EVALUATION OF ANTIBACTERIAL PROPERTY OF SOME MEMBERS OF ACANTHACEA FAMILY USED IN TRADITIONAL MEDICINE

REPORT OF MINOR PROJECT SUBMITTED TO THE UNIVERSITY GRANTS

COMMISSION, NEW DELHI

Submitted by

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CHAPTER I

INTRODUCTION

Microorganisms like bacteria, viruses, fungi and protozoa are responsible for causing many infectious diseases. Infectious diseases account for approximately 50% of all deaths in tropical countries. In industrial nations despite the progress made in the understanding of microorganisms and their control, incidents of epidemics due to drug resistant microbes and the emergence of hitherto unknown disease causing microbes pose enormous public health concerns. Death from infectious diseases, ranked fifth in 1981 became the third leading cause of death in 1992 (Pinner *etal*,1996). Furthermore the most dramatic increases occurred in the 25 to 40 year old age group (Pinner *et al*, 1996).

After the discovery of disease causing microorganisms, man made an attempt to find a way to control the causative microorganisms. So his interest turned to the pathogens that harmful to mankind and it also made him realize that every organism has its own capacity to protect against its enemies in various ways. These organisms may produce metabolic waste products that change the condition in a media, such as pH, osmotic pressure and surface tension. This causes the less tolerant organisms to grow in unfavourable conditions. It may elaborate specific toxic substances that may interfere with the metabolism of other organisms, such that they are either killed or prevented from multiplying. These specific toxic substances elaborated by living organisms are called antibiotics and the phenomenon is referred to as antibiosis.

The discovery of penicillin by Alexander Fleming was a milestone in the treatment of infections. Penicillin, because of the impetus of World War 11, was the first antibiotic to be produced on large scale, and it still is one of the best antibiotics available. It was

extracted from *Penicillin notatum*. Subsequently many other antibiotics were extracted from other organisms and most infectious diseases could be controlled. But unfortunately, over the years many new infections emerged and the already existing germs developed drug resistance. Moreover most of the antibiotics had deleterious side effects. So search was conducted for more effective and safer sources of antimicrobials. Plants were considered to be a treasure house of biologically active antimicrobial compounds and were used to cure many ailments since ancient times in all cultures of the world.

Medicinal plants are gifts of nature and are used to cure limitless numbers of disease among human beings. They have genuine utility and many people depend on it for primary health care. The WHO has been advocating the needs for orthodox medical practitioners to interact with traditional herbal healers with a view to identifying and exploiting aspects that provide safe and effective remedies for ailments caused by microbes and diseases caused due to other reasons.

Traditionally used medicinal plants produce a variety of compounds of known therapeutic properties. The antimicrobial properties of medicinal plants are being increasingly reported from different parts of the world. It is expected that plants extracts showing target sites other than those used by antibiotics will be active against drug – resistant microbial pathogen (Ahmad , 2001)

Plants are the source of many powerful drugs ; they contain certain compounds and toxic substances that could bring an inhibitory effect upon the harmful organisms. The plants produce a great variety of secondary products. These include essential oils, alkaloids, saponins and fatty acids etc which are useful in the field of pharmaceutical, agrochemical, fragrance and food industries. These compounds are

important for defence of plant against pathogenic organisms and herbivores. Many phyto-chemicals act

as protoxins for insects which further modify these compounds and incorporate them into their own defence.

The effect of plant extracts on bacteria has been studied by a very large number of researches in different parts of the world (Reddy, 2001). The demand for drugs from plant source is continuously increasing which necessitates screening medicinal plants with promising biological activity (Sumathi, 2010) A vast amount of research has been carried out in this context. Dabur *et al* (2007) carried out a survey to assess antimicrobial activity of some Indian medicinal plants. Ethnomedicinal plants used by Nilgiri tribals were screened for antibacterial property by Sasikumar *et al.,* (2007) The antibacterial activity of herbal products used in Pakistan was studied by Walter *et al*., (2011).

The Acanthaceae is a large dicot family and many of its plants are used in traditional systems of medicine. Some species of the Acanthaceae family present antimicrobial and antibacterial activities (Meurer,1996) and the chemical studies within this family was related to the isolation of alkaloids, lignins, flavonoids, terpenoids and phenylpropanoid glycosides (Berrondo 2003).

For the present study six plants were selected viz., *Justicia simplex* D. Don., *Asystasia gangentica* T.And., *Strobilathus heyneanus* Nees., *Andrographis paniculata* Nees., *Adathoda vasica* Nees. and *Justicia gendarussa* Linn.

Justicia simplex. : A diffuse slender plant with many divaricate branches and ovate- lanceolate leaves. Flowers white, calyx 5 partite; corolla 2-lipped; stamens 2; ovary bi- celled; fruit a papery capsule with villous apex. The plant is used as a diuretic,

stomachic, expectorant and anthelminthic by the hill tribes of Bangla Desh. The leaves contain a bitter and slightly toxic alkaloid and some aromatic amines .

