gliricidia sepium* possess powerful larvicidal effect against Anopheles mosquito larvae [35]. Its leaf extracts also possess antibacterial activity against *E.coli* and nematicidal property against *Meloidogyne incodnita* [36]. Its flowers possess effective anti-inflammatory activity [37]. In the present study, its leaves were tested for determining their efficacy in antibacterial activity against *Klebsiella pneumoniae* and *Escherichia coli*, the result was a promising and found that the activity was equivalent to the

activity of Tetracycline 30 mcg against *Klebsiella pneumoniae* and *Escherichia coli*. Previous report shows that the leaf of *Gliricidia sepium* contains antibacterial activity against *E.coli*, the present report suggests that its leaf also possesses activity against *Klebsiella pneumoniae* and the activity is equivalent to the activity of Tetracycline 30 mcg. Therefore, this study suggests that the leaves of *Gliricidia sepium* can be considered for the purification of active principles from it for developing an efficient and specific (narrow spectrum) antibiotic against *Klebsiella pneumoniae*.

Cerbera odollam Gaertn. belongs to the family Apocynaceae. It is also known as pong pong tree. Its fruits are apple like in appearance. 'Cerberin' is a toxic chemical present in it and this chemical inhibits the functions of the heart. Its fruits have bio-insecticide and deodorant properties [38]. It is also used in the treatment of hydrophobia. The wood of *Cerbera odollam* has some application in the treatment of paralysis and its latex is an effective purgative agent [39, 40]. Report showed that the leaf extract of *Cerbera odollam* is an effective anticancer agent [41]. Its extracts also possess antioxidant and antifungal activities [42]. One report shows that the hexane extracts of its flower, fruit, leaf, wood and bark have antibacterial activity against *Bacillus subtilis* [43]. The present study also suggests that the crude methanol extract of leaf of *Cerbera odollam* possess antibacterial activity against *Escherichia coli* and the activity was equivalent to the activity of Tetracycline 30 mcg against *Escherichia coli*. Therefore, this plant can be considered as a natural alternative for Tetracycline 30mcg against *Escherichia coli* infections; therefore, further studies are suggesting for the isolation of narrow spectrum antibiotic from the leaves of *Cerbera odollam* against *Staphylococcus aureus*.

3.6. CONCLUSION

Twenty five plants collected from various regions of Upper Kuttanad of Alappuzha District of Kerala State were tested for antibacterial activity against standard MTCC bacterial strains by disc diffusion method. The activity was also compared with standard antibiotics. The results of the study revealed that acetone and methanol extracts of eleven of them showed various ranges of activity, but the effect of acetone extracts was not a promising one. Among the active methanol extracts, the whole plant extracts of *Ludwigia parviflora* with a crude concentration of 10 mg/disc was very active against all the tested strains. Its activity was higher than that of the activity of Tetracycline 30 mcg against *Staphylococcus aureus*, *Pseudomonas aeruginosa, Klebsiella pneumoniae* and *Escherichia coli*. It also showed slightly higher activity than the common antibiotic, Gentamicin 10 mcg against the *Pseudomonas aeruginosa*. Therefore, it can be concluded that the crude methanol extract of whole plant of *Ludwigia parviflora* with a concentration of 10 mg/disc is equivalent to Gentamicin and is also more powerful than Tetracycline. Hence, the methanol extract of whole plant of *Ludwigia parviflora* is a natural alternative to the common antibiotics such as Tetracycline and Gentamicin that are commonly used against the infection caused by *Staphylococcus aureus*, *Pseudomonas aeruginosa, Klebsiella pneumoniae* and *Escherichia coli*. Its MIC study revealed that a concentration of 3mg/ml was the minimum inhibitory concentration against *Staphylococcus aureus* which is also a promising result in antibacterial studies. The methanolic extract of whole plant of *Pouzolzia zeylanica* with a crude concentration of 10 mg/disc was active against *Staphylococcus aureus* and the activity was equivalent to that of Tetracycline 30 mcg. Similarly, the crude methanol extract of leaf of *Gliricidia sepium* at a concentration of 10 mg/disc showed activity against *Klebsiella pneumoniae* and *Escherichia coli* and the activity was found

equivalent to the activity of Tetracycline 30 mcg against *Klebsiella pneumoniae* and *Escherichia coli*. The crude methanol extract of leaf of *Cerbera odollam* was also effective to inhibit the growth of *Escherichia coli*. Its activity was also equivalent to the activity of Tetracycline 30 mcg against *Escherichia coli*. Hence, these three plant extracts of *Pouzolzia zeylanica*, *Gliricidia sepium* and *Cerbera odollam* can be utilized for developing natural alternative for the antibiotic, Tetracycline 30 mcg.

