

UNIVERSITY GRANTS COMMISSION

BAHADUR SHAH ZAFAR MARG

NEW DELHI – 110 002

Utilization certificate

Minor Research Project

Certified that the grant of **Rs. 80000/- (Rupees Eighty thousand only)** received from the University Grants Commission under the scheme of support for Minor Research Project entitled **Antibacterial study of some ethnomedicinal plants of Danta Forest, North Gujarat** vide UGC letter No. **F. 47-2128/11(WRO)** dated **29 Feb 2012** has been fully utilized for the purpose for which it was sanctioned and in accordance with the terms and conditions laid down by the University Grants Commission.

**SIGNATURE OF THE
PRINCIPAL
INVESTIGATOR**

REGISTRAR/PRINCIPAL

**STAUTORY
AUDITOR**

SIGNATURE OF THE COINVESTIGATOR

UNIVERSITY GRANTS COMMISSION
BAHADUR SHAH ZAFAR MARG
NEW DELHI – 110 002.

Final Report of the work done on the Minor Research Project.

1. Project report Final
2. UGC Reference No. F. 47-2128/11(WRO)
3. Period of report: from April 2012 to March 2014
4. Title of research project **Antibacterial study of some ethnomedicinal plants of Danta Forest, North Gujarat**
5. (a) Name of the Principal Investigator Mr. Mohmedyasin F Mansuri
(b) Deptt. and University/College where work has progressed
Department of Microbiology
Smt. S. M. Panchal Science College, Talod.
Dist. Sabarkantha. Gujarat 383215
6. Effective date of starting of the project 01.04.2012
7. Grant approved and expenditure incurred during the period of the report:
 - a. Total amount approved Rs. 80000. (Rupees Eighty thousand only)
 - b. Total expenditure Rs. 84428. (Rupees Eighty four thousand and twenty eight only)
 - c. Report of the work done: (Please attach a separate sheet)
 - i. Brief objective of the project: The objective of the project was to develop an understanding and insight into the study of antimicrobial activity of ethnomedicinal plants of Danta forest region based on the application of the plants for the treatment of tribal people by the local medicine men of the forest region.
 - ii. Work done so far and results achieved and publications, if any, resulting from the work (Give details of the papers and names of the journals in which it has been published or accepted for publication)

One presentation was done in 53rd Annual Conference of AMI- 2012 at KIIT University, Bhubnaeshwar, Odisha.

Title: Study of antibacterial activity of some medicinal plants against pathogens causing urinary tract infections.

Manuscripts are being readied for publication and are listed as chapters

iii. Has the progress been according to original plan of work and towards achieving the objective. if not, state reasons **Yes**

iv. Please indicate the difficulties, if any, experienced in implementing the project.. nil.

v. If project has not been completed, please indicate the approximate time by which it is likely to be completed. A summary of the work done for the period (Annual basis) may please be sent to the Commission on a separate sheet **Project completed**

vi. If the project has been completed, please enclose a summary of the findings of the study. Two bound copies of the final report of work done may also be sent to the Commission

vii. Any other information which would help in evaluation of work done on the project. At the completion of the project, the first report should indicate the output, such as (a) Manpower trained (b) Ph. D. awarded (c) Publication of results (d) other impact, if any

**SIGNATURE OF THE PRINCIPAL
INVESTIGATOR**

SIGNATURE OF THE COINVESTIGATOR

REGISTRAR/PRINCIPAL

**UNIVERSITY GRANTS COMMISSION
BAHADUR SHAH ZAFAR MARG
NEW DELHI – 110 002**

**STATEMENT OF EXPENDITURE IN RESPECT OF MAJOR/MINOR
RESEARCH PROJECT**

1. Name of Principal Investigator: Mr. Mohmedyasir F Mansuri

2. Deptt. of University/College:

Department of Microbiology,

SMT. S. M. PANCHAL SCIENCE COLLEGE, TALOD.

DIST. SABARKANTHA. GUJARAT 383215

3. UGC approval No. and Date: F. 47-2128/11(WRO) 29 Feb 2012

4. Title of the Research Project **Antibacterial study of some ethnomedicinal plants of Danta Forest, North Gujarat**

5. Effective date of starting the project 01.04. 2012

6. a. Period of Expenditure: From 01.04. 2012 to 31.03.2012

b. Details of Expenditure

S.No.	Item	Amount Approved Rs.	Expenditure Incurred Rs.
i.	Books & Journals	5000	5128
ii.	Equipment	30000	34397
iii.	Contingency	5000	4700
iv.	Field Work/Travel (Details in the proforma at Annexure- VI).	10000	10000
v.	Hiring Services	0	0
vi.	Chemicals & Glassware	30000	30203
vii.	Overhead	0	0
viii.	Any other items (Please specify)	0	0

c . Staff

None

Date of Appointment: Not Applicable

1. It is certified that the appointment(s) have been made in accordance with the terms and conditions laid down by the Commission.
2. It as a result of check or audit objective, some irregularly is noticed, later date, action will be taken to refund, adjust or regularize the objected amounts.
3. Payment @ revised rates shall be made with arrears on the availability of additional funds.
4. It is certified that the grant of **Rs. 80000 (Rupees Eighty thousand only)** received from the University Grants Commission under the scheme of support for Major Research Project entitled **Antibacterial study of some ethnomedicinal plants of Danta Forest, North Gujarat** vide UGC letter No. **F. 47-2128/11(WRO)** dated **29 Feb 2012** has been fully utilized for the purpose for which it was sanctioned and in accordance with the terms and conditions laid down by the University Grants Commission.

**SIGNATURE OF PRINCIPAL
INVESTIGATOR**

REGISTRAR/PRINCIPAL

SIGNATURE OF THE COINVESTIGATOR

**UNIVERSITY GRANTS COMMISSION
BAHADUR SHAH ZAFAR MARG
NEW DELHI – 110 002**

STATEMENT OF EXPENDITURE INCURRED ON FIELD WORK

Name of the Principal Investigator : Mohmedyasin F Mansuri

Name of the Place visited	Duration of the Visit		Mode of Journey	Expenditure Incurred (Rs.)
	From	To		
Danta Town	14.04.12		Own car	1000
Mal Forest	28.04.12		Own car	1000
Kuvarsi village	05.05.12		Own car	1000
Harivav village	31.10.12		Own car	1000
Dhareda village	06.08.13		Own car	1000
Navavas village	24.08.13		Own car	1000
Mal forest	31.08.13		Own car	1000
Trishul Ghat	31.10.13		Own car	1000
Amba ghatta Forest	06.09.13		Own car	1000
Pataliya village	02.11.13		Own car	1000

Certified that the above expenditure is in accordance with the UGC norms for Major Research Projects

**SIGNATURE OF PRINCIPAL
INVESTIGATOR**

REGISTRAR/PRINCIPAL

SIGNATURE OF THE CO-INVESTIGATOR

**UNIVERSITY GRANTS COMMISSION
BAHADUR SHAH ZAFAR MARG
NEW DELHI – 110 002**

ACCEPTANCE CERTIFICATE FOR RESEARCH PROJECT

Name _____

No.F. _____ dated _____

Title of the Project _____

1. The research project is not being supported by any other funding agency.
2. The terms and conditions related to the grant are acceptable to the Principal Investigator and University/College/Institution.
3. At present, I have no research project approved by UGC and the accounts for the previous project, if any have been settled.
4. The College/University is fit to receive financial assistance from UGC and is included in the list prepared by the UGC.
5. The Principal Investigator is a retired teacher and eligible to receive honorarium as he/she is neither getting any honorarium from any agency nor is he/she gainfully employed anywhere.
6. His/her date of birth is _____
7. The date of implementation of the project is _____

Principal Investigator

Co-Investigator

Registrar/Principal

University/College

Dated:

**UNIVERSITY GRANTS COMMISSION
BAHADUR SHAH ZAFAR MARG
NEW DELHI – 110 002**

**PROFORMA FOR SUBMISSION OF INFORMATION AT THE TIME OF SENDING THE
FINAL REPORT OF THE WORK DONE ON THE PROJECT**

1. NAME AND ADDRESS OF THE PRINCIPAL INVESTIGATOR

Mohmedyasir F Mansuri.
Plot No: 552/1,
Sector 29, Gandhinagar, 382029.

2. NAME AND ADDRESS OF THE INSTITUTION –

Department of Microbiology,
Smt.S. M. Panchal Science College, Talod.
Dist. Sabarkantha. Gujarat. 383215.

3. UGC APPROVAL NO. AND DATE ... F. 47-2128/11(WRO). 29 Feb 2012

4. DATE OF IMPLEMENTATION ...01.04.2012

5. TENURE OF THE PROJECT ... 2 years

6. TOTAL GRANT ALLOCATED ...Rs. 80000/- (Rupees Eighty thousand only)

7. TOTAL GRANT RECEIVED ...Rs. 57500/- (Rupees Fifty thousand five hundred only)

8. FINAL EXPENDITURE ...Rs. 84428/- (Rupees Eighty four thousand four hundred and twenty eighty only)

9. TITLE OF THE PROJECT ... Antibacterial study of some ethnomedicinal plants of Danta Forest, North Gujarat

10. OBJECTIVES OF THE PROJECT ...

To study antibacterial activity of plants extracts obtained from plant specimens
To study the susceptibility and resistance pattern of bacterial pathogens against ethnomedicinal plants
To develop an insight into further application of the findings for future study

11. WHETHER OBJECTIVES WERE ACHIEVED ...

Bacterial pathogens obtained from IMTECH culture collection centre, Chandigarh as well Medical College, Ahmedabad were used for their inhibition by extracts of plants of ethnomedicinal importance. These pathogens have been isolated from diseased subjects and have thus proven to be live pathogens capable of causing diseases. The plant extracts prepared in various solvents were applied to bacterial pathogens and based on their inhibition or growth observed and the results were obtained. The results were scrutinized for developing an understanding for future further study.

12. ACHIEVEMENTS FROM THE PROJECT ...

1. The project provided the opportunity to carry out research work with large expenditure for research which could be afforded only by the financial assistance.
2. The project provided the platform to pursue research in an area of interest
3. It enabled paper presentation and publication opportunities for career advancement.

13. SUMMARY OF THE FINDINGS

Microorganisms have been both beneficial and harmful to the mankind. The diseases caused by bacteria have been studied and cured by us since olden times.

But this is never ending process and thus it is necessary to keep finding new medicines of cure. Nature provides us for the cure in its herbs, shrubs and trees. We have been using them since time immemorial.

Present day scenario as evident from scientific as well as social media has raised great concern of development of resistance of pathogens to almost many of the drugs used. To counter this, improved drugs are being developed, at the same time it has become also necessary to search for new ones.

Nature provides for many of such medicines that can be used effectively to treat diseases. Plant samples like leaf, stem, flower, etc obtained from Danta forest were collected, washed, dried and powdered. Their extracts were prepared in water, ethanol, and other solvents. Bacterial pathogens were obtained from Microbial culture collection centre Chandigarh and Ahmedabad Municipal Medical College. They were used and plant extracts so prepared were applied to the growth of these bacterial pathogens to study their inhibition or growth in their presence. Many pathogens were observed to be inhibited successfully by applying the extracts. This was observed depending on the zone of inhibition obtained. More than 70 plants were evaluated for their antibacterial capability. Almost all of them were able to control growth of at least one pathogen. Some of them were effective on more than one and few were effective in inhibiting almost all of the pathogens studied. This work thus provides a report on successful use of medicinal plants to control infections by present day pathogens as the bacteria used were either isolated from infected patients or have a history of causing disease. Although this study cannot advocate the use of the plant extracts directly for therapeutic purpose, it supports further study for potential application.

This study also provides an understanding that even those pathogens that could be developing resistance to antibiotics can be treated using natural extracts. Resistance to natural products does not develop as like the allopathic antibiotics. This form of treatment is also safe from development of resistance and thus this therapy should be used in conjunction with allopathic treatment if not used as sole treatment procedure. Local medicine men of forest region with their ancestral profession have been giving this form of treatment to tribal population but the work carried out is risky as not all drugs are effective and may cause toxicity and adverse reaction which in worst cases can take a toll of life of the patient.

The scientific research of this kind can provide a platform of understanding of risk to benefit aspect of disease treatment. Further research on isolation of active ingredient and its purification and toxicity testing can enhance our knowledge for potential use of the medicines.

14. CONTRIBUTION TO THE SOCIETY

This work at large can benefit the society by providing valuable information regarding disease treatment. This work provides platform for further study of extracts that have been successfully applied to control growth of bacterial pathogens on synthetic media. This work paves the way for further study of disease control in animal models and human trials after studying their toxicity and ADME properties. Society will be benefited as the findings can help find a cure for treatment of infections caused by resistant bacterial pathogens.

15. WHETHER ANY PH.D. ENROLLED/PRODUCED ... None
OUT OF THE PROJECT

16. NO. OF PUBLICATIONS OUT OF THE PROJECT... one presentation was done in 53rd
Annual Conference of AMI- 2012 at KIIT University, Bhubnaeshwar, Odisha.
Manuscripts are being readied for publication in suitable scientific journals and are listed as
chapters

(PRINCIPAL INVESTIGATOR)
(CO-INVESTIGATOR)

(REGISTRAR/PRINCIPAL)

CHAPTER NO: 1

INTRODUCTION

Introduction

Nature always stands as a golden mark to exemplify the outstanding phenomenon of symbiosis. The biotic and abiotic elements of nature are all interdependent. The plants are indispensable to men for his life. There are three important necessities of life food, clothing and shelter and a host of other useful products are supplied to him by the plant kingdom. Nature has provided a complete store house of remedies to cure all elements of mankind. The knowledge of the drug has accumulated over thousands of years as a result of man's inquisitive nature so that today we possess many effective means of ensuring health-care.

The human being appears to be afflicted with more disease than any other animal species. There can be little doubt then that he, very early, sought to alleviate his suffering from injury and disease by taking advantage of plant growing around him. In the past, a vast store of knowledge concerning therapeutic properties of different plants has accumulated. All phylla of plants viz .Thallophyta, Bryophyta, Pteridophyta and Spermatophyta (of which conservative estimates place the total number of known species at approximately 3,35,000) contain species that yield official and unofficial products of medicinal importance. By far the greatest numbers of these are derived from plants and include 300 or more recognised families of Spermatophyta.