Asystasia gangentica , commonly known as Chinese violet is a perennial herb with branched quandrangular stem often rooting at the lower nodes. The leaves are opposite and ovate to lanceolate. Inflorescence is a terminal raceme; bracts opposite and small; bracteoles minute; calyx deeply 5- partite, lobes equal; corolla tube cylindric below, inflated above, ventricose or funnel shaped, 5- lobed, lobes sub- equal, imbricate in bud; stamens 4, didynamous; ovary 2- celled; fruit an elliptical capsule. This plant is used as a leaf vegetable and fodder in many parts of the world. The sap is applied to wounds and sores. It is used as an analgesic .In Africa it is used to cure snake- bite and stomach ache. In India sap is used to treat rheumatism. Leaves and flowers are used as intestinal astringent.

Strobilanthes heynianus is a small shrub with pale blue flowers. The leaves are toothed; flowers in interrupted bracteates spikes; caalyx deeply 5- partite, lobes narrow; corolla larger, upper part ventricose, lower part cylindric, 5- lobed, twisted to left in bud. Stamens didynamous; ovary 2- celled; fruit a 2- 4 seeded capsule. The plant finds use in the treatment of skin diseases and diabetes. It is a blood purifier also.

Adthoda vasica is a dense shrub with foetid smell and large yellowish green entire leaves. Flowers are white with red or yellow streaks and are sessile in the axils of opposite bracts in dense axillary and terminal spikes.Bract herbaceous and large while bracteoles are small and narrower. Calyx 5-partite,lobes imbricate corolla tube short and 2- lipped, stamens 2mand ovary 2- celled. Fruit is a clavate capsule with solid base.

The leaves of this plant contain 45-95% vascicine, which is used for developing the mucolytic drug Bromhexine. Vascicine stimulates respiratory activity and is used as a

bronchodilator in treatment of coughs and colds. The leaves are also used as a poultice in the joints to relieve cramps.

Andrographis paniculata is a small plant with tetragonous stem. The leaves are lanceolate and acute at both ends. Flowers are bracteolate; bracts small, bracteoles absent, flowers in elongated racemes. Calyx 5- partite, lobes narrow; corolla small, spotted with brown- purple dots, tubular at the base and prominently 2- lipped ; upper lobe slightly bifid. Stamens 2, anthers bearded at the base. Ovary 2- celled, fruit an ellipsoidal capsule.

This plant is an immune-stimulant and has anti bacterial, antiviral, antiparasitic and tonic properties. The active chemical andrographolide present in the plant extracts help to stop clumping of blood platelets. It is also used to cure leprosy.

Justicia gendarussa is a small shrub with flowers in spikes. The bracts are small and linear ; bracteoles absent. Calyx 5-partite,lobes narrow; corolla tube bilipped, white with purple spots, upper lip bilobed, lower 3- lobed. Stamens 2, ovary 2-celled, fruit a glabrous capsule.

It is useful in asthma, rheumatism and colic of children. It is a natural remedy for respiratory tract diseases and also provides relief from inflammation, arthritis, migraine fever and constipation. It also provides immunity against human Influenza Virus (H1N1).It prevents formation of new blood vessels from existing ones and hence thought to be potentially useful in the treatment of cancer.

Four pathogenic bacteria were selected as test organisms to assess the antibacterial activity of the different plants under study.

Staphylococcus aureus is a facultative anaerobic gram positive coccus. It is the causative agent of many suppurative processes ranging from localized abscesses which

can occur any where in the body to fatal septicaemia and pneumonia. It is distinguished by its golden yellow pigment.

Escherichia coli is a facultative anaerobic gram negative rod shaped bacteria which is a part of the normal floral of intestinal tract. Certain strains can cause a moderate to severe gastroenteritis in humans and animals. It is used as an indicator organism to detect faecal contamination of water.

Pseudomonas aeruginosa is an aerobic gram negative non spore forming rods producing a furanose blue pigment pyocyanin. It is a common saprophyte but some time is found in unhealed wounds. It has been implicated in some out breaks of diarrhoea especially among new born children.This organism was frequently reported to have developed multidrug resistance to many of the antibiotic. Therefore it is not surprising to learn that *P. aeruginosa* is the least responding bacterial strain to the tested plant extraction in many studies.. (Dogan *et al*.2010)

Klebsiella aerogenes is also a bacterium of coliformgroup. It consists of non motile capsulated rods. Most strains are saprophytic but they are sometimes detected as commensals in human and animal intestine. They display an opportunistic pathogenic ability in the respiratory tract. The present study considered effect of plant extract on growth of bacteria by measuring turbidity after a specified period of time (end point detection). In the second set of experiments antibacterial activity was assessed by well method.