3.7. REFERENCES

1. Rosina Khan, Barira Islam, Mohd Akram, Shazi Shakil, Anis Ahmad, S. Manazir Ali, Mashiatullah Siddiqui and Asad U. Khan. Antimicrobial activity of five herbal extracts against multi drug resistant (MDR) strains of bacteria and fungus of clinical origin. *Molecules*. 2009; 14: 586-597.
2. K. O. Akinyemi, O. K. Oluwa, E. O. Omomigbehin. Antimicrobial activity of crude extracts of three medicinal plants used in south-west Nigerian folk medicine on some food borne bacterial pathogens. *Afr. J. Traditional, Complementary and Alternative Medicines*. 2006; 3 (4): 13 – 22.
3. Abu Shanab B, Adwan G, Abu-Safiya D, Adwan K and Abu Shanab M. Antibacterial activity of *Rhus Coriaria* L. extracts growing in Palestine. *J. Islamic University of Gaza*. 2005; 13(2):147-153.
4. Lee S.B., Cha K.H., Kim S.N., Altantsetseg S., Shatar S., Sarangerel O.and Nho C.W. The antimicrobial activity of essential oil from *Dracocephalum foetidum* against pathogenic microorganisms. *J. Microbiol.* 2007; 45: 53-57.
5. Betoni J.E.C., Mantovani R.P., Barbosa L.N., Di Stasi L.C.and Fernandes A. Synergism between plant extract and antimicrobial drugs used on *Staphylococcus aureus* diseases. *Mem. Inst. Oswaldo Cruz*. 2006; 101: 387-390.
6. S. Paudel, Thakur Magrati and J. R. Lamichhane. Antimicrobial activity of wild olive crude extracts *in vitro*. *International Journal of Pharma Sciences and Research*. 2011; 2 (3): 110-113.

7. Hero Farhad Salah Akrayi & Zirak Fage Ahmed Abdullrahman. Screening in vitro and in vivo the antibacterial activity of *Rhus coriaria* extract against *S. aureus*. IJRRAS. 2013; 15 (3): 390-397.
8. Firdaus Jahan, Rubina Lawrence, Vinod Kumar and Mohd Junaid. Evaluation of antimicrobial activity of plant extracts on antibiotic-susceptible and resistant *Staphylococcus aureus* strains. *Journal of Chemical and Pharmaceutical Research*. 2011; 3(4): 777-789.
9. Gislene G. F. Nascimento, Juliana Locatelli1, Paulo C. Freitas and Giuliana L. Silva. Antibacterial activity of plant extracts and phytochemicals on antibiotic resistant bacteria. *Brazilian Journal of Microbiology*. 2000; 31:247-256.
10. Amit Kapoor, Gurdeep Kaur and Rajinder Kaur. Antimicrobial activity of different herbal plants extracts: a review. *World Journal of Pharmacy and Pharmaceutical Sciences*. 2015; 4 (7): 422-459.
11. Selvamohan T., V. Ramadas S. and Shibila Selva Kishore. Antimicrobial activity of selected medicinal plants against some selected human pathogenic bacteria. *Adv. Appl. Sci. Res*. 2012; 3(5): 3374-3381.
12. Abhishek Mathur, Rakshanda Bhat, G.B.K.S. Prasad, V.K. Dua, Satish K. Verma and Pavan K. Agarwal. Antimicrobial activity of plants traditionally used as medicines against some pathogens. *Rasayan J Chem*. 2010; 3 (4): 615-620.
13. Amjad Khalil. Antimicrobial activity of ethanol leaf extracts of *Catharanthus roseus* from Saudi Arabia. *2012 2nd International Conference on Environment Science and Biotechnology IPCBEE*. 2012; 48: 6-11.