The history of herbal medicines is as old as human civilization. The documents, many of which are of great antiquity, revealed that plants were used medicinally in China, India, Egypt, and Greece long before the beginning of the Christian era. One of the most famous surviving remnants is Papyrus Ebers, a scroll some 60 feet long and a foot wide, dating back to the 16th century before Christ. The text of documents is dominated by more than 800 formulated and 700 different drugs. The drugs such as acasia, castor oil and fennel are mentioned along with apparent references to such compound as Iron oxide, Sodium chloride, Sodium carbonate, and Sulphur. Most of the medicinally active substances identified in the 19 th and the 20th centuries were used in the form of crude extracts. In China, many medicinal plants had been in use since 5000 B.C. the oldest known herbal is Pen-t'sao written by emperor Shen Nung around 3000 B.C. It contains 365 drugs, one for each day of the year. Indian also, worked meticulously to examine and classified the herbals which they came across, into groups called Guans. Charaka made 50 groups of ten herbs each of which, according to him, would suffice an ordinary physician's need. Similarly, Sushruta arranged 760 herbs in 7 distinct sets based on some of their common properties. A larger portion of the Indian population even today depends on the Indian system of medicine-Ayurveda, "An ancient science of life". The well-known treatises in Ayurveda are Charaksamhita and Sushrutasamhita. (Kokate CK, 2008)

Medicinal plants still play an important role in emerging and developing countries of Asia, both in preventive and curative treatments, despite advances in modern western medicine.

One of the oldest repositories of human knowledge, the Rigveda (4500-4600 BC) mentioned the use of medicinal plants for the treatment of one or other disease. In the long struggle to overcome the powerful force of nature, the human beings have always turned to plants.

The potential of higher plants as source for new drug is still largely unexplored. Among the estimated 250,000-500,000 plants species, only a small percentage has been investigated phytochemically and the fraction submitted to biological or

pharmacological screening is even smaller. Thus, any phytochemical investigation of a given plant will reveal only a very narrow spectrum of its constituents. Historically pharmacological screening of compounds of natural or synthetic origin has been the source of innumerable therapeutic agents. Random screening as tool in discovering new biologically active molecules has been most productive in the area of antibiotics. (Mahesh B et al., 2008), (Gerhartc W et al., 1985), (Kroschwitz JI et al., 1992)

Even now, contrary to common belief, drug from higher plants continue to occupy an important niche in modern medicine. On a global basis, at least 130 drugs, all single chemical entities extracted from higher plants, or modified further synthetically, are currently in use, though some of them are now being made synthetically for economic reasons. (Newman DJ et al., 2000)

Medicinal plants represent a rich source of antimicrobial agents. Plants are used medicinally in different countries and are sources of many potent and powerful drugs (Srivastava JJ et al., 1996)

Different extracts from traditional medicinal plants have been tested to identify the source of the therapeutic effect.

A wide range of medicinal plant parts is used for extracts as raw drugs and they possess varied medicinal properties.

The different parts are used include root, stem, flower, fruits, twigs exudates and modified plant organs.

While some of these raw drugs are collected in smaller quantities by the local communities and folk healers for local used, many other raw drugs are collected in larger quantities and traded in the market as the raw material for many herbal industries (Uniyal SK et al., 2006)

As a result some natural products have been approved as new antibacterial drugs, but there is still an urgent need to identify novel substances that are active towards pathogens with high resistance (Parekh J et al., 2007), (Crugg GM et al., 1997).

The discovery of antibiotics has decreased the spread and severity of a wide variety of diseases. However, as a result of their indiscriminate use, the efficiency of many antibiotics is being threatened by the emergence of microbial resistance to existing chemotherapeutic agents (Wamidh HT et al., 2010), (Cowan MM,1999).

Bacteria and fungi are evolving numerous mechanisms to evade antimicrobial agents and the resistance to old and new antibiotics is rising in medical practice (Chanda S et al., 2007). Bacterial strains such as methicillin- resistant *Staphylococcus aureus* (MRSA), penicillin-resistant *Streptococcus pneumoniae* (PRSP), and vancomycin-resistant *Enterococci* (VRE), in addition to the development of multidrug-resistant (MDR) bacterial strains are the few examples that have made the search for new and novel antimicrobial substances among the first priorities within the quest for such material (Alamis AL et al., 2005). Not to mention the fact that the use of some antibiotics is associated with side-effects, including allergy, immune-suppression, and hypersensitivity (Ahmed I et al., 1998).

For all these reasons, there is a pressing need to identify new and novel antimicrobial agents that would help in alleviating the problems of emerging resistant bacterial and fungal pathogens. Plants derived natural products represent an attractive source of

antimicrobial agents since they are natural and affordable, especially for rural societies in poor developing countries and also used in different formulations by different cultures as part of their material media heritage (Ghosh A et al., 2008). Also plant derived agents may have different mechanisms of action than conventional drugs, and this could be of clinical importance in health care improvement (Eloff JN, 1998).

Plants are rich sources of many bioactive secondary metabolites that have the potential to treat different afflictions. Examples of these compounds include Flavonoids, Phenol, Phenol glycosides, unsaturated lactones, Sulphur compounds, saponins, cyanogenic glycosides and glucosinolates (Quiroga EN et al., 2001).

The tribal people mainly of the Bhil tribe and its sub tribes like Parghi, Damor, Bubadiya, Bhemiyat and other reside in the forest. Due to their occupational and migratory habits they remain away from local health centres and thus availability of standard therapies of diseases is limited. Being there in the forest since many generations with small scale agriculture practice and relying on forest resources for their livelihood, some of them have inherited the practice of applying local herbs and plants for treating their ailments. They represent the ethno medicinal practitioners of the area and are referred to as “medicine men”.

Local tribes as well as people from the surrounding villages come to them regularly for getting their diseases treated. These “medicine men” use the herbal therapy for treating diseases that could be related to names like cholera, tuberculosis, syphilis, gonorrhoea, leprosy, dysentery, typhoid, hepatitis, and common cold and fever among others.

Among the many human microbe interactions, the one that takes its toll on human health is the human - pathogen interaction, which is a never ending battle in which humans develop newer drugs and combinations to combat the pathogen and the latter develops drug resistance to keep inflicting injuries to humans. Diseases caused by pathogens like Multidrug Resistance *Staphylococcus aureus* (MRSA), enteroinvasive and enterotoxigenic *E.coli* (O157: H7) are yet to be conquered. Thus the onus lies on us to keep a tab of their resistance and pathogenicity patterns and keep on developing ways and means of their control. It is in the light of these events, newer drugs and therapies for combatting diseases are discovered. The never ending quest for better and more potent drugs have made use of advanced methods of computer aided designing and development of drugs like the in silico drug design. The fundamental base of such an approach lies in finding a natural antimicrobial chemical and the simulation of this substance. Traditional herbal therapy not only controls the disease but also improves the overall immunity and health status of an individual, but in this jet era, it is considered to be old and slow, so to sharpen the edge, the active principles of herbal drugs are identified, the active principles are isolated and modifications are performed to improve the efficacy and ultimately dosage forms are developed.

This science is a never ending field of study that requires extensive and continuous study by the scientific fraternity of mankind.

Danta Forest

Gujarat State can be divided into four major biogeographic zones viz. Semiarid, Deserts, Western Ghat Mountains and Mangrove rich Coastal belts. These four zones support a wide range of flora covering about 2200 species of plants (Anonymous, 1996). Banaskantha district, in northern part of the Gujarat State, lies between 230.35' to 240.34' north latitudes and 710.00' to 730.00' east longitudes.

Danta taluka has got two forest ranges viz. Danta range forest and Ambaji range forest. The former one is situated on eastern part of the district. Danta range forest covers about 220 Sq. Km. area and is divided into four rounds viz. such as Danta round (59.08 Sq. Km.), Rangpur (50.17 Sq. Km.), Gorad (68.04 Sq. Km.) and Motasada (42.69 Sq. Km.).

Forest being a part of Aravalli hills with, rivers and rivulets passing through. Biodiversity of this area is very rich with flowering and non-flowering plant species. However, drastic reduction in the vegetation cover has been noticed in the recent years due to extensive cutting for timber, fuel and fodder. Danta is a small town having many granite, marble and stone crushing factories in forest areas. The state and rural roads make a good network of communication to link different villages.

The type of forest is dry deciduous scrub with scattered patches of trees. Being part of Aravalli hills, it has got varied altitudes from 150-600 m. Danta, Dhareda, Gorad, Vagol, Pamodara, Chori, Jasvantgarh, etc. are the areas having dense forest. The seasonal rivers/rivulets such as Banas, Sabarmati, Sanali, Kidi-Mankodi, Vasi (Arjuni), Motasada, Tundia, Ukanchali, Dhamnai, Saraswati (Kugaraka), etc. are running through this range forest and forming marshy vegetation.

The range is consisting of diverse topographical features and landscapes, having hills, forests, village grooves, stretch of barren plains, low rising and falling rocky hills and densely gullied topography near major river banks. The type of forest is dry deciduous scrub with scattered patches of trees. Being part of Aravalli hills, it has got varied altitudes from 150-600 m. Some of the hills are Harivoz, Gorad bhankhro, Divatiyo dungar, Chamunda ghat, Trishul ghat, Kuvarasi ghat, Amba ghat, etc. Among all, Harivoz is the tallest hill of about 600m heights. Danta, Dhareda, Gorad, Vagol, Pamodara, Chori, Jasvantgarh, etc. are the areas having dense forest. The seasonal rivers/rivulets such as Banas, Sabarmati, Vasi (Arjuni), Saraswati (Kugaraka), etc. are running through this range forest and forming marshy vegetation. Some annual/perennial water falls, namely Harivav, Piplavali vav, Dhareda, Vagol, etc are also found in this region. Some of the interior forest areas of Danta range are undisturbed and having rich vegetation.

The following tribes such as, Rabari, Thakarada, Meman, Chaudhari, Muman, Rajput, Raval, Harijan, Bajaniya, Madari etc. are residing mainly in villages where as Bubadiya, Parghi, Taral, Bhemiya, Dharngi, Khair, Makvana, Dabhi, Solanki, Parmar, Khamar, Rohisa, Rathod, Mansi, Damor etc. are the sub tribes of Bhil community residing in the forests.

Chamunda ghat, Trishul ghat, Kuvarasi ghat, Amba ghat etc. are the important hills which support good forest vegetation comprising of various strata.

The vegetation consists of woody species, rare trees, trees planted under special forestry program, common and rare herbs and shrubs, parasitic plants, and cultivated

varieties. The flora consists of many species and is a comprehensive list but a few names are included here such as, *Aegle marmelos*, *Delonix elata*, *Zizyphus oenoplia*, *Terminalia bellirica*, *Bauhinia tomentosa*, *Tridax procumbens*, *Ficus benghalensis*, *Evolvulus alsinoides*, *Bombax ceiba*, *Emblica officinalis*, *Oroxylum indicum*, *Moringa concanensis*, *Ailanthus excelsa*, *Derris indica*, *Eucalyptus globulus*, *Ficus carica*, *Gliricidia sepium*, *Gmelina arborea*, *Leucaena leucocephala*, *Mangifera indica*, *Acalypha indica*, *Argemone mexicana*, *Cynodon dactylon*, *Euphorbia hirta*, *Evolvulus alsinoides*, *Hibiscus ovalifolius*, *Dactyloctenium aegyptium*, *Cleome gynandra*, *Abrus precatorius*, *Capparis decidua*, *Pedaliium murex*, *Cuscuta reflexa*, *Crataeva nurvala*, *Limonia acidissima*, *Solanum surattense*, *Vitex negundo*, *Chlorophytum borivilianum*, *Commiphora wightii*, *Abutilon indicum*, etc.

CHAPTER NO: 2
MATERIALS
AND
METHODS

Collection of Plant Material

Plants were collected from various locations within Danta forest. Care was taken to collect disease free, healthy specimens of leaves, stem flower, etc. The taxonomic identity of each plant was authenticated by Dr. K.C. Patel, Department of Biology, Smt. S. M. Panchal Science College, Talod.

The leaves, stem, flowers were washed thoroughly in running tap water. The samples were dried in hot air oven. The specimens were powdered and stored in plastic bags in the refrigerator until the next step of extraction.

Process of Extraction

The extraction was carried out using solvents, water, methanol, ethanol, petroleum ether, and acetone. Extraction procedures were employed like, cold extraction, hot extraction and soxhlet extraction. The complete procedures are detailed in further chapters as part of the research manuscripts.

Microorganisms and media.

Bacterial pathogens capable of causing diseases in humans representing infectious agents of various physiological systems of human body are used for the study. They were collected from two centres viz., A.M.C.M.E.T Medical College, Ahmedabad and IMTECH, Chandigarh. The media used for their cultivation and growth includes, LB broth, Mueller Hinton agar, and Nutrient agar media. The list of the microorganisms is given below in a tabular form below.

Preparation of the McFarland standard:

Add 0.5 ml 0.048 M BaCl₂ (1.17%w/v BaCl₂. 2H₂O) to 99.5 ml of 0.18 M H₂SO₄ (1%v/v) with constant stirring. The standard was distributed into screw cap tubes of the same size and with the same volume as those used in growing the broth cultures. The tubes sealed tightly to prevent loss by evaporation. Stored protected from light at room temperature. The turbidity standard was vigorously agitated on a vortex mixture before use. Standard may be stored for up to 6 months, after which time they should be discarded. Alternatively, prepared standards can be purchased (Jennifer M. Andrews, 2001)

Antibacterial study (Antibacterial sensitivity testing using disc diffusion method):

Suspensions of bacteria prepared in normal saline under aseptic conditions. 0.1 mL of this suspension was transferred on the surface of the agar medium plates and spread with the help of a sterile spreader.

Preparation of specimen discs

Circular discs of 6mm diameter were prepared from Whatman filter paper no. 1. They were impregnated with the prepared dilutions of the plant specimen material and placed on the surface of the previously inoculated agar plates. The plates were then incubated in a physiological incubator at 37°C for 24hrs. The antibacterial inhibition is measured as zone of inhibition in mm. surrounding the disc.

Following is the list of pathogenic bacteria used for the study.

Serial no: 1 to 5 were acquired from Medical college, Ahmedabad whereas serial no: 6 to 10 were procured from IMTECH, Chandigarh.

The bacteria were either isolated from human patients or were proven pathogens that could cause disease.