CHAPTER II

REVIEW OF LITERATURE

Man has been involved in the study of diseases since time immemorial. The main thrust of these studies involves finding suitable control measures to overcome these diseases. The discovery of penicillin by Alexander Fleming and the subsequent discovery of other antibiotics led scientists to believe that man has emerged victorious in the fight against disease. This belief was dealt a blow when many new diseases emerged, and diseases thought to be completely eradicated resurfaced. The main reason for these new outbreaks was the acquisition of antibiotic resistance by the infectious agents.

In recent times, focus on plant research has increased all over the world and a large body of evidence has collected to show the immense potential of medicinal plants used in various traditional systems. Many plants that are traditionally used for medicinal purposes have proven therapeutical effects. Various attempts are being made to isolate the active principles in these plants and use them for preparation of various medicines.

The plant chemicals that have disease preventive properties are called phytochemicals. There are more than a thousand known phytochemicals. Plants produce these phytochemicals for self-defence. But recent researches demonstrate that they can protect humans against disease. Many phytochemicals have curative, anticarcinogenic, antioxidant and antibacterial properties. Plants produce a great variety of secondary metabolites.

Ghosh (2000) conducted an extensive study on polyamines and alkaloids and found that the antimicrobial activity of plant extracts can be mainly attributed to the presences of alkaloids. There are many plants which are used for medicinal purposes, and these belong to different angiosperm families. Proper documentation and identification of these plants should be done to provide scientific basic to studies on medicinal properties of plants.

Man's desire to find toxic substances against the pathogens and use of plants as medicine is as old as civilization itself. In ancient days the practice of using plants as curative agents was mainly based on the morphological feature of the plants that showed similarity with human organs. During the age of the herbals, which lasted from 1470-1670, a parallel development that happened was the doctrine of signature. This was based on the belief that plant and plant parts resembling human body parts or organs may be used as cure for diseases of such parts. This is mainly based on superstitions and traditions inherited from the middle ages. This definitely aroused an interest in searching for more medicinal plants. The names of some plants still owe their origin to this doctrine. For example, the plants that resembled the shape of liver were used for treatment of liver diseases, e.g. bryophytes, or liverworts, Such examples can also be found in the herbals and in ancient Indian writings, and all classification of plants were practical to begin with (Sivarajan, 1996).

The discovery of different antibiotics led to a decline in the use of natural plants products in the last century. Indiscriminate use of synthetic drugs has given rise to many other health problems, and this led to a regeneration in the study of useful medicinal plants and their products. Traditional medicine is a store house of information regarding the medicinal property of many plants – but often there is no written record of these- the only way of transmission from one generation to another is by way of recitation

or rote. It is the duty of the present scientific community to document this ‘folk knowledge’ and provide the required scientific proof.

The plant extracts contain certain compounds that areinhibitory to the growth of pathogens. Plants produce a great variety of secondary metabolites. These include essential oils, alkaloids, saponins, fatty acids etc. which are useful in the field of pharmaceuticals, agro chemicals, fragrance and food industries. Taditionally used medicinal plants produce a number of compounds of known therapeutic properties. In recent years antimicrobial activity of medicinal plants are being increasingly reported from different partsof the world. Vichkanova (1971) tested the effect of saponin compounds extracted from 18 different plants. From the extracts, 9 of the 62 preparationswere active mainly against fungi and protozoa. Later, antimicrobial activity of other secondary metabolites was studied by Abdou (1972). Khrissagar and Mehra (1972) evaluated antimicrobial activity of ferns. Mitcher (1978)screened 54 species of plants and got positive antimicrobial results.

Andrographis paniculata was shown to have antibacterial property by Mishra *et al.,(*2007). *Acanthus ilicifolius* collected from Karnataka mangroves was shown to have antimicrobial activity by Khajure and Rathod, (2010). *In vitro* antibacterial studies in *Andrographis paniculata* by AnielKumar and coworkers yielded positive results. Antifungal components were isolated from *Brillantsia lamium* (Tamokou, 2009). Rashmi and Mathew (2012) found that the antimicrobial activity of Justicia adhathoda could be attributed to the presence of vasicine. Pharmochemical characterization and antibacterial activity of *Asystasia gangetica* was carried out by Daffodil *et al.* (2013). They found that the whole plant contained various bioactive compounds.

It is expected that plant extracts showing target sites other than those used by antibiotics will be active against drug- resistant microbial pathogens. However very little information is available on such activity of medicinal plants (Ahmad and Beg,2001). In addition to their antimicrobial activity, plant extracts also possess other medicinal properties. Many of them have toxic, diuretic, anti-diabetic, anti-carcinogenic and laxative properties, to name a few (Ilango *et al*., 2009).