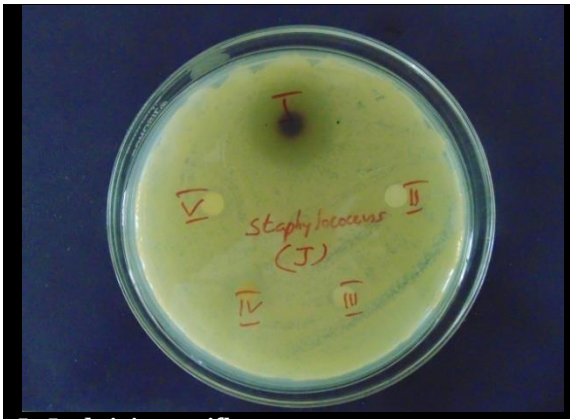
14. M. Mashiar Rahman, M. Mominul Islam Sheikh, Shamima Akhtar Sharmin, M. Soriful Islam, M. Atikur Rahman, M. Mizanur Rahman and M. F. Alam. Antibacterial activity of leaf juice and extracts of *Moringa oleifera* Lam. against some human pathogenic bacteria. *CMU. J. Nat. Sci*. 2009; 8(2): 219-227.
15. Priscila Ikeda Ushimaru, Mariama Tomaz Nogueira da Silva, Luiz Claudio Di Stasi, Luciano Barbosa and Ary Fernandes Junior. Antibacterial activity of medicinal plant extracts. *Brazilian Journal of Microbiology*. 2007; 38:717-719.
16. Sarah M. Wigmore, Mani Naiker and David C. Bean. Antimicrobial activity of extracts from native plants of temperate Australia. *Pharmacogn. Commn*. 2016; 6(2): 80-84.
17. Atikya Farjana, Nagma Zerin and Md. Shahidul Kabir. Antimicrobial activity of medicinal plant leaf extracts against pathogenic bacteria. *Asian Pacific Journal of Tropical Disease*. 2014; 4 (2): S920-S923.
18. Antara Sen and Amla Batra. Evaluation of antimicrobial activity of different solvent extracts of medicinal plant: *Melia azedarach* L. *International Journal of Current Pharmaceutical Research*. 2012; 4 (2): 67-73.
19. Ali Sadeghian, Ahmad Ghorbani, Ahmad Mohamadi-Nejad and Hassan Rakhshandeh. Antimicrobial activity of aqueous and methanolic extracts of Pomegranate fruit skin. *Avicenna Journal of Phytomedicine*. 2011; 1 (2): 67-73.
20. Adegoke AA, Iberi PA, Akinpelu DA, Aiyegoro OA and Mboto CI. Studies on phytochemical screening and antimicrobial potentials of *Phyllanthus amarus* against multiple antibiotic resistant bacteria. *International Journal of Applied Research in Natural Products*. 2010; 3 (3): 6-12.

21. Anjana Sharma, Rani Verma and Padmini Ramteke. Antibacterial activity of some medicinal plants used by tribals against UTI causing pathogens. *World Applied Sciences Journal*. 2009; 7 (3): 332-339.
22. Shibumon George and Benny PJ. Antibacterial potency of fruit extracts of *Flacourtia inermis* against multidrug resistant strains and comparison of its activity with that of standard antibiotics. *International Journal of Pharmaceutical Science and Biotechnology*. 2010; 1(2):96-99.
23. Shibumon George, Benny PJ, Sunny Kuriakose and Cincy George. Antibiotic activity of 2, 3-dihydroxybenzoic acid isolated from *Flacourtia inermis* fruit against multidrug resistant bacteria. *Asian Journal of Pharmaceutical and Clinical Research*. 2011; 4(1): 126- 130.
24. Kirby, M.D.K., Bauer, A.W., Sherris, J. C and Turck, M. Antibiotic susceptibility testing by standardized single disc diffusion method. *Am J Clin Pathol*. 1966; 45, 493-496.
25. Voravuthikunchai S.P. and Limsuwan S. Medicinal plant extracts as anti- *E. coli* 0157: H 7 agents and their effects on bacterial aggregation. *J. Food. Prot*. 2006; 69 (10): 2336-2341.
26. Hayam M. Ibrahim and Ferial M. Abu-Salem. Antibacterial activity of some medicinal plant extracts. *International Journal of Biological, Biomolecular, Agricultural, Food and Biotechnological Engineering*. 2014; 8 (10): 1168 – 1173.
27. Kong JM, Goh NK, Chia LS, Chia TF. Recent Advances in traditional plant drugs and orchids. *Acta Pharmacol*. 2003; 24:7-21.
28. Datta SC and Banerjee AK. Useful weeds of west Bengal rice fields. *Economic Botany*. 1979; 32: 297-310.