NO: NAME OF THE PATHOGENS

1. Staphylococcus albus
2. Staphylococcus citreus
3. Klebsiella pneumonia
4. Proteus vulgaris
5. Pseudomonas aeruginosa
6. Staphylococcus aureus (MTCC 96)
7. Escherichia coli (MTCC 443)
8. Shigella flexneri (MTCC 1457)
9. Bordetella bronchiseptica (MTCC 6838)
10. Hemophilus influenza (MTCC 3826)

The bacteria used for the study represent disease causing ability in various human body systems viz., respiratory tract, blood vascular system, urinary and reproductive system, nervous system, gastrointestinal system.

The list of plants, specimens of which were taken for study is as below:

No:	NAME OF PLANTS	No:	NAME OF PLANTS
1.	<i>Salvadora persica</i>	37.	<i>Alangium salvifolium</i>
2.	<i>Limonia acidissima</i>	38.	<i>Acalypha indica</i>
3.	<i>Aegle marmelos</i>	39.	<i>Madhuca longifolia</i>
4.	<i>Ficus carica</i>	40.	<i>Abrus precatorius</i>
5.	<i>Carissa carandas</i>	41.	<i>Ailanthus excelsa</i>
6.	<i>Cocculus hirsutus</i>	42.	<i>Amaranthus spinosus</i>
7.	<i>Tecomella undulata</i>	43.	<i>Delonix elata</i>
8.	<i>Lawsonia inermis</i>	44.	<i>Martynia annua</i>
9.	<i>Calotropis procera</i>	45.	<i>Oroxylum indicum</i>
10.	<i>Agave Americana</i>	46.	<i>Pedaliu murex</i>
11.	<i>Heliotropium supinum</i>	47.	<i>Amaranthus viridis</i>
12.	<i>Ocimum sanctum</i>	48.	<i>Ficus religiosa</i>
13.	<i>Acacia chundra</i>	49.	<i>Gmelina arborea</i>
14.	<i>Cassia fistula</i>	50.	<i>Tinospora cordifolia</i>
15.	<i>Derris indica</i>	51.	<i>Abutilon indicum</i>
16.	<i>Mangifera indica</i>	52.	<i>Ficus benghalensis</i>
17.	<i>Adhatoda vasica</i>	53.	<i>Euphorbia hirta</i>
18.	<i>Argemone mexicana</i>	54.	<i>Chlorophytum borivilianum</i>
19.	<i>Terminalia bellirica</i>	55.	<i>Commiphora wightii</i>
20.	<i>Capparis decidua</i>	56.	<i>Evolvulus alsinoides</i>
21.	<i>Azadirachta indica</i>	57.	<i>Acacia nilotica</i>
22.	<i>Ficus racemosa</i>	58.	<i>Vitex negundo</i>
23.	<i>Tridax procumbens</i>	59.	<i>Butea monosperma</i>
24.	<i>Syzygium cumini</i>	60.	<i>Holarrhena antidysentrica</i>
25.	<i>Moringa concanensis</i>	61.	<i>Emblca officinalis</i>
26.	<i>Jatropha curcas</i>	62.	<i>Cuscuta reflexa</i>
27.	<i>Bauhinia tomentosa</i>	63.	<i>Ficus virens</i>
28.	<i>Achyranthes aspera</i>	64.	<i>Annona squamosa</i>
29.	<i>Anisomeles indica</i>	65.	<i>Triticum aestivum</i>
30.	<i>Cynodon dactylon</i>	66.	<i>Lannea coromandelica</i>
31.	<i>Ziziphus oenoplia</i>	67.	<i>Solanum surattense</i>
32.	<i>Zizyphus glabrata</i>	68.	<i>Euphorbia nivulia</i>
33.	<i>Kirganelia reticulata</i>	69.	<i>Crataeva nurvala</i>
34.	<i>Cleome gynandra</i>	70.	<i>Hemigraphis latebrosa</i>
35.	<i>Cissampelos pareira</i>	71.	<i>Melilotus indica</i>
36.	<i>Bombax ceiba</i>		

CHAPTER NO: 3

Study of Antibacterial activity of some medicinal plants against pathogens causing Urinary Tract Infections.

Study of Antibacterial activity of some medicinal plants against pathogens causing Urinary Tract Infections.

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INTRODUCTION:

On a global basis, at least 130 drugs, all single chemical entities extracted from higher plants, or modified further synthetically, are currently in use, though some of them are now being made synthetically for economic reasons.

Medicinal plants represent a rich source of antimicrobial agents. Plants are used medicinally in different countries and are sources of many potent and powerful drugs.

Different extracts from traditional medicinal plants have been tested to identify the source of the therapeutic effect.

Bacteria and fungi are evolving numerous mechanisms to evade antimicrobial agents and the resistance to old and new antibiotics is rising in medical practice.

Thus, there is a pressing need to identify novel antimicrobial agents that would help in alleviating the problems of emerging resistant bacterial and fungal pathogens.

Plants are rich sources of many bioactive secondary metabolites that have the potential to treat different afflictions. Examples of these compounds include Flavonoids, Phenol, Phenol glycosides, Unsaturated lactones, Sulphur compounds, Saponins, Cyanogenic Glycosides and Glucosinolates.

Acute infection of the urinary tract fall into two general anatomic categories: lower tract infection (urethritis and cystitis) and upper tract infection (acute pyelonephritis, prostatitis, and intrarenal and perinephric abscesses).

Infection at various sites may occur together or independently and many either are asymptomatic or present as one of the clinical syndromes.

Extracts of medicinal plants like, *Tinospora cordifolia*, *Abutilon indicum*, *Ficus benghalensis*, *Euphorbia hirta*, *Chlorophytum borivillianum*, *Commiphora wightii*, *Evolvulus alsinoides*, and *Anisomeles indica* have been prepared in Acetone, methanol and water. Their antibacterial potential was studied on cultures of *Staphylococcus aureus*, *Staphylococcus albus*, *Staphylococcus citreus*, *Klebsiella pneumonia*, *Proteus vulgaris* and *Pseudomonas aeruginosa*. Within the study undertaken, extracts of *Ficus benghalensis* were effective on *K. pneumoniae* and *S. albus*, the detailed pattern of susceptibility and resistance will be presented.

MATERIALS AND METHODS:

Collection of plant materials

Plants were collected from the Danta Forest Region, North Gujarat. They were identified with the help of Dr. K.C.Patel, Department of Botany, Smt. S.M.Panchal Science College, Talod.

Preparation of extracts

The plants were cleaned and washed with sterile distilled water then kept in room temperature for proper drying. The 5 gm of dried plant powder (leaves, stem, fruits, etc.) is successively with 50 ml of water and organic solvents viz. petroleum ether, acetone, methanol (from higher polarity to lower polarity) separately in 500 ml sterile conical flask and covered with cotton wool and shaken vigorously for 48 hrs at room temperature. The mixture was then filtered using a Whatman No. 1 filter paper. The filtrate was evaporated at 50°C on a water bath to obtain crude extract. The same procedure was followed for all solvent extraction.

Test Microorganisms

Cultures of *Staphylococcus aureus*, *Staphylococcus albus*, *Staphylococcus citreus*, *Klebsiella pneumonia*, *Proteus vulgaris* and *Pseudomonas aeruginosa* were obtained from infectious disease patients from A.M.C.M.E.T College, L.G Hospital Compound, Ahmedabad. The isolated bacteria were identified by standard biochemical test. The organisms were maintained on agar slopes at 4°C and sub cultured for 24 hr before use.

Preparation of the McFarland standard

Add 0.5mL of 0.048M BaCl₂ to 99.5mL of 0.18M H₂SO₄ (1%v/v) with constant stirring. Distribute the standard into screw cap tubes of the same size with the same volume as those used in growing the broth cultures. Seal the tubes tightly to prevent loss by evaporation. Store protected from light at room temperature. Vigorously agitate the turbidity standard on a vortex mixture before use. Standards may be stores up to 6 months, after which they should be discarded.

Bacterial susceptibility testing

Kirby-Bauer method was followed for disc diffusion assay.

Isolated colonies of the bacteria were cultured into tubes containing 10ml of sterile nutrient broth and incubated at room temperature till the cultures turbidity reached 0.5 McFarland standards and aseptically swabbed on the surface of sterile Nutrient Agar plates. Filter paper discs of 6mm diameter were prepared and sterilized. Using Acetone, Methanol, Water and Petroleum Ether dipped discs served as a positive control, while the standard Hi-media antibiotic discs served as a negative control were aseptically placed over sterile Nutrient Agar plates seeded with respective test organisms. 100 µg/µl of original crude extract were aseptically transferred to these discs. The loaded disc was placed on the surface of medium and the compound was allowed to diffuse for 5 min at 37°C. The plates were incubated inverted position at 37°C or 24 hrs and each extract was tested on three replicate plates. At the end of incubation inhibition zone formed around the disc were measured in mm (millimeter) and the results were recorded.

Results:

Antibacterial Activity of Standard Antibiotics Against Pathogens

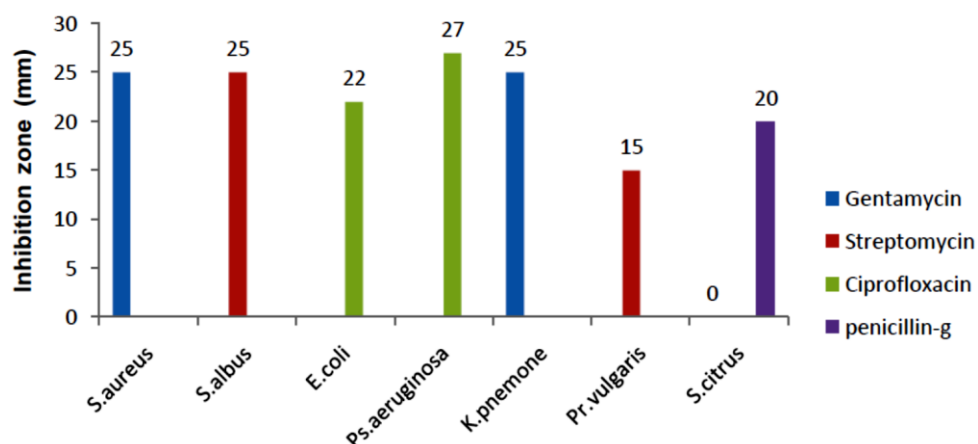


Fig: 1

Study of Diff. Extracts(Solvents) of Plant Specimens

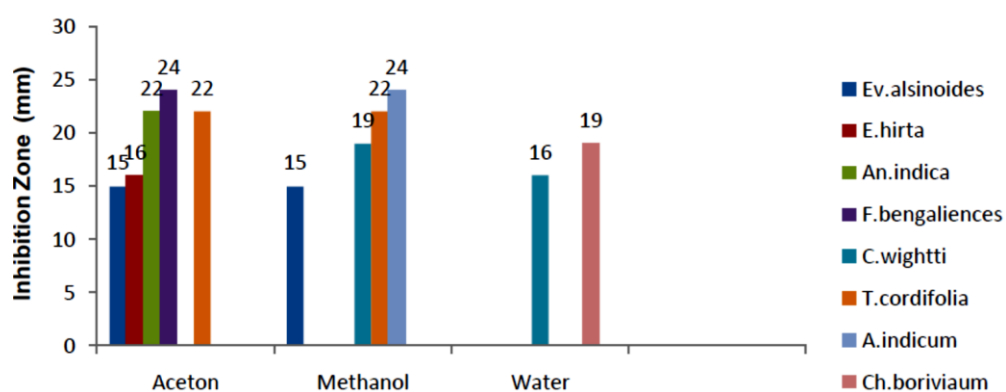


Fig: 2

Antibacterial Activity Of Plant Extracts Against *E. coli*

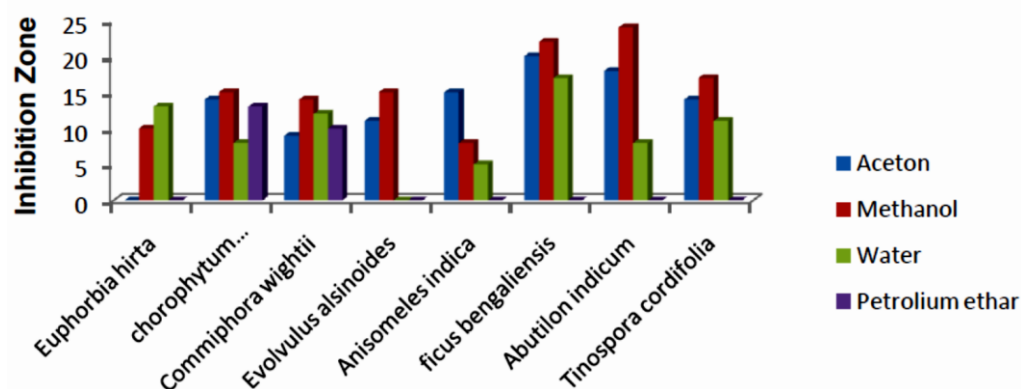


Fig: 3

Antibacterial Activity of Plant Extracts Against *P.vulgaris*

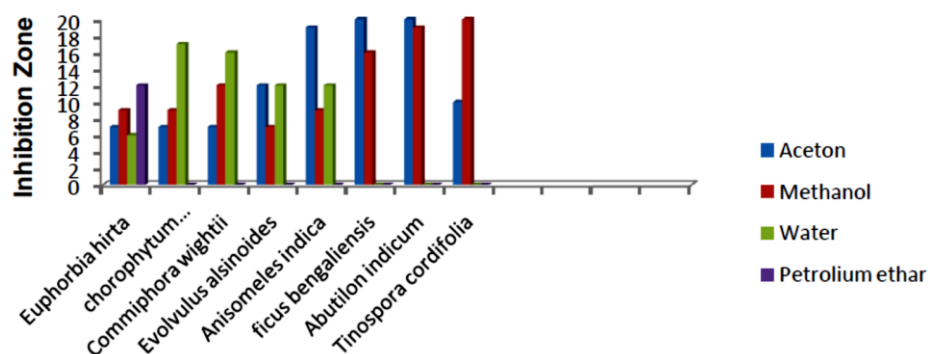


Fig: 4

Antibacterial activity of Plant Extract Against *P.aeruginosa*

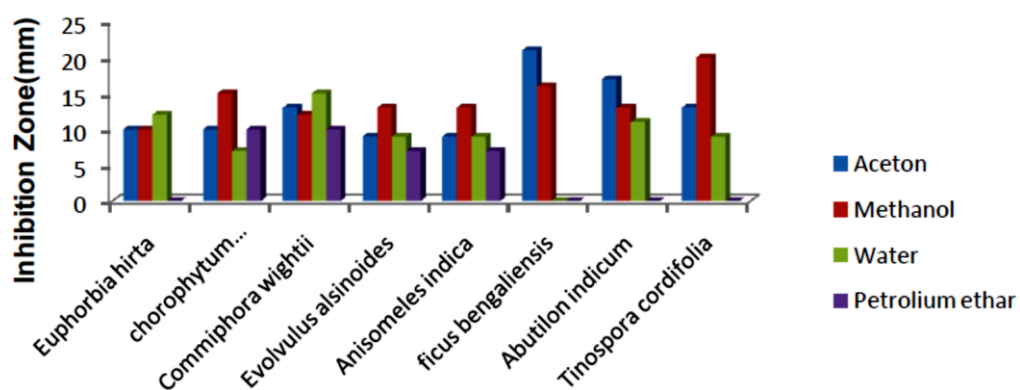


Fig: 5

Antibacterial Activity of Plant Extract Against *S. citrus*

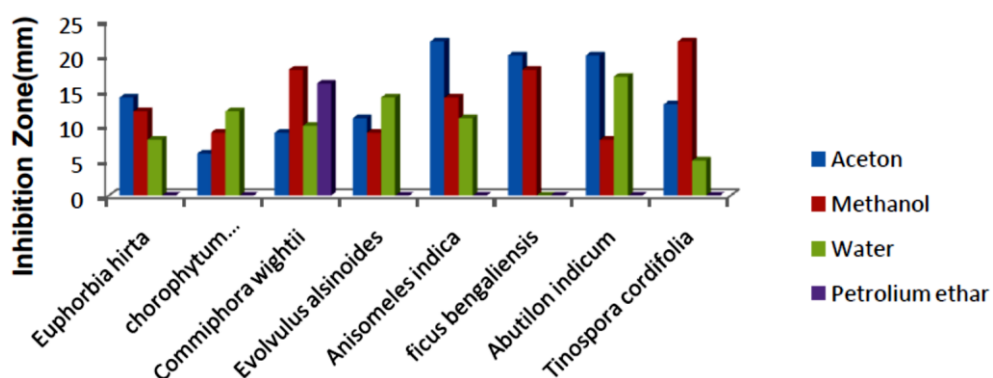


Fig: 6

Antibacterial Activity of Plant Extracts Against *K. pneumoniae*

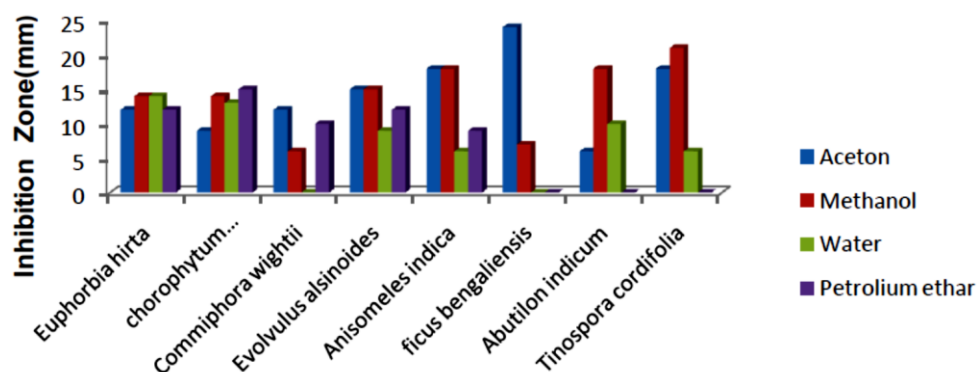


Fig: 7

Antibacterial Activity of Plant Extracts Against *S.albus*

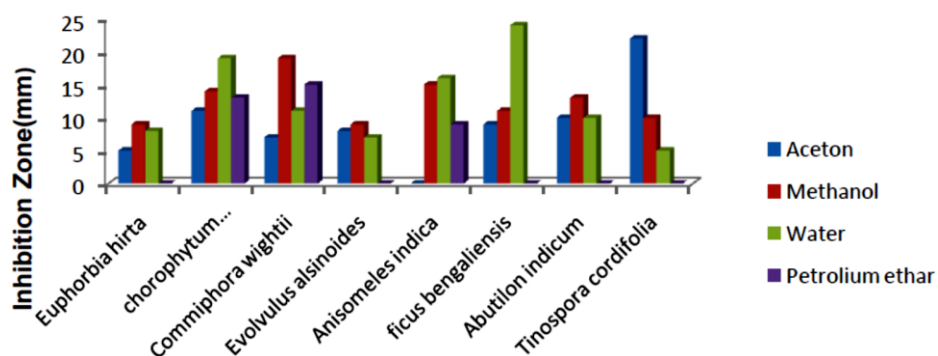


Fig: 8

Antibacterial Activity of Plant Extract Against *S. aureus*

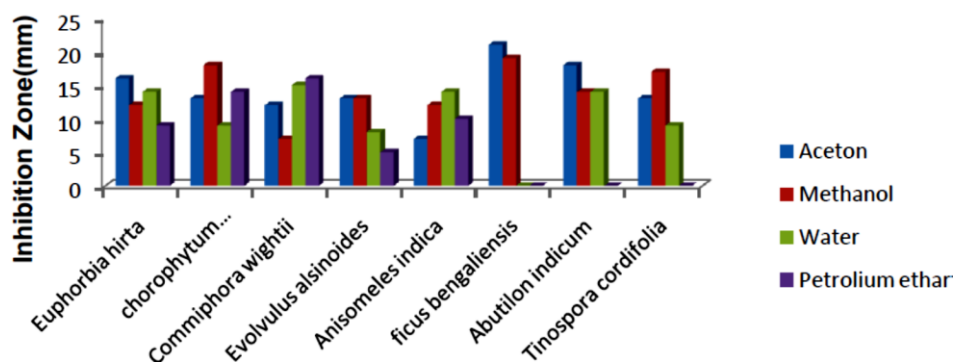


Fig: 9

In this study, various parts of eight plants and standard antibiotic discs were evaluated for their antimicrobial activity against seven microorganisms.

The antibiotic Gentamycin (25 mm), Streptomycin & Ciprofloxacin (18 mm) were the most effective against *S. aureus*. Streptomycin (25 mm) & Gentamycin (20 mm) were the most effective against *S. albus*. While *E. coli* was susceptible with Bacitracin (13 mm), Ciprofloxacin(22 mm).The antibiotic Ciprofloxacin (27 mm) was most effective against *P. aeruginosa*, Gentamycin(25 mm) was most effective against *K. pneumoniae*, Streptomycin(15 mm) was most effective against *P. vulgaris* & Penicillin G was the most effective against *S.citrus* (Fig. 1).

Ficus benghaliensis showed maximum antibacterial activity against *Klebsiella pneumoniae* in acetone extract & *S. albus* in aqueous extract. [Highest zone size: 24 mm] (Fig. 2,7).

Methanol extract of *Abutilon indicum* showed maximum activity against *E. coli* [Highest zone size: 24 mm] (Fig. 3). *P. vulgaris* was inhibited by *Tinospora cordifolia* in the methanol extract [Highest zone size:20 mm] (Fig. 4). *Ps. aeruginosa* was inhibited by *Ficus benghaliensis* and *Tinospora cordifolia* (Fig. 5). Acetone extract of *Anisomeles indica* showed maximum antibacterial activity against *S. citrus* [Highest zone size: 22 mm] (Fig. 6). Acetone & methanol extract of *Evolvulus alsinoides* showed highest activity against *K. pneumoniae* [Highest zone size: 15 mm] (Fig. 7). *T. cordifolia* showed highest activity against *S. albus* in acetone extract & *S. citrus* in methanol extract [Highest zone size: 22 mm] (Fig. 8, 6). Acetone extract of *Euphorbia hirta* showed highest antibacterial activity against *S. aureus* [Highest zone size: 16 mm] (Fig. 9). Aqueous extract of *Chorophytum borivillianum* showed maximum activity against *S. albus* [Highest zone size: 19 mm] (Fig. 8). Methanol extract of *Commiphora wightii* showed maximum activity against *S. albus* [Highest zone size: 19 mm] (Fig. 8).

S. albus showed maximum susceptibility with *Chorophytum borivillianum*, *Ficus benghaliensis* in aqueous extract (24 mm) & *T. cordifolia* in acetone extract(22mm). *K.pneumoniae* showed highest susceptibility with *F. benghaliensis* (24 mm), *E. alsinoides* in acetone extract & methanol extract. *E. coli* showed maximum inhibition with *Abutilon indicum* (stem) in methanol extract (24 mm). *S. citrus* showed highest susceptibility with *T. cordifolia* & *A. indica* in acetone extract (22 mm). *S. aureus* showed maximum inhibition with *E. hirta* in acetone extract (16 mm).

Discussion:

Extract of the total eight plants undertaken for study, 2 plants showed highest activity against three pathogens. The acetone extract of *Ficus benghaliensis* were the most effective against *K.pneumoniae*, aqueous extract of *Ficus benghaliensis* against *S. albus*. Methanol extract of *Abutilon indicum* were the most effective against *E. coli*. *S.albus* was the most susceptible with the alcoholic extract of *Chlorophytum borivillianum* , *Ficus benghaliensis* & *Tinospora cordifolia*.

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CHAPTER NO: 4

**Antibacterial activity of some
traditional medicinal plants used by
Bhil peoples of Danta forest, North
Gujarat.**

Antibacterial activity of some traditional medicinal plants used by Bhil peoples of Danta forest, North Gujarat.

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Introduction

Danta forest of North Gujarat region provides a source of food, shelter, means of livelihood to people habituated in and around the region. The people employ the services of local medicine men for curing their ailments. The following text provides an insight into the understanding of the how flora found in the region can be useful in treating bacterial disease. This study cannot be used per se for the treatment of diseases but it tries to explain the possible reason for curing bacterial diseases using the locally available plants and its parts like leaf, stem, roots, seeds, and flowers etc. This forest is inhabited by many tribes like, Bhil, Bubadiya, Meman, Parghi etc. Bhil is a hunter gatherer type of tribe, which leaves almost solely on forest products. It follows many ancient customs and rituals. Black magic and other arts are common to them. With the government programs, this tribe has now evolved into more mainstream. Education has been an important factor in this tribal development. Like other tribes, this one also depends on its native medicine men for diseases and ailments.

Following is the list of the plants used and their medicinal uses:

Calotropis procera

Calotropis procera plays an important role in improving soil fertility and improved soil water holding capacity. The root bark is febrifuge, anthelmintic, depurative, expectorant, and laxative. The powdered root promotes gastric secretions and useful in asthma, bronchitis, and dyspepsia. Madar root-bark is very largely used in India as a treatment for elephantiasis, leprosy, and in chronic eczema. It is also used as antidote substance and for abortive purposes.

Agave Americana

In the region of Tequila, agaves are called mezcales, and the high-alcohol product of their distillation is called mescal. In mezcal and tequila production, the sugars are extracted from the pinas (or hearts) by heating them in the ovens, rather than by collecting aguamiel from the plant's cut stalk.

Ocimum sanctum

The free leaves, its juice and volatile oil are used for various purposes. The oil is antibacterial and insecticidal. The leaves are used as stimulant, aromatic, antidiarrheal, spasmolytic and diaphoretic. The juice is used as an antiperiodic and as a constituent of several preparations skin diseases and also to ear-ache. Infusion of

the leaves is used as a stomachic. The drug is good immune-modulatory agent. (Kokate C.K. 2007).

Tecomella undulata

Tecomella undulata has got medicinal properties as well. The bark obtained from the stem is used as a remedy for syphilis. It is also used in curing urinary disorders, enlargement of spleen, gonorrhoea, leucoderma and liver diseases. Seeds are used against abscess. Traditionally in Musakhel, Pakistan its flower used as Hepatitis.

Acacia chundra

A mixture of flower tops, cumic, milk and sugar is useful in gonorrhoea. A mixture of catechu and myrrh (kathol) is usually prescribed as a tonic and as a galactagogue to women after confinement. Kheersal is used as a remedy for chest diseases, especially for the treatment of asthma, cough and sore throat.

Cassia fistula

The root is considered a purgative, and self-medication or any use without medicinal supervision is strongly advised against in Ayurvedic texts. Though its use in herbalism has been attested to for millennia, there has been rather little research in modern times.

Derris indica

While the oil and residue of the plant are toxic and will induce nausea and vomiting if ingested, the fruits and sprouts, along with the seeds, are used in many traditional remedies. Juices from the plant, as well as the oil, are antiseptic and resistant to pests.

Mangifera indica

Mangifera in (a pharmacologically active flavonoid, a natural xanthone C-glycoside) is extracted from Mango at high concentrations from the young leaves, bark, and from old leaves. Mango trees grow best in full sun on fertile, well drained soils and should have ample moisture.

Adhatoda vasica

Adhatoda vasica Nees (Vasaka) is used in various chest affections and enjoys wide reputation as an expectorant in the indigenous system of medicine. It was used also by traditional midwives at the time of delivery. In chronic bronchitis and asthma it is said to be very useful. The juice of the leaves is used in diarrhoea and dysentery and powdered leaves in malaria in southern India. Juice from the leaves and the decoction of the leaves and roots are helpful in asthma, bronchitis and chronic coughs and breathlessness.

Argemone Mexicana

The Seri of Sonora, Mexico use the entire plant both fresh and dried. An infusion is made to relieve kidney pain, to help expel a torn placenta, and in general to help cleanse the body after parturition. Use in Hispanic cultures includes as a sedative and analgesic tea, including for use to help alleviate migraine headaches. The seeds are taken as a laxative.

Terminalia bellirica

The fruits are much applied in local medicine, for instance in Java and India. Unripe fruits are purgative, whereas ripe fruits are astringent and often employed in a mixture with chebulicmyrobalan in cases diarrhoea, haemorrhoids and dropsy.

Capparis decudua

Its spicy fruits are used for preparing vegetables, curry and fine pickles and can attract helpful insectivores; the plant is used in folk medicine and herbalism.

Azadirachta indica

In India, the plant is variously known as “Sacred Tree,” “Heal All,” “Nature’s Drugstore,” “Village Pharmacy” and “Panacea for all diseases”. Products made from neem trees have been used in India for over two millennia for their medicinal properties: neem products are believed to be anthelmintic, antifungal, antidiabetic, antibacterial, antiviral, contraceptive and sedative. Neem oil is also used for healthy hair, to improve liver function, detoxify the blood, and balance blood sugar levels, and is considered to have no side effects. Safety issues: There has been reported that neem oil can cause some form of toxic encephalopathy and ophthalmopathy if consumed in large quantities.