The family Acanthaceae (or Acanthus family) is a taxon of dicotyledonous flowering plants containing almost 250 genera and about 2500 species. Most are tropical herbs, shrubs, or twining vines; some are epiphytes. Only a few species are distributed in temperate regions. The four main centres of distribution are Indonesia and Malaysia, Africa, Brazil and Central America. The representatives of the family can be found in nearly every habitat, including dense or open forests, in scrublands, on wet fields and valleys, at the sea coast and in marine areas, and in swamps and as an element of mangrove woods. The Acanthaceae family is an important source of therapeutic drugs, and the ethno pharmacology knowledge of this family requires urgent documentation as several of its species are near extinction (Correa and Alcantara , 2012). They reviewed the chemical constituents and biological activity of Brazilian species of *Justicia. Justicia* is the largest genus of Acanthaceae with about 600 species. Many genera of Acanthaceae are used in traditional medicine (The Ayurvedic Pharmacopeia of India, 1999). Jadhav (2009) evaluated the ethno medicinal plants used in leaf therapy by tribes of Ratlam. Vivek Kumar *et al* (2011) conducted pharmacognostic studies on *Asystasia dalzelliana*.

Different species of the genus *Hygrophilla* were found to be useful medicinally, (Shanmugasundaran and Venkataram, 2006; VijayaKumar *et al*, 2006; Patra e*t al* 2008; and Sarfaraj Hussain *et al* 2009). Another valuable plant is *Adhathoda vasika*

or ‘Vasa’ (The Ayurvedic Pharmacopeia of India, 1999). Antimicrobial activity of many other Acanthaceae genera was evaluated by Chopra *et al*, (1956)- *Andrographis paniculata, Asteracantha* sps, *Justicia* sps, to name a few. The antibacterial activity of leaf extract of *Justicia gendarussa* was studied by Reddy *et al(2013).* Their study suggests that this plant may represent a new source of a stable biologically active antibacterial compound which can be used in modern medicine.

It can be safely suggested that the role of traditional medicines in resolving health problems is invaluable on a global level. Medicinal plants continue to provide valuable therapeutic agents, in both modern and alternative forms of medicines. Traditional knowledge will be lost over time if concerted efforts are not made to document and valuable information.

CHAPTER II

MATERIALS AND METHODS

A. PLANTS SELECTED

Six plants belonging to the family Acanthaceae which were available locally were selected for testing their antibacterial activity. The list of the plants used for this study is given in Table-I.

Table - I

Serial Number	Botanical Name	Vernacular Name	Part used for the studies
1	*Justicia simplex*	Shrimp plant	Leaves
2	*Asystasia gangetica*	Kaka poovu,	Leaves
3	*Strobilathus heyneanus*	Karimkurinji	Leaves
4	*Adathoda vasica*	Adalotakam	Leaves
5	*Andrographis paniculata*	Kiriyaat	Leaves
6	*Justicia gendarussa*	Vathakolli	Leaves

B. BACTERIAL ISOLATES

\Four bacterial isolates were used as test organisms. The bacteria used for this study were: *Escherichia coli, Klebsiella aerogenes, Pseudomonas aeruginosa* and *Staphylococcus aureus* .

The cultures of the bacteria were done on Nutrient –Agar medium prepared according to the composition given below.

Beef extract - 3.0g

Peptone - 5.0g

NaCl - 5.0g

Agar-agar - 20g

Distilled water - 1000ml

pH - 6.5

The prepared medium was poured into test tubes up to about 1/3rd level and the test tubes were plugged with cotton wool and sterilized in an autoclave for 15 minutes at 120C and 15lbs pressure. The test tubes were kept in a slanting position and the medium allowed to solidify. These agar slants were then

used for sub culturing the bacteria. To study the effect of plant extract on the growth of the bacteria , these were cultured in Nutrient broth medium prepared according to the following compositions.

Peptone - 5g

Beef extract - 3g

Distilled water - 1000ml

C. **EFFECT OF PLANT EXTRACT ON GROWTH OF BACTERIA IN BROTH MEDIUM**

PREPARATION OF PLANT EXTRACT

The plant materials were washed in distilled water and then blotted dry with blotting paper. 100grams of the plant material was weighed and ground in a mortar and pestle to a fine paste using 10-50 ml of Nutrient Broth medium. The extract was then filtered through a fine muslin cloth. The extract thus obtained was then made up to 250ml with Nutrient Broth, so that the final concentration is about 2.50mg plant extract/ ml of the medium. This was taken as the stock extract. From this stock solution, different concentrations of plant extract were prepared by diluting with the required amount of Nutrient Broth medium.

5ml of each concentration was taken in a test tube. For each concentration a replicate was taken. The test tubes were plugged with cotton wool and sterilized in an autoclave. Each test tube was inoculated at 30+/-2^0 Cfor 24hrs. After 24hrs the turbidity of cultures were evaluated by measuring the optical density at 600nm using a colorimeter. Un-inoculated medium having plant extract of same concentration was taken as blank in each case.