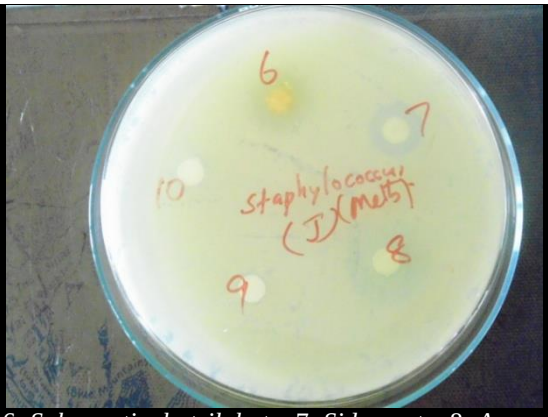
29. Mohammad Shamim Qureshi, A Venkateshwar Reddy, G.S. Kumar and Lubna Nousheen. Ethnobotanical study on medicinal plants used by traditional health practitioners and villagers of Garhphulghar Gram, Chhattisgarh, India. *Asian Journal of Pharmaceutical Research*. 2017; 7 (2): 98-105.
30. Shyamal Kanti Mallick. Floristic study of some weeds found in the rice field of Palita of the district Burdwan, West Bengal. *Remarking*. 2015; 2(5): 27-29.
31. Selvamuthu B, Seetharaman S, Indra V and Daisy A. Antibacterial activity of methanolic extract of *Ludwigia perennis* leaves. *World Journal of Pharmacy and Pharmaceutical Sciences*. 2016; 5 (7): 1186-1193.
32. Nguyen Ngoc Bao Chau, Ly Thi Minh Hien and Dang Thi Tinh. Bioefficacy of leaf extracts from *Pouzolzia zeylanica* (L.) Benn against diamondback moth *Plutella xylostella* in Vietnam. *Journal of Science Ho Chi Minh City Open University*. 2017; 7(2), 44-50.
33. Swati Paul and Dibyajyoti Saha. *In Vitro* screening of cytotoxic activities of ethanolic extract of *Pouzolzia zeylanica* (L.) Benn. *International Journal of Pharmaceutical Innovations*. 2012; 2(1): 52-55.
34. Dibyajyoti Saha, Swati Paul and Srikanta Chowdhury. Antibacterial activity of ethanol extract of *Pouzolzia zeylanica* (L.) Benn. *International Journal of Pharmaceutical Innovations*. 2012; 2(1): 1-5.
35. Jiby John Mathew, Prem Jose Vazhacharickal, Sajeshkumar N.K and Jesmi Sunil. Larvicidal acitivity of *Gliricidia sepium* leaf extracts on mosquito larvae and its lethal effect on non targeted organisms. *CIB Tech Journal of Biotechnology*. 2015; 4 (2): 13-19.

36. T. Jasmine, R. Meenakshi Sundaram, M. Poojitha, G. Swarnalatha, J. Padmaja, M. Rupesh Kumar and K. Bhaskar Reddy. Medicinal properties of *Gliricidia sepium*: a review. *International Journal of Current Pharmaceutical & Clinical Research*. 2017; 7(1): 35-39.
37. Kola Phani Kumar, Vadite Siva Naik, V.Bhuvan Chandra, R. Lavanya, K. Narendra Kumar, V.Bhagyasree, B.Soumya and Lakshmi Sudeepthi N. Evaluation of *In Vitro* and *In Vivo* anti-inflammatory activity of aqueous extract of *Gliricidia sepium* flowers in rats. *International Journal of Pharmacognosy and Phytochemical Research*. 2014; 6(3): 477-481.
38. Md. Siddiqul Islam and Zebunnesa Ahmed. A pharmacological and phytochemical review of *Cerbera odollam* a plant with significant ethnomedicinal value. *European Journal of Pharmaceutical and Medical Research*. 2017; 4(12): 19-21.
39. Kuddus M.R., Rumi F., Masud M.M. and Hasan C.M. Phytochemical screening and antioxidant activity studies of *Cerbera odollam* Gaertn. *Int J Pharma Bioscience*. 2011; 2: 413-418.
40. Naskar K.R. and Bakshi D.G. Some of the important medicinal plants of Apocynaceae from West Bengal. *Bull. Bot. Soc. Bengal*. 1981; 35: 7-14.
41. Syarifah M.S., Nurhanan M.Y., Haffiz J.M., Ilham A.M., Getha K., Asiah O., Norhayati I., Sahira H.L. and Suryani S.A. Potential anticancer compound from *Cerbera odollam*. *Journal of Tropical Forest Science*. 2011; 89-96.
42. Abinash Sahoo, Thankamani Marar. Phytochemical analysis, antioxidant assay and antimicrobial activity in leaf extracts of *Cerbera odollam* Gaertn. *Pharmacogn J*. 2018; 10(2): 285-292.
43. M.H.M. Amini, R. Hashim, N.S. Sulaiman, O. Sulaiman,S.F. Sulaiman, F. Abood, F. Kawamura, R. Wahab, M. Mohamed and M.S.M. Rasat. Antibacterial activity of different biomass components of *Cerbera odollam* and their potential to be used as new preservative for wood based products. *Applied Mechanics and Materials*. 2015; 754-755:1040-1044.