Ficus racemosa

The bark of tree is said to have healing power. In countries like India, the bark is rubbed on a stone with water to make a paste and the paste is applied over the skin which is having boils or mosquito bites. Allow the paste to dry on the skin and reapply after a few hours. For people whose skin is especially sensitive to insect bites; this is a very simple home remedy.

Materials and Methods:

Solvents:

Distilled water and methanol were used for the extraction method.

Extraction method:

Hot bath Extraction:

5gm of powdered and 50ml solvent (distilled water and methanol) was mixed in flask. This mixture was put of evaporate condition in the water bath for 3 or 4hr and boiled. After 3 or 4hr. it was cooled and filtered the Whatman No. 3 filter paper. Filtrate was took in the beaker and it was put in the water bath for dried.

Soxhlation Extraction:

The plant material is continuously flushed with fresh solvent. But the fresh solvent is formed by boiling the solvent containing the extracted analyte. The total amount of solvent is limited. In spite of what is something though, a soxhlation extraction can be far from complete, due to channelling or the presence of air in the semi-permeable containing the plant material.

Suitability of the soxhlet method: for heat stable substances only, and also for active constituents which are less soluble in the menstrum in the absence of heat.

After soxhlation method, each extract is concentrated by distilling off the solvent and then evaporating the solvent to dryness on a water bath. The extract obtained with the solvent is weighed.

Microorganism and media.

Five bacterial strains Gram positive – *Staphylococcus aureus* (MTCC 96), Gram negative – *Escherichia coli* (MTTCC 443), *Shigella flexneri* (MTCC 1457), *Bordetella bronchiseptica* (MTCC 6838), and *Hemophilus influenza* (MTCC 3826) were selected as test cultures.

Cultures were obtained from IMTECH Chandigarh.

Preparation of inoculum:

Pure cultures were maintained on Nutrient Agar slant. Inoculum was prepared by suspending the growth from Nutrient agar slant in normal saline water. Periodic transfers were made on fresh media every month. The turbidity of culture was adjusted to that comparison with a 0.5 McFarland standard. (Jayshree D. Patel, 2009)

Preparation of the McFarland standard:

Add 0.5 ml 0.048 M BaCl₂ (1.17%w/v BaCl₂. 2H₂O) to 99.5 ml of 0.18 M H₂SO₄ (1%v/v) with constant stirring. Distribute the standard into screw cap tubes of the same size and with the same volume as those used in growing the broth cultures. Seal the tubes tightly to prevent loss by evaporation. Store protected from light at room temperature. Vigorously agitate the turbidity standard on a vortex mixture before use. Standard may be stored for up to 6 months, after which time they should be discarded. Alternatively, prepared standards can be purchased (Jennifer M. Andrews, 2001)

Preparation of Dilutions:

Stock solution = 200 mg extract in 1 ml distilled water.

1. 25 ug/ml = 1 ml stock in 7 ml distilled water
2. 50 ug/ml = 1 ml stock in 3 ml distilled water
3. 75 ug/ml = 3 ml stock in 1 ml distilled water
4. 100 ug/ml = 2 ml stock in 2 ml distilled water
5. 125 ug/ml = 2 ml stock in 3 ml distilled water

Antimicrobial assay:

Antibacterial sensitivity testing using disc diffusion method:

Circular disc of 6 mm diameter were made from the Whatman no 1 filter paper. Discs were impregnated with equal volume (50 ug/ml) of each plant extract at four different concentrations (0.05 g/ml, 0.2 g/ml & 4 g/ml). The discs were aseptically placed over plates of Muller Hinton agar seeded with each of test pathogens. The plates were incubated in an incubator at 37°C for 24 hours at the end of which the zone of inhibition was measured in mm surrounding the disc.

Results and Discussion

Table No. 01

Organism	Solvent	Dilution ug/ml	Zone of inhibition (mm)						
			<i>Acacia chundra</i>		<i>Argemone mexicana</i>		<i>Mangifera indica</i>		
<i>E. coli</i>	Methanol		Leaf	Stem	Leaf	Stem	Leaf	Stem	Flower
		25	9	7	21	9	0	0	0
		50	11	9	23	10	0	0	0
		75	14	12	26	13	0	0	
		100	15	15	27	15	0	0	0
		125	18	16	30	18	0	0	0
	D/water	25	0	0	36	0	8	27	0
		50	0	0	30	0	13	30	0
		75	0	0	36	0	20	32	0
		100	0	0	30	3	20	35	0
		125	0	0	30	0	22	37	0
<i>S. aureus</i>	Methanol	25	12	14	32	15	8	0	0
		50	14	16	32	18	10	7	0
		75	18	17	34	21	11	8	0
		100	20	20	8	24	13	10	0
		125	24	22	12	27	16	12	0
	D/water	25	10	12	15	17	0	30	0
		50	12	13	18	19	15	31	0
		75	13	14	21	21	0	34	0
		100	0	16	24	25	17	35	0
		125	15	20	27	27	18	38	0
	Methanol	25	9	7	17	12	0	0	0

<i>B. bronchiseptica</i>		50	14	13	19	14	0	0	0
		75	16	14	21	16	0	0	0
		100	18	16	25	17	0	0	0
		125	21	20	27	19	0	0	0
		25	10	7	12	9	0	20	0
	D/water	50	11	9	14	13	10	24	0
		75	12	11	16	15	15	29	0
		100	15	13	17	17	18	30	0
		125	18	16	19	20	21	31	0
		25	0	20	21	10	7	0	0
<i>S. flexneri</i>	Methanol	50	8	22	25	14	9	0	0
		75	10	25	27	17	12	8	0
		100	13	30	12	20	16	10	0
		125	15	27	14	10	19	13	0
		25	7	9	16	14	13	0	0
	D/water	50	8	12	17	17	15	0	0
		75	10	15	19	20	20	9	0
		100	11	17	9	25	20	10	0
		125	14	20	13	15	23	12	0
		25	10	12	15	17	11	9	0
<i>H. influenzae</i>	Methanol	50	15	13	17	19	14	12	0
		75	15	16	20	21	15	14	0
		100	17	19	10	25	18	16	0
		125	22	23	14	10	21	19	0
		25	12	18	17	12	7	25	0
	D/water	50	15	20	23	13	9	28	0
		75	18	25	24	15	12	29	0
		100	20	28	24	19	14	35	0
		125	25	30	27	8	18	40	0
		25	10	12	15	17	11	9	0

Organism	Solvent	Dilution ug/ml	<i>Cassia fisula</i>		<i>Derris indica</i>		<i>Adhatoda vassica</i>	
<i>E. coli</i>	Methanol		Leaf	Stem	Leaf	Stem	Leaf	Stem
		25	9	0	0	0	0	0
		50	12	0	0	0	0	0
		75	15	0	8	0	0	0
		100	17	0	10	0	0	0
		125	20	0	12	0	0	0
	D/water	25	0	0	8	0	0	0
		50	0	0	13	9	0	0
		75	0	0	8	10	0	0
		100	0	7	13	15	0	0
		125	7	9	17	18	0	0

<i>S. aureus</i>	Methanol	25	21	10	20	8	25	12
		50	23	15	12	10	30	16
		75	25	17	15	12	32	18
		100	28	19	20	15	33	21
		125	30	22	22	17	35	24
	D/water	25	32	21	25	8	28	30
		50	34	28	10	11	31	34
		75	37	32	13	13	38	36
		100	38	34	18	16	40	38
		125	40	38	21	19	0	40
<i>B. bronchiseptica</i>	Methanol	25	8	11	26	10	0	15
		50	9	13	9	13	0	16
		75	12	16	12	16	12	18
		100	14	18	14	17	14	20
		125	16	19	19	20	16	21
	D/water	25	0	0	13	8	8	7
		50	7	8	15	10	9	9
		75	11	9	17	12	10	12
		100	13	11	20	15	13	16
		125	14	13	23	18	15	19
<i>S. flexneri</i>	Methanol	25	11	9	26	17	14	10
		50	13	13	28	19	15	12
		75	16	15	30	20	18	15
		100	19	17	32	23	19	17
		125	21	19	35	25	25	19
	D/water	25	0	0	15	10	31	26
		50	0	0	16	12	32	28
		75	0	8	19	16	36	37
		100	0	10	21	19	0	38
		125	0	12	24	21	43	41
<i>H. influenzae</i>	Methanol	25	21	15	19	15	21	19
		50	22	18	20	18	22	20
		75	25	19	23	19	25	24
		100	25	23	25	20	25	26
		125	27	24	30	23	27	29
	D/water	25	21	20	14	13	21	24
		50	24	25	16	16	23	25
		75	28	27	19	17	27	27
		100	29	29	20	18	25	26
		125	31	30	25	22	30	28

Organism	Solvent	Dilution	<i>Ficus racemosa</i>		<i>Azadirachta indica</i>		<i>Terminalia bellirica</i>	
			Leaf	Stem	Leaf	Stem	Leaf	Stem
<i>E. coli</i>	Methanol	25	7	0	14	9	0	8
		50	8	0	17	11	0	9
		75	9	0	20	14	0	11
		100	12	0	21	18	0	13
		125	15	0	22	20	0	15
	D/water	25	15	8	0	7	0	0
		50	18	12	0	8	0	0
		75	21	15	0	9	0	0
		100	25	17	0	10	0	0
		125	30	19	0	12	0	0
<i>S. aureus</i>	Methanol	25	21	10	23	10	20	15
		50	22	12	30	10	21	16
		75	21	14	33	11	23	18
		100	25	16	35	12	25	20
		125	30	18	37	14	27	24
	D/water	25	9	15	7	11	14	11
		50	18	18	9	12	16	13
		75	21	20	10	15	17	15
		100	25	23	10	17	19	17
		125	27	26	12	19	21	18
<i>B. bronchiseptica</i>	Methanol	25	11	9	8	8	7	0
		50	12	11	10	12	9	0
		75	18	13	12	14	11	8
		100	19	15	13	16	12	10
		125	20	17	15	18	15	13
	D/water	25	19	13	9	9	10	8
		50	20	17	16	14	13	10
		75	21	19	17	20	14	12
		100	25	20	22	22	16	13
		125	30	22	29	24	18	14
<i>S. flexneri</i>	Methanol	25	16	13	22	12	10	9
		50	17	15	24	13	12	11
		75	20	17	26	15	14	12
		100	21	19	28	18	15	12
		125	25	21	28	20	17	15
	D/water	25	12	9	8	12	15	10
		50	15	10	8	14	18	13
		75	18	13	10	15	20	17
		100	18	15	15	18	23	18
		125	20	19	18	22	26	20
<i>H. influenzae</i>	Methanol	25	21	15	15	15	13	9

		50	24	17	19	21	14	12
		75	28	19	19	22	16	15
		100	30	21	21	23	18	17
		125	30	23	23	24	19	19
	D/water	25	21	19	14	20	15	11
		50	19	21	16	24	16	13
		75	20	23	18	28	17	15
		100	24	25	20	30	19	17
		125	24	27	23	35	21	18

Zone of inhibition (mm)						
Organism	Solvent	Dilution ug/ml	<i>Capparis decidua</i>	<i>Heliotropium supinum</i>		<i>Agave americana</i>
<i>E. coli</i>	Methanol		Leaf	Leaf	Stem	Leaf
		25	0	8	0	7
		50	0	10	0	12
		75	0	12	0	14
		100	0	14	0	16
		125	0	15	0	19
	D/water	25	0	0	0	7
		50	8	0	0	9
		75	9	0	0	12
		100	10	0	0	15
		125	10	0	0	19
<i>S. aureus</i>	Methanol	25	16	12	9	0
		50	18	14	11	8
		75	22	15	13	10
		100	24	17	15	11
		125	26	19	17	15
	D/water	25	0	20	7	0
		50	8	22	7	7
		75	8	28	9	9
		100	9	30	11	10
		125	11	32	12	12
<i>B. bronchisepti ca</i>	Methanol	25	9	9	7	0
		50	11	10	9	16
		75	13	12	11	19
		100	15	14	13	21
		125	18	16	15	21
	D/water	25	15	12	0	13
		50	19	14	0	16
		75	21	14	0	17
		100	25	16	0	18

		125	30	18	0	20
<i>S. flexneri</i>	Methanol	25	17	14	10	17
		50	18	15	12	17
		75	20	17	14	19
		100	22	17	14	19
		125	24	19	17	18
	D/water	25	9	9	12	9
		50	10	8	15	10
		75	13	10	20	12
		100	17	13	21	14
		125	19	15	23	16
<i>H. influenzae</i>	Methanol	25	11	22	19	14
		50	13	22	20	17
		75	15	25	22	19
		100	18	30	24	26
		125	20	30	25	27
	D/water	25	20	20	12	20
		50	24	24	13	21
		75	25	25	16	24
		100	28	29	20	24
		125	30	30	17	30

Organism	Solvent	Dilution ug/ml	<i>Tecomella undulata</i>		<i>Ocimum sanctum</i>		<i>Calotropis procera</i>		
<i>E. coli</i>	Methanol		Leaf	Stem	Leaf	Stem	Leaf	Stem	Flower
		25	0	0	12	0	7	0	0
		50	9	9	15	0	9	0	0
		75	12	10	15	0	10	0	0
		100	14	13	18	0	15	0	0
		125	14	17	20	0	17	0	0
	D/water	25	0	0	12	0	0	9	0
		50	0	0	15	0	8	11	0
		75	0	0	18	0	9	13	0
		100	0	0	18	0	10	15	0
		125	0	0	20	0	12	17	0
<i>S. aureus</i>	Methanol	25	0	18	25	23	0	9	0
		50	7	17	27	25	12	10	0
		75	12	20	28	26	15	12	0
		100	15	21	28	28	17	14	0
		125	17	25	32	30	19	16	0
	D/water	25	7	20	27	22	15	12	0
		50	12	24	30	24	17	14	0

		75	14	21	31	26	18	15	0
		100	13	28	31	28	19	17	0
		125	16	30	33	30	21	18	0
<i>B. bronchiseptica</i>	Methanol	25	19	8	15	12	20	9	0
		50	20	9	18	13	21	10	0
		75	21	15	20	15	23	12	0
		100	21	17	22	17	25	16	0
		125	25	19	25	19	26	20	0
	D/water	25	12	23	10	0	14	10	0
		50	15	24	15	8	16	12	0
		75	18	26	13	10	18	13	0
		100	20	27	18	12	20	15	0
		125	28	29	23	14	21	17	0
<i>S. flexneri</i>	Methanol	25	13	10	15	10	13	10	0
		50	15	12	18	13	14	12	0
		75	17	17	19	15	17	19	0
		100	19	20	20	17	19	20	0
		125	20	25	25	19	20	25	0
	D/water	25	10	8	16	9	15	10	0
		50	11	12	18	13	17	12	0
		75	13	15	19	15	18	14	0
		100	13	19	20	16	19	15	0
		125	15	21	27	18	20	16	0
<i>H. influenzae</i>	Methanol	25	21	19	25	20	24	19	0
		50	25	19	27	22	23	20	0
		75	25	22	29	24	24	22	0
		100	28	26	30	25	22	25	0
		125	30	27	31	27	28	30	0
	D/water	25	12	14	21	15	14	12	0
		50	14	16	28	17	16	13	0
		75	14	18	27	21	18	15	0
		100	16	20	29	25	20	17	0
		125	18	25	33	27	21	18	0

Plants showing highest zone size:

Among the plants taken up for the study, *Adhatoda vasica* gave highest zone sizes.