CHAPTER III

RESULTS

Four bacteria, *Escherichia coli, Klebsiella aerogenes, Pseudomonas aeruginosa and Staphylococcus aureus* were selected as test organisms to evaluate the antimicrobial activity of the six plants belonging to the family Acanthaceae. The plants selected for this study were ., *Justicia simplex* D. Don., *Asystasia gangentica* T.And., *Strobilathus heyneanus* Nees., *Andrographis paniculata* Nees., *Adathoda vasica* Nees. and *Justicia gendarussa* Linn.

Significant results were obtained on testing growth of bacteria in plant extract supplemented broth medium and compared with growth in unsupplemented Nutrient broth. Growth was measured by turbidity analysis.

1. ASYSTASIA GANGETICA Leaves of this plant were ground in a motar and pestle using nutrient broth medium, and from the stock solution concentration different dilutions were prepared.

The following results were obtained.

Escherichia coli – when *E. coli* was grown in plain Nutrient broth medium an optical density of 0.39nwas obtained at 600nm. This was taken as the control reading for this organism in all further experiments.

Growth of *E. coli* was inhibited to different degrees at the various concentrations studied. 28% inhibition was obtained at 25mg/ml concentration which increased to 97% at 400mg/ml concentration. There was thus a graded increase in inhibition of this bacterium by *Asystasia* extract, except at 200mg/ml concentration. This may be attributed to some error in experimentation.

Klebsiella aerogenes- yielded an OD of 0.6 at 600nm when grown in plant extract free Nutrient broth medium. There was a drastic inhibition of 92% growth of this bacteria even at concentration as low as 25mg/ml, and when the concentration was increased above this level there was a complete cessation of growth.

Pseudomonas aeruginosa **–** gave an OD of 0.3 as control reading. On growing this bacteria in plant extract supplemented medium it was observed that growth was not affected at 25mg/ml concentration. On further increasing the concentration of *Asystasia* extract, a graded increase in growth inhibition ranging from 10% to 43% was observed.

***Staphylococcus aureus*-** The optical density of 24 hour old culture of this bacteria grown in plant extract free broth medium was 0.5; this was taken as the control reading. The

result obtained with this organism showed that there was a maximum growth inhibition of 78%; the surprising thing was that the highest inhibition was found at the lowest concentration of plant extract added. On increasing plant extract concentration; slight decrease in percentage of inhibition was observed. The results are presented in Table II.

TABLE-II : EFFECT OF *ASYSTASIA GANGETICA* ON GROWTH OF BACTERIA

NO	Conc. (mg/ ml)	*E.coli*		*K. aerogenes*		*P.aeruginosa*		*S. aureus*	
		O.D.	Inhibition %	O.D.	Inhibition %	O.D.	Inhibition %	O.D.	Inhibition %
1	25	0.28	28	0.05	92	0.31	0	0.11	78
2	50	0.24	38	0	100	0.27	10	0.12	76
3	100	0.21	46	0	100	0.22	27	0.14	72
4	200	0.22	44	0	100	0.19	36	0.17	66
5	300	0.05	87	0	100	0.20	33	0.19	62
6	400	0.01	97	0	100	0.17	43	0.20	60

2 . JUSTICIA SIMPLEX The leaf extract of this plant was found to be a potent inhibitor of the bacteria as evidenced by the following results (Table III)

Escherichia coli At the lowest concentration, i.e 25mg/ml no inhibition of growth was observed. On raising the concentration further a proportionate increase in rate of

inhibition of bacterial growth was observed and at 400mg/ml concentration 87% inhibition was obtained.

Klebsiella aerogenes Lower concentration of this plant brought about more than 60% inhibition of bacterial growth. 93% inhibition was observed at 200mg/ml and above this concentration no growth of bacteria was observed.

Pseudomonas aeraginosa The three lower concentrations of extract studied, i.e, 25,50 and 100mg/ml not only failed to inhibit growth, but also showed a slight beneficial effect. At 200mg/ml concentration only 27% growth inhibition was observed, but at 300mg/ml concentration it steeply increased to 94%. At 400mg/ml concentration growth was almost completely inhibited (99%).

Staphylococcus aureus 66% growth inhibition of this organism was found even at 25mg/ml plant extract concentration. The percentage of growth inhibition showed an increase with increase in concentration ; growth completely stopped at concentration above 100mg/ml.

TABLE-III : EFFECT OF *JUSTICIA SIMPLEX* ON GROWTH OF BACTERIA

No	Conc. (mg/ml)	*E.coli*		*K. aerogenes*		*P.aeruginosa*		*S. aureus*	
		O.D.	Inhibition %	O.D.	Inhibition %	O.D.	Inhibition %	O.D.	Inhibition %
1	25	0.39	0	0.23	62	0.46	0	0.17	66
2	50	0.33	15	0.22	63	0.44	0	0.13	74
3	100	0.29	26	0.17	72	0.35	0	0.04	92
4	200	0.13	67	0.04	93	0.22	27	0	100
5	300	0.11	72	0	100	0.08	94	0	100

6	400	0.05	87	0	100	0.04	99	0	100

3. STROBILANTHUS HEYNIANUS Significant results were obtained on growing bacteria in medium supplemented with leaf extract of this plant (Table IV)

Escherichia coli Growth of this bacterium was completely inhibited at 400mg/ml, lower concentration also were inhibitory at different levels.