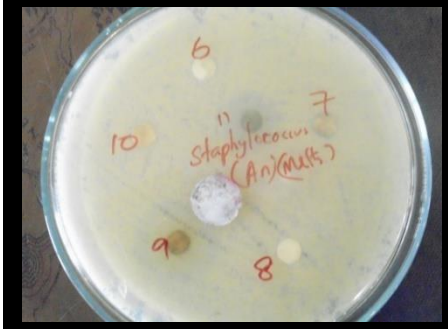
Photograph of antibacterial activity of methanolic extract of active plants & antibiotic susceptibility test:



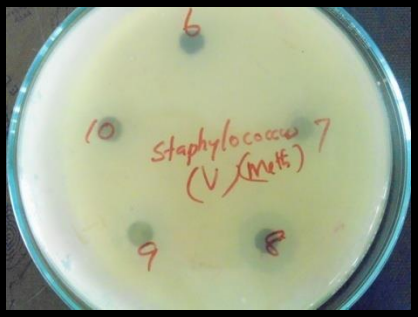
I:-*Ludwigia parviflora*



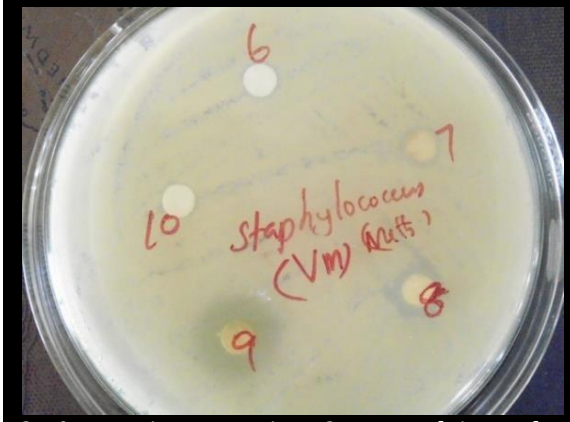
6: *Sphagneticola trilobata*, 7: *Sida acuta*, 8: *Aerva lanata*



11: *Senna alata*

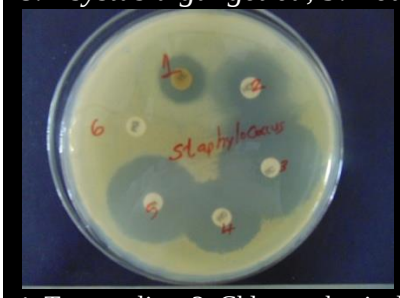


8: *Ocimum basalicum*



8: *Asystasia gangetica*, 9: *Pouzolzia zeylanica*

1: Tetracycline; 2: Chloramphenicol; 3: Ciprofloxacin; 4: Gentamicin; 5: Amikacin; 6: Penicillin G



1: Tetracycline; 2: Chloramphenicol; 3: Ciprofloxacin; 4: Gentamicin; 5: Amikacin; 6: Penicillin G

1: Tetracycline; 2: Chloramphenicol; 3: Ciprofloxacin; 4: Gentamicin; 5: Amikacin; 6: Penicillin G