Acacia chundra

The leaf methanol extract inhibited *E. coli* (18mm), *S. aureus* (24mm), *B. bronchiseptica* (21mm), *S. flexneri* (15mm) and *H. influenzae* (22mm).

Leaf aqua extract inhibited *S. aureus* (15mm), *B. bronchiseptica* (18mm), *S. flexneri* (14mm) and *H. influenzae* (25mm).

Stem methanol extract inhibited *E. coli* (16mm), *S. aureus* (22mm), *B. bronchiseptica* (16mm), *S. flexneri* (20mm) and *H. influenzae* (23mm).
Stem aqua extract inhibited *S. aureus* (20mm) *B. bronchiseptica* (27mm), *S. flexneri* (20mm) and *H. influenzae* (23mm).

Argemone mexicana

The leaf methanol extract inhibited *E. coli* (30mm), *S. aureus* (20mm), *B. bronchiseptica* (23mm), *S. flexneri* (22mm) and *H. influenzae* (32mm).
Leaf aqua extract inhibited *E. coli* (34mm) *S. aureus* (22mm), *B. bronchiseptica* (25mm), *S. flexneri* (24mm) and *H. influenzae* (30mm).
Stem methanol extract inhibited *E. coli* (18mm), *S. aureus* (27mm), *B. bronchiseptica* (19mm), *S. flexneri* (25mm) and *H. influenzae* (19mm).
Stem aqua extract inhibited *S. aureus* (27mm), *B. bronchiseptica* (20mm), *S. flexneri* (25mm) and *H. influenzae* (17mm).

Mangifera indica

The leaf methanol extract inhibited all but *E. coli* and *B. bronchiseptica*.
Leaf aqua extract inhibited *E. coli* (22mm) *S. aureus* (18mm), *B. bronchiseptica* (21mm), *S. flexneri* (23mm) and *H. influenzae* (18mm).
Stem methanol extract inhibited all but *E. coli* and *B. bronchiseptica*.
Leaf aqua extract inhibited *E. coli* (37mm) *S. aureus* (38mm), *B. bronchiseptica* (31mm), *S. flexneri* (12mm) and *H. influenzae* (40mm).
Flower aqua and methanol extract inhibited none of the organisms.

Cassia fistula

The leaf methanol extract inhibited *E. coli* (20mm), *S. aureus* (30mm), *B. bronchiseptica* (16mm), *S. flexneri* (21 mm) and *H. influenzae* (27mm).
Leaf aqua extract inhibited *E. coli* (07mm) *S. aureus* (40mm), *B. bronchiseptica* (14mm) and *H. influenzae* (31 mm).
Stem methanol extract inhibited *S. aureus* (22mm), *B. bronchiseptica* (19mm), *S. flexneri* (19mm) and *H. influenzae* (24mm).
Stem aqua extract inhibited *E. coli* (09mm), *S. aureus* (38mm), *B. bronchiseptica* (13mm), *S. flexneri* (12mm) and *H. influenzae* (30mm).

Derris indica

The leaf methanol extract inhibited *E. coli* (12mm), *S. aureus* (25mm), *B. bronchiseptica* (19mm), *S. flexneri* (35mm) and *H. influenzae* (30mm).
Leaf aqua extract inhibited *E. coli* (21mm) *S. aureus* (26mm), *B. bronchiseptica* (23mm), *S. flexneri* (24mm) and *H. influenzae* (25mm).
Stem methanol extract inhibited *S. aureus* (11mm), *B. bronchiseptica* (20mm), *S. flexneri* (25mm) and *H. influenzae* (23mm).
Stem aqua extract inhibited *E. coli* (18mm), *S. aureus* (19mm) *B. bronchiseptica* (18mm), *S. flexneri* (21mm) and *H. influenzae* (22mm).

Adhatoda vasica

The Leaf methanol extract inhibited *S. aureus* (35mm), *B. bronchiseptica* (16mm), *S. flexneri* (19mm) and *H. influenzae* (27mm).

Leaf aqua extract inhibited *S. aureus* (40mm), *B. bronchiseptica* (15mm), *S. flexneri* (43mm) and *H. influenzae* (30mm).

Stem methanol extract inhibited *S. aureus* (24mm), *B. bronchiseptica* (2\mm), *S. flexneri* (17mm) and *H. influenzae* (29mm).

Stem aqua extract inhibited *E. coli* (08mm), *S. aureus* (40mm), *B. bronchiseptica* (19mm), *S. flexneri* (41mm) and *H. influenzae* (28mm).

Ficus racemosa

The Leaf methanol extract inhibited *E. coli* (15mm), *S. aureus* (30mm), *B. bronchiseptica* (20mm), *S. flexneri* (25mm) and *H. influenzae* (30mm).

Leaf aqua extract inhibited *E. coli* (30mm), *S. aureus* (21mm), *B. bronchiseptica* (30mm), *S. flexneri* (20mm) and *H. influenzae* (24mm).

Stem methanol extract inhibited *S. aureus* (18mm), *B. bronchiseptica* (17mm), *S. flexneri* (21mm) and *H. influenzae* (23mm).

Stem aqua extract inhibited *E. coli* (19mm), *S. aureus* (26mm), *B. bronchiseptica* (22mm), *S. flexneri* (19mm) and *H. influenzae* (21mm).

Azadirachta indica

The leaf methanol extract inhibited *E. coli* (22mm), *S. aureus* (37mm), *B. bronchiseptica* (15mm), *S. flexneri* (28mm) and *H. influenzae* (23mm).

Leaf aqua extract inhibited *S. aureus* (17mm), *B. bronchiseptica* (29mm), *S. flexneri* (18mm) and *H. influenzae* (23mm).

Stem methanol extract inhibited *E. coli* (20mm), *S. aureus* (14mm), *B. bronchiseptica* (18mm), *S. flexneri* (20mm) and *H. influenzae* (24mm).

Stem aqua extract inhibited *E. coli* (12mm), *S. aureus* (19mm), *B. bronchiseptica* (24mm), *S. flexneri* (22mm) and *H. influenzae* (35mm).

Terminalia bellirica

Leaf methanol extract inhibited *S. aureus* (27mm), *B. bronchiseptica* (15mm), *S. flexneri* (17mm) and *H. influenzae* (19mm).

Leaf aqua extract inhibited *S. aureus* (21mm), *B. bronchiseptica* (15mm), *S. flexneri* (26mm) and *H. influenzae* (21 mm).

Stem methanol extract inhibited *E. coli* (15 mm), *S. aureus* (24mm), *B. bronchiseptica* (13mm), *S. flexneri* (15mm) and *H. influenzae* (19mm).

Stem aqua extract inhibited *S. aureus* (18mm), *B. bronchiseptica* (14mm), *S. flexneri* (20mm) and *H. influenzae* (18mm).

Capparis decidua

Stem methanol extract inhibited *S. aureus* (26mm), *B. bronchiseptica* (18mm), *S. flexneri* (24mm) and *H. influenzae* (20mm).

Stem aqua extract inhibited *E. coli* (10mm), *S. aureus* (11mm), *B. bronchiseptica* (30 mm), *S. flexneri* (19mm) and *H. influenzae* (30mm).

Heliotropium supinum

Leaf methanol extract inhibited *E. coli* (15mm), *S. aureus* (19mm), *B. bronchiseptica* (16mm), *S. flexneri* (19mm) and *H. influenzae* (30mm).

Leaf aqua extract inhibited *S. aureus* (32mm), *B. bronchiseptica* (18mm), *S. flexneri* (15mm) and *H. influenzae* (30mm).

Stem methanol extract inhibited *S. aureus* (32mm), *B. bronchiseptica* (18mm), *S. flexneri* (15mm) and *H. influenzae* (25mm).

Stem aqua extract inhibited all but *E. coli* and *B. bronchiseptica*.

Agave Americana

Leaf methanol extract inhibited *E. coli* (19mm), *S. aureus* (15mm), *B. bronchiseptica* (21 mm), *S. flexneri* (18mm) and *H. influenzae* (27mm).

Leaf aqua extract inhibited *E. coli* (19mm), *S. aureus* (12mm), *B. bronchiseptica*(20mm), *S. flexneri* (16mm) and *H. influenzae* (30mm).

Calotropis procera

Leaf methanol extract inhibited *E. coli* (15mm), *S. aureus*(30mm), *B. bronchiseptica*(20mm), *S. flexneri* (25mm) and *H. influenzae* (30mm).

Leaf aqua extract inhibited *E. coli* (30mm), *S. aureus*(21mm), *B. bronchiseptica* (30mm), *S. flexneri* (20mm) and *H. influenzae* (24mm).

Stem methanol extract inhibited *S. aureus* (18mm), *B. bronchiseptica* (11mm), *S. flexneri* (21mm) and *H. influenzae* (23mm).

Stem aqua extract inhibited *E. coli* (19mm), *S. aureus* (26mm), *B. bronchiseptica* (22mm), *S. flexneri* (19mm) and *H. influenzae* (27mm).

Ocimum sanctum

Leaf methanol extract inhibited *E. coli* (20mm), *S. aureus* (32mm), *B. bronchiseptica* (25mm), *S. flexneri* (25mm) and *H. influenzae* (31mm).

Leaf aqua extract inhibited *E. coli* (20mm), *S. aureus* (33mm), *B. bronchiseptica* (23mm), *S. flexneri* (27mm) and *H. influenzae* (33mm).

Stem methanol extract inhibited *S. aureus* (30mm), *B. bronchiseptica* (19mm), *S. flexneri* (19mm) and *H. influenzae* (27mm).

Stem aqua extract inhibited *S. aureus* (30mm), *B. bronchiseptica* (14mm), *S. flexneri* (18mm) and *H. influenzae* (27mm).

Tecomella undulata

Leaf methanol extract inhibited *E. coli* (14mm), *S. aureus* (11mm), *B. bronchiseptica* (25mm), *S. flexneri* (20mm) and *H. influenzae* (30mm).

Leaf aqua extract inhibited *S. aureus* (11mm), *B. bronchiseptica* (25mm), *S. flexneri* (20mm) and *H. influenzae* (30mm).

Stem methanol extract inhibited *E. coli* (17mm), *S. aureus* (25mm), *B. bronchiseptica* (19mm), *S. flexneri* (25mm) and *H. influenzae* (27mm).

Leaf aqua extract inhibited *S. aureus* (30mm), *B. bronchiseptica* (29mm), *S. flexneri* (21mm) and *H. influenzae* (25mm).

Result of bacterial inhibition by plants:

Among the bacteria studied, *H. influenzae* was the most sensitive bacteria being inhibited by all plants taken up for study whereas *E. coli* was the most resistant as it could be inhibited by 14 plants from the total study of 15 plants.

S. aureus and *S. flexneri* were highly sensitive in terms of zone of inhibition among the plant extracts taken up for the study.

B. bronchiseptica was highly sensitive to *Calotropis procera*, *Mangifera indica*, *Heliotropium supinum*.

Potential Application of Plant Extract to inhibition of pathogen (in mm)

Plant	Bacteria	Solvent	Zone	
<i>Acacia chundra</i>	Stem	<i>H. influenzae</i>	Aqua	30
<i>Argemone mexicana</i>	Leaf	<i>E. coli</i>	Aqua	34
<i>Argemone mexicana</i>	Leaf	<i>H. influenzae</i>	Methanol	32
<i>Mangifera indica</i>	Stem	<i>E. coli</i>	Aqua	37
<i>Mangifera indica</i>	Stem	<i>S. aureus</i>	Aqua	38
<i>Mangifera indica</i>	Stem	<i>B. borditella</i>	Aqua	31
<i>Mangifera indica</i>	Stem	<i>H. influenzae</i>	Aqua	40
<i>Cassia fistula</i>	Leaf	<i>S. aureus</i>	Aqua	40
<i>Cassia fistula</i>	Stem	<i>S. aureus</i>	Aqua	38
<i>Cassia fistula</i>	Leaf	<i>H. influenzae</i>	Aqua	31
<i>Adhatoda vasica</i>	Leaf	<i>S. aureus</i>	Aqua	40
<i>Adhatoda vasica</i>	Leaf	<i>S. flexneri</i>	Aqua	43
<i>Adhatoda vasica</i>	Leaf	<i>H. influenzae</i>	Aqua	30
<i>Ficus racemosa</i>	Leaf	<i>E. coli</i>	Aqua	30
<i>Ficus racemosa</i>	Leaf	<i>H. influenzae</i>	Methanol	30
<i>Azadirachta indica</i>	Leaf	<i>B. borditella</i>	Methanol	37
<i>Azadirachta indica</i>	Stem	<i>H. influenzae</i>	Aqua	35
<i>Capparis decidua</i>	Stem	<i>B. borditella</i>	Aqua	30
<i>Capparis decidua</i>	Stem	<i>H. influenzae</i>	Aqua	30
<i>Heliotropium supinum</i>	Leaf	<i>S. aureus</i>	Aqua	32
<i>Aegle marmelos</i>	Leaf	<i>H. influenzae</i>	Aqua	30
<i>Agave Americana</i>	Leaf	<i>H. influenzae</i>	Aqua	30
<i>Ocimum sanctum</i>	Leaf	<i>H. influenzae</i>	Aqua	33
<i>Tecomella undulata</i>	Leaf	<i>S. aureus</i>	Aqua	30

Results of standard antibiotic disc study:

Among the eight antibiotic disc used, *S. flexneri* was inhibited by Tetracycline while *H. influenzae* resisted all antibiotic discs. Whereas *E. coli* inhibited by Erythromycin, Tobramycin, and Kanamycin. *B. bronchiseptica* inhibited by Tobramycin and Tetracycline. *S. aureus* also inhibited by Tobramycin and Tetracycline.