Klebsiella aerogenes Growth of this organism was drastically inhibited by *Strobilanthus*. More than 65% inhibition was observed even at 25mg/ml and50mg/ml and at concentrations above this complete lack of bacterial growth, i.e, 100% inhibition was observed.

Pseudomonas aeruginosa Complete inhibition of growth of this organism was observed at concentration above 100mg/ml.; different degrees of inhibition ranging from 20%-70% were observed at the three lower concentrations studied.

Staphylococcus aureus This organism was the most affected by the extract of *Strobilanthus*. Even at 25mg/ml concentration; 92% of growth inhibition took place,and above 50mg/ml concentration, the bacterium was not able to grow at all. 100% inhibition was observed at concentrations higher than 50mg/ml.

TABLE-IV : EFFECT OF *STROBILANTHES HEYNIANUS* ON GROWTH OF BACTERIA

NO	Conc. (mg/ml)	*E.coli*		*K. aerogenes*		*P.aeruginosa*		*S. aureus*	
		O.D.	Inhibition %	O.D.	Inhibition %	O.D.	Inhibition %	O.D.	Inhibition %

1	25	0.36	8	0.21	65	0.24	20	0.04	92
2	50	0.17	56	0.19	68	0.23	25	0.03	94
3	100	0.04	90	0	100	0.09	70	0	100
4	200	0	100	0	100	0	100	0	100
5	300	0.1	73	0	100	0	100	0	100
6	400	0	100	0	100	0	100	0	100

4. *ADATHODA VASICA* proved to be very effective in controlling growth of all the tested bacteria.

Escherichia coli –.

Growth of *E. coli* was inhibited at all the concentrations studied. Only 18% inhibition was obtained at 25mg/ml concentration which increased to 82% at 100mg/ml concentration; above this concentration complete absence of growth was observed.

Klebsiella aerogenes**-** There was 58% growth inhibition of this bacteria even at concentration as low as 25mg/ml, and when the concentration was increased above 50 mg/ml there was a complete cessation of growth.

Pseudomonas aeraginosa 33% inhibition of growth was found at 25mg/ml concentration. On further increasing the concentration of *Adathoda* extract, an increase in growth inhibition was observed; at 300 mg/ml growth completely stopped.

Staphylococcus aureus**-** At the three lower concentrations inhibition of growth observed was in the range 20- 50 % which increased to 80% at 200mg/ ml . Complete growth suppression was obtained in the two highest concentrations. . The results are presented in Table V

TABLE- V : EFFECT OF *ADATHODA VASICA* ON GROWTH OF BACTERIA

NO	Conc (mg/ml)	*E.coli*		*K. aerogenes*		*P.aeruginosa*		*S. aureus*	
		O.D.	Inhibition %	O.D.	Inhibition %	O.D.	Inhibition %	O.D.	Inhibition %
1	25	0.32	18	0.25	91	0.2	33	0.4	20
2	50	0.20	49	0.07	99	0.15	50	0.3	40
3	100	0.07	82	0	100	0.10	66	0.25	50
4	200	0	100	0	100	0.09	70	0.1	80
5	300	0	100	0	100	0	100	0	100
6	400	0	100	0	100	0	100	0	100

5. ANDROGRAPHIS PANICULATA The leaf extract of this plant was found to be highly inhibitory to growth of the bacteria as evidenced by the following results (Table VI). Complete inhibition was observed in all the bacteria at higher concentrations .

Escherichia coli At the lowest concentration, i.e 25mg/ml only26% inhibition of growth was observed. On raising the concentration further a proportionate increase in rate of

inhibition of bacterial growth was observed and at 300mg/ml and 400mg/ml concentration 100% inhibition was obtained.

Klebsiella aerogenes Lower concentration of this plant brought about more than 50% inhibition of bacterial growth. 100% inhibition was observed at 300mg/ml and above .

Pseudomonas aeraginosa At 25mg/ml concentration 50% growth inhibition was observed, and above 100mg/ml growth was completely arrested. ***Staphylococcus aureus*** Only 36% growth inhibition of this organism was found at 25mg/ml plant extract concentration. The percentage of growth inhibition showed an increase with increase in concentration ; growth almost completely stopped at 400mg/ml concentration.

TABLE-VI : EFFECT OF *ANDROGRAPHIS PANICULATA* ON GROWTH OF BACTERIA

NO	Conc (mg/ml)	*E.coli*		*K. aerogenes*		*P.aeruginosa*		*S. aureus*	
		O.D.	Inhibition %	O.D.	Inhibition %	O.D.	Inhibition %	O.D.	Inhibition %
1	25	0.29	26	0.3	50	0.15	50	0.32	36
2	50	0.25	36	0.2	66	0.07	77	0.30	40
3	100	0.19	51	0.15	75	0	100	0.27	46
4	200	0.07	82	0.1	83	0	100	0.15	70
5	300	0	100	0	100	0	100	0.07	98
6	400	0	100	0	100	0	100	0.01	99

6. JUSTICIA GENDARUSSA significantly reduced growth of bacteria in medium supplemented with leaf extract (Table IV)

Escherichia coli Growth of this bacterium was inhibited in the range 10- 77%.

Klebsiella aerogenes Growth of this organism was inhibited by more than half at the concentrations above 100 mg/ml; maximum inhibition attained was 93%.

Pseudomonas aeruginosa Almost complete inhibition of growth of this organism was observed at a concentration of 400mg/ml. No inhibition was observed at 25 mg/ml.

Staphylococcus aureus was only moderately affected by justicia extracts at lower concentrations; even at 400mg/ml only 66% inhibition was observed.

TABLE-VII : EFFECT OF *JUSTICIA GENDARUSSA*ON GROWTH OF BACTERIA

NO	Conc. (mg/ml)	*E.coli*		*K. aerogenes*		*P.aeruginosa*		*S. aureus*	
		O.D.	Inhibition %	O.D.	Inhibition %	O.D.	Inhibition %	O.D.	Inhibition %
1	25	0.35	10	0.4	33	0.3	0	0.48	4
2	50	0.32	18	0.32	47	0.25	17	0.43	14
3	100	0.29	26	0.28	53	0.19	37	0.38	24
4	200	0.24	38	0.13	78	0.12	60	0.35	30
5	300	0.18	54	0.08	87	0.08	73	0.25	50
6	400	0.09	77	0.04	93	0.03	90	0.17	66

CHAPTER V

DISCUSSION

The present era has witnessed tremendous advancements in the fields of technology, science and medicine, but, even today man has failed to control the dramatic rise in the spread of many infectious diseases. WHO (2002a) stated that infectious diseases remain the second leading cause of death world - wide. After the discovery of antibiotics in the last century, they were considered miracle drugs and were instrumental in bringing about cure of various infectious diseases for decades. However the recent failure of antibiotics due to the dramatic emergence of multidrug- resistant pathogens, and rapid spread of new infections urge the health organizations and pharmaceutical industries all over the world to change their strategy and search for antibiotics from other sources.

Plants are the storehouses of biochemical and pharmaceutical compounds. Medicinal plants are rich in numerous variety of secondary metabolites with antimicrobial properties - saponins, tannins, alkaloids, flavanoids, sesquiterpenes, phenols and phorbol

(Lewis and Ausubel, 2006). Plant compounds are safer substitutes for synthetically produced antibiotics and antimicrobials.

The family Acanthaceae includes many medicinal plants which are traditionally exploited for pharmaceuticals- *Adathoda vasica , Hemigraphis colorata, Justicia gendarussa* etc., to name a few (The Ayurvedic Pharmacoepia of India, 1999). This study evaluated the antibacterial property of *Asystasia gangetica, Justicia simplex* and *Strobilanthes heynianus*, belonging to the family Acanthaceaceae. These plants were selected because in our country not many studies on their medicinal properties have been conducted and their therapeutic potential has not been fully exploited.

Most studies on antimicrobials employ the Kirby- Bauer disc diffusion technique or the agar- well diffusion method; in this study in addition to the agar well method , turbidity analysis was used to quantitate the inhibition of bacterial growth. Four pathogenic bacterial isolates were used as test organisms. Bacteria were grown in medium having graded concentrations of the respective plant extract. Nutrient broth medium was taken as medium of dilution.

Asystasia gangetica extracts of different concentrations were effective in controlling the growth of *E. coli, K. aerogenes* and *P. aeruginosa*. The organism which was inhibited maximally was *K. aerogenes*; 91% growth was reduced even at the lowest concentration (25mg/ml) and growth of this organism could be completely stopped by increasing the concentration to 50mg/ml. In *E. coli* 400mg/ml concentration brought about a 93% decrease in growth over the control. Only 43% inhibition of *P. aeruginosa* was obtained even in the highest concentration studied. Results contrary to that obtained from the above three organisms were observed in the case of *S. aureus.* Instead of inhibiting growth all concentrations of the plant extract were found to promote growth of this

organism (Table II). This contradictory result may be due to the masking of the inhibitory factor present in *Asystasia* extract by some growth stimulatory compound which stimulates growth of S. aureus. Further studies have to be conducted to find out the reason for this phenomenon. *Asystasia gangetica* is used as a leaf vegetable and fodder in South Africa and South-east Asia (Tilloo *et al.,* 2012). In our state this plant enjoys wide distribution in open uncultivated areas. Potential uses of this plant have yet to be exploited in our area. Certain folk remedies in Tamil Nadu use extracts of this plant for curing diabetes and asthma (Tilloo *et al*., 2012).