Conclusion of std. antibiotic disc

None of the antibiotics used could inhibit all of the bacteria whereas *H. influenzae* was most resistant among all.

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CHAPTER NO: 5

**Evaluation of antibacterial activity of
some traditional medicinal plants used
by Parghi peoples of Danta forest,
North Gujarat.**

Evaluation of antibacterial activity of some traditional medicinal plants used by Parghi peoples of Danta forest, North Gujarat.

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INTRODUCTION

The plants are basic to man for his life. The three paramount necessities of life – nourishment, dress and asylum – and a group of other suitable items are supplied to him by the plant kingdom. Nature has given a complete storage facility of solutions to cure all afflictions of humanity. The learning of medications has collected over many years as a consequence of man's curious nature so that today we have numerous viable methods for guaranteeing social insurance. All phyla of plants viz. Thallophyta, Bryophyta, Pteridophyta and spermatophyte, (of which preservationist evaluations put the aggregate number of known species at roughly 335000) hold species that yield official and informal results of medicinal significance. Charaka made fifty assemblies of ten herbs each of which, as stated by him, might suffice a customary doctor's need. Correspondingly, Sushruta organized 760 herbs in 7 unique sets dependent upon some of their normal properties. An expansive share of the Indian populace even today relies on upon the Indian System of Medicine – Ayurveda, 'An old investigation of life. The well-known treatises in Ayurveda are Charaka Samhita and Sushruta Samhita (Kokate, 2010).

Present day drug or allopathy has bit by bit created throughout the years of investigative and observational deliberations of researchers. On the other hand, the support of its advancement stays in the bases of accepted pharmaceutical and helps. The old shrewdness has been the support of up to date medication and will stay as one vital wellspring of future drug and therapeutics. (Asis. B, 2010).

A large portion of the world population, especially in developing countries, depends on the traditionally systems of medicine to treat a variety of diseases. Several hundred genera of plants are used medicinally. The World Health Organization (WHO) reported that 80% of the world population relies chiefly on traditional medicine and a major part of the traditional therapies, which involve the use of plant extracts or their active constituents. Due to indiscriminate use of antimicrobial drugs, microorganisms have developed resistance to many antibiotics and that has created immense clinical problems in the treatment of infectious diseases. In the present scenario of emergence of drugs resistance in human pathogenic organisms, there is a need to develop alternative antimicrobial drugs for the treatment of infectious diseases. (Kantalak P, 2002).

Human population dwelling in the forest depend on loacal medicine men for their treatment of diseases. Parghi tribe is one of the underdeveloped population of Danta forest region. Many of its kind follows a basic hunter gatherer type of living. With ongoing education program, their development into mainstream has been possible. But the old customs of black magic and woodoo still exist. They depend on plant products not only for their food, clothing and shelter, but also for their survival from fatal diseases.

Acalypha indica L.

It is useful in bronchitis, asthma, pneumonia and rheumatism, its roots and leaves have laxative properties. Juice of leaves is considered an efficient emetic, that is a medicine for causing vomiting. A product of fresh leaves is useful in ulcers. The juice extracted from the leaves, mixed with lime and applied on skin to cure diseases caused by Ringworm. Fresh juice of leaves mixed with oil and salt is used for *Rheumatoid arthritis* and to cure Scabies. Powdered leaves are used to cure bedsores and infected wounds. The active medicinal compounds like Acalyphine and Triacetoneamine are extracted from this plant. They contain cyanogenic glucoside and alkaloids. The paste of the leaves can be applied to burns (Raamachandran, J.).

Alangium salvifolium

Root bark is emetic, febrifuge, purgative, anthelmintic, diaphoretic, antipyretic; useful in fever and piles. It is also used in leprosy, syphilitic and other skin diseases. Leaves are useful in poultice in rheumatic pains. Fruits are laxative, expectorant, carminative, anthelmintic, alexiteric; useful in inflammation, burning of the body, spermatorrhoea, gleet, acute fever and lumbago (Ravi Shankar Pandey, 2012).

Bombax ceiba

Flower petal boiled in water, mixed with nappee (crushed and processed small fish) and chilli to prepare chutney. Cotton is used for pillow and blanket. Flowers are boiled in water is eaten as salad and also cooked as vegetable. Paste prepared from crushed flowers mixed with fruit shell of ripe banana and applied around the boils for boil suppuration. Flower paste applied to boils, itches and affected sore. Petal removed from the flower and rest of the part is cooked as vegetable. Juice prepared from the crushed root is taken in the morning to increase sex in male and clear bowels (Bawn, 1998).

Cissampelos pareira

Cissampelos pareira leaves and stems, to cure gastro-intestinal complaints such as diarrhoea, dysentery, ulcers, colic, intestinal worms and digestive complaints, and also urogenital problems such as menstrual problems, venereal diseases, infertility, uterine bleeding and threatening miscarriage. A rhizome decoction or pounded leaves are also widely taken or externally applied as a febrifuge and stomachic, and against cough, heart trouble, rheumatism, jaundice, snake bites and skin infections such as sores, boils, scabies and childhood eczema. (Baerts M. & Lehmann J, 2006).

Cleome gynandra

The tender leaves, young shoots and occasionally flowers are eaten boiled as potherb, relish, stew or side dish. The leaves are utilized in fresh form or dried as powder. Sometimes the leaves are bitter and then cooked with milk and/or with other leafy vegetables such as cowpea leaves, amaranth. In other areas the leaves are boiled and the cooking water is discarded. In several countries, pounded groundnut paste (peanut butter) is added to improve the flavour. The seeds may be used as a substitute for mustard. In several communities, boiled spider plant leaves are traditionally given to mothers before and after delivery of a child, and in other situations where blood has been lost, e.g. to warriors. Similarly, an infusion of the leaves is used to treat anaemia.

The leaves and seeds are used medicinally as rubefacient and vesicant, and to treat rheumatism, externally as well as internally. (Chayamarit K 1993).

Kirganelia reticulata

The leaves are employed as a diuretic and cooling medicine. Juice of the leaves is used in diarrhoea of infants. Pills made in combination with the leaf juice, camphor and cubebs are allowed to dissolve in the mouth as a remedy for spongy and bleeding gums. Fruits are astringent to the bowels; useful in inflammations. Decoction of the bark is considered alterative and attenuant. Marma of Chittagong Hill Tracts gives root juice for treating Malaria; it is given in dysentery in Chittagong (Yusuf et al.2009).

Zizyphus glabrata

Many of these indigenous medicinal plants are used as spices and food plants. They are also sometimes added to foods meant for pregnant and nursing mothers for medicinal purposes. Medicinal plants contain some organic compounds which provide definite physiological action on the human body and these bioactive substances include tannins, alkaloids, carbohydrates, terpenoids, steroids and flavonoids, (Kalaivani T, Kanchana G, Rubalakshmi G). Wood is used in making agricultural implements and poles. The leaves are tied with flowers into garland as an offering to the gods. (Shah, G.L., 1978).

Zizyphus oenoplia

The berries are edible and the bark is used for tanning. The plant produces cyclopeptide alkaloids known as *Zizyphus oenoplia* and has a long history of use as an herbal medicine. In India the root is used in Ayurvedic medicine. The Konkani people of Maharashtra use the chewed leaves as a dressing for wounds. In Burma the stem bark is used as a mouthwash for sore throats, for dysentery. (Der Pharmacia Lettre, 2010: 2(1) 87-93).

Cynodon dactylon

Cynodon dactylon is used as a folk remedy for diarrhoea, bronchitis, anasarca, calculus, dropsy, haemorrhage, urogenital disorders, cough, headache, sores, cancer, carbuncles, convulsions, cramps, cystitis, dysentery, epilepsy, haemorrhoids, leucoderma, hypertension, hysteria, asthma, tumors, measles, rubella, snakebite, stones, warts, wounds, eye disorders weak vision and Antidandruff. Oral administration of the juice of the plant with honey 2-3 times a day for few days effectively treats menorrhagia. (Shah, G.L., 1978).

Anisomeles indica

Having the properties of medicine, In China and India, *Anisomeles Indica* is used to treat gastric dysfunction, inflammatory disorders, and hypertension. A decoction from the pre-flowering stage leaves and stems shown to has anti-histamine, free radical scavenging, membrane stabilizing, and cyclooxygenase-I inhibitory activities. (Shah, G.L., 1978).

Achyranthes aspera

It is one of the 21 leaves used in the Ganesh Patra Pooja done regularly on Ganesh Chaturthi day. In Uttar Pradesh the plant is used for a great many medicinal purposes, especially in obstetrics and gynecology, including abortion, induction of labor, and cessation of postpartum bleeding. (Shah GL, 1978).

Bauhinia tomentosa

The root bark is administered or use for the inflammation of liver; leaves, buds and flowers are prescribed for dysentery and diarrhoea; fruit is diuretic. Seeds are eaten for their aphrodisiac action and made into a paste with vinegar as an efficacious application to wounds inflicted by poisonous animals, snakes and scorpions. Bruised bark ground with rice water into a paste is externally applied to tumors and wounds such as scrofulous (Rhama and Madhvan 2012).

Jatropha curcas

Medicinal plants like *J. curcas* have played a major role in the treatment of various diseases including bacterial and fungal infections. The extracts of many *Jatropha* species including *J. curcas* displayed potent cytotoxic, anti-tumor and anti-microbial activities in different assays (Arekemase M et al 2011).

Moringa concanensis

Moringa is believed to have multiple medicinal qualities. For example, the barks, roots, leaves and flowers of *Moringa* tree are used in traditional medicine and folk remedies in many countries. The stem bark is used to relieve bloating and the gum is used to headache and dental problems (Moshood A et al 2000).

Syzygium cumini

S. cumini has been widely used for the treatment of various diseases in traditional and folk medicine. Unani system of medicine describes the use of the plant in liver tonic, enrich blood, strengthen teeth and gums and form good lotion for removing ringworm infection of the head (Panchavarnakili N et al 2012).

Tridax procumbens

It possess wound healing activity and promotes hair growth. *Tridax procumbens* L. also dispensed as “Bhringraj”, which is well known Ayurvedic medicine for liver disorders. The leaf juice possess antiseptic, insecticidal and antiparasitic properties. It is also used to check haemorrhage from cuts, bruises and wounds. (Kumar A et al 2009).

Materials and Methods

Plant specimens were collected from Danta forest region, identified with the help of Dr. K.C.Patel, Dept. of Botany.

Fresh, disease free, leaves, stems, flowers were collected, washed, dried, and powdered with a mixer for use.

Solvent used for sample preparation:

The samples were prepared in distilled water and methanol. The prepared samples were used for the extraction method.

Extraction method

The methods and materials for the extraction are the same as those mentioned in the previous manuscripts.

Microorganism and media

Five bacterial strains Gram positive – *Staphylococcus aureus* (MTCC 96), Gram negative – *Escherichia coli* (MTCC 443), *Shigella flexneri* (MTCC 1457), *Bordetella bronchiseptica* (MTCC 6838), and *Haemophilus influenzae* (MTCC 3826) were selected as test cultures. Media used Nutrient medium.

Antimicrobial assay

As described previously (page no: 23)

Results and Discussion

Table No. 01

Zone of inhibition(mm)								
Organism	Solvent	Dilution µg/ml	<i>A. indica</i>		<i>A.salvifolium</i>		<i>B. ceiba</i>	
			Leaf	Stem	Leaf	Stem	Bark	Flower
<i>S. aureus</i>	Methanol	100	0	0	0	0	30	20
		200	0	0	0	0	30	22
		300	0	0	0	0	32	20
		400	0	0	0	0	34	30
	Distilled water	100	22	0	0	0	0	0
		200	25	0	0	0	0	0
		300	28	0	0	0	0	0
		400	30	0	0	0	0	0
<i>E. coli</i>	Methanol	100	0	0	0	0	32	18
		200	0	0	0	00	34	30
		300	0	0	0	0	36	25
		400	0	0	0	0	39	32
	Distilled water	100	0	0	0	0	0	0
		200	0	0	0	0	0	0
		300	0	0	0	0	0	0
		400	12	0	10	0	0	0
		100	0	0	0	0	30	12
		200	0	0	0	0	31	21
		300	0	0	0	0	37	23
		400	0	0	0	0	39	27

	<i>Distilled water</i>	100	8	0	0	0	0	0
		200	8	0	0	0	0	0
		300	10	0	0	0	0	0
		400	15	0	0	0	0	0
<i>B. bronchiseptica</i>	Methanol	100	0	0	0	0	30	0
		200	0	0	0	0	30	14
		300	0	0	0	0	37	18
		400	0	0	0	0	39	28
	<i>Distilled water</i>	100	0	0	0	0	0	0
		200	0	0	7	0	0	0
		300	8	0	9	0	0	0
		400	0	0	15	0	0	0
<i>H. influenzae</i>	Methanol	100	0	0	0	0	15	0
		200	0	0	0	0	25	0
		300	0	0	0	0	26	0
		400	0	0	0	0	35	0
	<i>Distilled water</i>	100	10	0	20	0	0	0
		200	20	0	25	0	0	0
		300	25	0	28	0	0	0
		400	28	0	30	0	0	0

Table No. 02

Organism	Solvent	Dilution	<i>C.paireira</i>		<i>C. gynandra</i>		<i>K. reticulata</i>	
			Leaf	Stem	Leaf	Stem	Leaf	Stem
<i>S. aureus</i>	Methanol	100	15	24	28	0	28	0
		200	16	26	30	0	30	0
		300	17	27	38	0	32	0
		400	17	29	39	0	34	0
	<i>Distilled water</i>	100	8	8	0	0	0	0
		200	11	23	0	0	0	0
		300	12	25	0	0	0	0
		400	15	25	0	0	0	0
<i>E. coli</i>	Methanol	100	0	5	32	30	10	8
		200	0	8	35	35	18	12
		300	0	10	36	39	20	20
		400	0	10	40	40	22	22
	<i>Distilled water</i>	100	0	0	0	0	0	0
		200	0	0	0	0	0	0
		300	0	0	0	0	0	0
		400	25	0	0	0	0	0