Justicia simplex is a common weed plant of our locality. The extract of this plant brought about complete inhibition of growth of *K. aerogenes* and *S. aureus*. A fair degree of inhibition was observed even at the lower concentrations. Lower concentrations of this plant did not affect the growth of *E.coli* and *P. aeruginosa.* Almost complete inhibition (99%) was present in *P. aeruginosa* at the highest treated concentration; at this concentration 86% inhibition of growth was observed in *E. coli.* These findings corroborate the use of this plant in traditional medicine in Tamil Nadu as diuretic, anthelminthic, stomachic, febrifuge etc.(Satyabhama *et al* ,2012).

Strobilanthes heynianus, a plant widely distributed in Kerala has many documented medicinal uses. This plant has anti-inflammatory and antimicrobial properties and is a part of many ayurvedic formulations for the treatment of rheumatism (Ravishanker *etal*, 1987). In the present study it was observed that this plant extract could completely inhibit the growth of all the four test organisms. The only difference among the organisms was in the minimum inhibitory concentrations required for complete cessation of growth. *S. aureus* was the organism which was the most affected by this organism. Even at the low concentration of 25mg/ml 92% reduction in growth was observed which increased to 100% at 100mg/ml concentration.

Adathoda vasica is a plant which finds use in many ayurvedic medicines, especially in medicines for curing respiratory disorders(The Ayurvedic Pharmacoepia of India, 1999).The result of this study showed that concentrations above 100mg/ml brought about complete suppression of growth of all the bacteria tested thus authenticating its use as an antibacterial drug.

Andrographis paniculata or Kaalmegh extract is bitter in taste and was found to stop growth of all the test bacteria at concentrations above 200 mg/ml. *Pseudomonas* was the maximally affected bacterium with complete growth inhibition even at 100 mg/ml concentration.

Justicia gendarussa is traditionally used to cure pains associated with arthritis. Only *Pseudomonas* could be effectively controlled by this plant extract, complete growth inhibition was not observed in any concentration. This indicates that this plant would not be much effective in controlling bacterial infections.

The overall results of this study suggests that of the six plants studied *Strobilanthes heynianus, Adathoda vasica* and *Andrographis paniculata* were the most effective in completely controlling the growth of all the bacterial isolates considered. *Asystasia gangetica* could not control growth of *S. aureus. Justicia simplex* also proved to be a potent antibacterial agent. *Justicia gendarussa* possessed only moderate antibacterial activity.

Further experimentation is required to understand the nature of the active ingredients in these plants. Methods to isolate , purify and characterize the actual antibacterial components of the crude plant extracts can be taken up for further studies. This is a promising field of research because plants provide safer, cheaper and more reliable weapons in the fight against disease.

Since times immemorial, plants have been resisting the continuous attack of pathogens by producing a wide array of secondary metabolites and it remains our duty to add these compounds in our weaponry in the fight against disease. The battle against infectious pathogens is never ending but we can beat them by returning to nature.

Knowledge about the medicinal value of plants are often passed from generation to generation orally and there is often a lack of proper documentation. So, with the passage of time this vast treasure of ancestral knowledge may be lost. It is necessary to record this knowledge for the future generations. Herbal lore of native people should be recorded in the written form so that it is not permanently lost. It is the duty of every citizen to preserve our flora on which is dependent all other forms of life. Even the seemingly insignificant 'weed' has a role to play. Several of our indigenous plants have been assessed as endangered, vulnerable and threatened due to over-harvesting in the wild and also due to habitat destruction.

CHAPTER VI

SUMMARY AND CONCLUSION

The antibacterial activity of leaf extracts of *Asystasia gangetica, Justicia simplex, Strobilanthes heyneanus, Adathoda vasica, Andrographis paniculata* and *Justicia gendarussa* against four pathogenic organisms was evaluated. Antibacterial activity was tested by growth measurement in plant extract fortified broth medium by turbidity analysis All the six plants showed potent antimicrobial activity. *A. gangetica* inhibited growth of all the bacterial isolates except *S. aureus*. Only about 50% reduction of growth of *P.aeruginosa* was brought about by a concentration as high as 400mg/ml. Complete absence of growth was observed in the case of *E.coli* and *K.aerogenes*. *J. simplex* inhibited the growth of all the bacteria studied; growth reduction of 86% was observed in *E.coli* while all the other bacteria were completely inhibited. *S. heyneanus ,Adathoda vasica* and *Andrographis paniculata* ,were the plants which showed uniformly high activity against all the tested bacterial isolates; complete growth inhibition was observed in all the cases.

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