<i>S. flexneri</i>	Methanol	100	20	20	6	19	0	15
		200	22	22	10	20	24	22
		300	23	25	11	26	25	24
		400	25	28	15	26	26	25
	<i>Distilled water</i>	100	20	20	0	0	0	0
		200	23	22	0	0	0	0
		300	24	26	0	0	0	0
		400	28	27	0	0	0	0
<i>B. bronchiseptica</i>	Methanol	100	0	0	30	30	10	0
		200	0	0	32	30	22	0
		300	0	0	34	35	20	10
		400	0	0	35	39	24	14
	<i>Distilled water</i>	100	0	0	0	0	0	0
		200	0	0	0	0	0	0
		300	6	0	0	0	0	0
		400	8	0	0	0	0	0
<i>H. influenzae</i>	Methanol	100	18	0	28	20	0	0
		200	19	0	32	25	14	0
		300	20	0	34	26	15	10
		400	21	0	25	34	16	14
	<i>Distilled water</i>	100	14	25	0	0	0	0
		200	14	25	0	0	0	0
		300	28	28	0	0	0	0
		400	20	30	0	0	0	0

Table No. 03

Organism	Solvent	Dilution	<i>Z.glabrata</i>		<i>Z.oenoplia</i>		<i>C.dactylon</i>	
			Leaf	Stem	Leaf	Stem	Leaf	Stem
<i>S. aureus</i>	Methanol	100	6	13	24	0	0	0
		200	7	16	24	8	0	0
		300	9	16	25	10	0	0
		400	9	15	25	16	0	0
	<i>Distilled water</i>	100	0	0	7	0	0	0
		200	0	0	8	0	0	0
		300	0	0	8	0	0	0
		400	0	0	9	0	0	0
<i>E. coli</i>	Methanol	100	24	23	32	0	16	0
		200	24	25	34	13	16	0
		300	25	24	34	16	17	0
		400	26	25	35	20	19	0

	<i>Distilled water</i>	100	9	0	27	0	16	0
		200	9	0	28	0	16	0
		300	10	0	30	0	17	0
		400	9	0	30	0	19	0
<i>S. flexneri</i>	Methanol	100	0	0	0	0	0	00
		200	0	10	0	0	0	0
		300	0	13	0	0	0	0
		400	0	14	0	0	0	0
	<i>Distilled water</i>	100	0	0	0	0	0	0
		200	0	0	0	0	0	0
		300	0	0	0	0	0	0
		400	0	0	0	0	0	0
<i>B. bronchiseptica</i>	Methanol	100	9	0	9	0	0	0
		200	9	0	10	0	0	0
		300	11	9	14	10	0	0
		400	11	7	15	20	00	0
	<i>Distilled water</i>	100	15	0	0	0	0	0
		200	16	0	0	0	0	0
		300	19	0	8	0	0	0
		400	18	0	9	0	0	00
<i>H. influenzae</i>	Methanol	100	0	0	22	8	0	0
		200	0	0	22	8	0	0
		300	0	0	25	9	0	0
		400	0	0	29	10	0	0
	<i>Distilled water</i>	100	0	0	0	0	0	0
		200	0	0	26	0	0	0
		300	0	0	27	0	0	0
		400	0	0	30	0	0	0

Table No. 04

Organism	Solvent	Dilution	<i>Z.glabrata</i>		<i>Z.oenoplia</i>		<i>C.dactylon</i>	
			Leaf	Stem	Leaf	Stem	Leaf	Stem
<i>S. aureus</i>	Methanol	100	0	10	29	5	0	0
		200	0	11	30	6	0	0
		300	0	13	30	7	0	0
		400	0	13	32	8	0	0
	<i>Distilled water</i>	100	0	0	10	9	0	00
		200	0	0	11	9	0	0
		300	0	0	13	10	0	0
		400	0	0	13	10	0	0
<i>E. coli</i>	Methanol	100	6	13	24	23	0	0

		200	8	13	25	23	0	0
		300	8	14	28	20	0	0
		400	9	15	30	26	0	0
	<i>Distilled water</i>	100	0	0	0	0	0	0
		200	0	0	0	0	0	0
		300	0	0	8	9	0	0
		400	0	0	8	10	0	0
<i>S. flexneri</i>	Methanol	100	0	0	26	11	0	0
		200	0	0	30	12	0	0
		300	0	0	30	13	0	0
		400	0	0	34	14	0	0
	<i>Distilled water</i>	100	0	0	0	0	0	0
		200	0	0	0	0	0	0
		300	0	0	0	0	0	0
		400	0	0	5	0	0	0
<i>B. bronchiseptica</i>	Methanol	100	0	7	0	0	0	0
		200	0	7	0	0	0	0
		300	0	8	0	0	0	0
		400	0	8	0	0	0	0
	<i>Distilled water</i>	100	0	0	8	8	0	0
		200	0	0	9	9	0	0
		300	0	0	9	9	0	0
		400	0	0	10	9	0	0
<i>H. influenzae</i>	Methanol	100	0	10	20	9	0	0
		200	0	11	22	10	0	0
		300	0	12	23	11	0	0
		400	0	13	25	12	0	0
	<i>Distilled water</i>	100	0	0	5	0	0	0
		200	0	0	7	0	0	0
		300	0	0	8	0	0	0
		400	0	0	10	0	0	0

Table No. 05

Organism	Solvent	Dilution	<i>B.tomentosa</i>		<i>J.curcus</i>		<i>M.concanesis</i>	
			Leaf	Stem	Leaf	Stem	Leaf	Stem
<i>S. aureus</i>	Methanol	100	0	0	0	8	14	8
		200	6	0	0	8	20	9
		300	0	0	0	9	6	9
		400	12	0	0	12	6	0
	<i>Distilled water</i>	100	0	0	7	0	14	0
		200	0	0	7	0	17	0

		300	0	0	8	0	14	0
		400	0	0	8	0	16	0
<i>E. coli</i>	Methanol	100	0	0	0	7	17	8
		200	12	0	0	8	22	0
		300	16	0	0	9	14	8
		400	18	0	0	11	17	10
	<i>Distilled water</i>	100	14	0	8	0	16	0
		200	17	0	9	0	4	0
		300	19	0	9	0	14	0
		400	20	0	8	0	15	0
<i>S. flexneri</i>	Methanol	100	12	0	0	9	22	0
		200	15	0	0	8	23	0
		300	16	0	0	7	25	8
		400	18	0	0	8	26	9
	<i>Distilled water</i>	100	11	0	8	0	0	0
		200	0	0	9	0	14	0
		300	0	0	11	0	22	0
		400	15	0	13	0	21	0
<i>B. bronchiseptica</i>	Methanol	100	0	0	0	0	0	8
		200	0	0	0	0	0	0
		300	0	0	0	0	0	8
		400	0	0	0	0	0	10
	<i>Distilled water</i>	100	0	0	0	0	0	0
		200	0	0	0	0	0	0
		300	0	0	0	0	0	0
		400	0	0	0	0	0	0
<i>H. influenzae</i>	Methanol	100	14	0	0	8	15	8
		200	16	0	0	0	17	8
		300	18	0	0	10	19	8
		400	20	0	0	0	19	14
	<i>Distilled water</i>	100	11	0	7	0	13	0
		200	15	00	9	0	16	0
		300	17	0	8	0	18	0
		400	0	0	10	0	18	0

Table No. 06

Organism	Solvent	Dilution	<i>S.cumini</i>		<i>T.procumbens</i>	
			Leaf	Stem	Leaf	Stem
<i>S. aureus</i>	Methanol	100	8	10	30	0
		200	9	11	22	0
		300	8	10	25	0

	<i>Distilled water</i>	400	12	12	26	0
		100	0	0	16	0
		200	0	0	18	0
		300	0	0	16	0
		400	0	0	22	0
<i>E. coli</i>	Methanol	100	8	9	0	0
		200	11	8	0	0
		300	10	9	0	0
		400	12	10	0	0
	<i>Distilled water</i>	100	0	0	10	0
		200	0	0	18	0
		300	0	0	0	0
		400	0	0	0	0
<i>S. flexneri</i>	Methanol	100	10	9	0	0
		200	11	8	20	0
		300	11	11	16	0
		400	12	10	26	0
	<i>Distilled water</i>	100	0	0	18	0
		200	0	0	20	0
		300	0	0	17	0
		400	0	0	22	0
<i>B. bronchiseptica</i>	Methanol	100	6	7	0	0
		200	11	9	0	0
		300	10	10	0	0
		400	11	10	0	0
	<i>Distilled water</i>	100	0	0	0	0
		200	0	0	0	0
		300	0	0	0	0
		400	0	0	0	0
<i>H. influenzae</i>	Methanol	100	7	8	0	0
		200	8	8	20	0
		300	11	9	15	0
		400	13	11	22	0
	<i>Distilled water</i>	100	0	0	0	0
		200	0	0	0	0
		300	0	0	0	0
		400	0	0	0	0

Plants showing highest zone size:

The antimicrobial activity of leaf D/W extract of *A. indica* is observed (30mm zone size) to be highest on *S. aureus* in comparison to test organism *E. coli*, *S. flexneri*, *B. bronchiseptica*, *H. influenzae*.

Leaf D/W extract of *A. salvifolium* observed (30mm zone size) to be highly inhibitory to *H. influenzae* in comparison to test organism *E. coli*, *S. flexneri*, *B. bronchiseptica*, *S. aureus*.

Bark methanol extract of *B. ceiba* is observed (39mm zone size) to be highly inhibitory to *S. flexneri*, *E. coli*, *B. bronchiseptica* in comparison to test organism *S. aureus*, *H. influenzae*.

The stem D/W extract of *C. pareira* is observed (30mm zone size) to be highly inhibitory to *H. influenza* in comparison to test organism *E. coli*, *S. flexneri*, *B. bronchiseptica*, *S. aureus*.

The antimicrobial activity of stem and leaf methanol extract of *C. gynandra* is observed (40mm zone size) to be highest on *E. coli* in comparison to test organism *S. aureus*, *S. flexneri*, *B. bronchiseptica*, *H. influenzae*.

K. reticulata leaf methanol extract is observed (34mm zone size) to be highly inhibitory to *S. aureus* in comparison to test organism *E. coli*, *S. flexneri*, *B. bronchiseptica*, *H. influenzae*.

Activity of leaf methanol extract of *Anisomelus indica* is observed to be highest on *H. influenzae* in comparison to test organisms and the antimicrobial activity of Leaf Methanol extract of *Anisomelus indica* is observed to be lowest zone on *S. flexneri*.

Activity of stem methanol extract of *Anchranthes aspera* is observed to be highest on *S. flexneri* in comparison to test organisms and the antimicrobial activity of flower, stem methanol extract of *Anchranthes aspera* is observed to be lowest zone on *B. bronchiseptica*.

Leaf methanol extract of *Zizyphus glabrata* is observed to be highly inhibitory to *E. coli* in comparison to test organisms and the antimicrobial activity of leaf, stem methanol and distilled water both extract of *Zizyphus glabrata* is observed be lowest zone on *H. influenzae*.

Zizyphus oenoplia leaf methanol extract of is observed to be *E. coli* in comparison to test organisms and The The antimicrobial activity of leaf, stem methanol and distilled water extract Of *Zizyphus oenoplia* is observed be lowest zone on *S. flexneri*.

Cynodon dactylon leaf methanol extract is observed to be highly inhibitory to *E. coli* in comparison to test organisms and the antimicrobial activity of leaf methanol and distilled water both Extract of *Cynodon dactylon* is observed to be lowest zone on *S. flexneri*, *H. influenzae*, *B. bronchiseptica*, *S. aureus*.

B. tomentosa leaf extract of is observed to be highly inhibitory to *E. coli* with distilled water extract and *H. influenzae* with methanol extract with 20mm zone size in comparison to test organism and moderate on *S. flexneri* distilled water extract with

11mm of zone size and *S. aureus* is resistant to *B. tomentosa* leaf distilled water extract.

The medicinal plant *S. cumini* leaf methanol extract has antimicrobial activity on *H. influenzae* with 13mm of zone size in comparison to all test organism and moderate observation on *S. aureus* with zone size of 9mm and lowest on *B. bronchiseptica* with 6mm of zone size.

S. cumini stem methanol extract show highest antimicrobial activity on *S. aureus* and *S. aureus* with 12mm of zone size in compare to test organism and lowest result observe on *B. bronchiseptica* with 7mm and moderate zone of Antimicrobial activity observe on *E. coli*, *B. bronchiseptica* and *S. flexneri* with 10mm of zone.

The antimicrobial activity of *M. concanensis* medicinal plant leaf extract observe to be highest on *S. flexneri* with methanol extract with zone size of 26mm in comparison to other test organism and moderate *H. influenzae* with distilled water extract with 13mm of zone and lowest result observe on *S. aureus* with methanol extract.

M. concanensis stem methanol extract observe to possess highest Antimicrobial activity on *H. influenzae* with 14mm of zone size in comparison to test organism and moderate on *B. bronchiseptica* with 14mm of zone size and lowest result observe on *E. coli* with 7mm of zone size.

The medicinal plant *J. curcus* leaf distilled water observed to possess highest antimicrobial activity on *S. flexneri* with zone size of 13mm and moderate activity on *H. influenzae* with 10mm zone and lowest on *S. aureus*.

J. curcus stem methanol extract observe highest Antimicrobial activity on *S. aureus* with 12mm of zone size and lowest observe on *E. coli* and *S. flexneri* with 7mm of zone size and moderate result observe on *H. influenzae* with 10mm.

The antimicrobial activity of *Tridax procumbens* leaf methanol and distilled water extract observe to be highest on *S. aureus* with methanol extract with 30mm of zone size and moderate on *H. influenzae* with methanol extract with 15mm of zone size and *H. influenzae* with distilled water extract and *E. coli* with methanol extract inhibit antimicrobial activity of *Tridax procumbens*.

Result of Bacterial inhibition by plants:

In terms of microbial sensitivity of the test culture, it is observed that *S. aureus* is highly sensitive to methanol extract *C. gynandra* moderately sensitive to leaf methanol extract of *K. reticulata* and *C. pareira* and it is resistant to stem methanol extract of *K. reticulata*.

E. coli is highly inhibited to methanol extract *C. gynandra* moderately sensitive to leaf methanol extract of *B. ceiba* and it is resistant to stem methanol extract of *C. pareira*.

S. flexneri is highly sensitive to methanol extract *B. ceiba* moderately sensitive to leaf methanol extract of *C. pareira* and it is resistant to stem methanol extract of *C. gynandra*.

It is observed that *B. bronchiseptica* highly sensitive to methanol extract *C. gynandra* and *B. ceiba* moderately sensitive to leaf methanol extract of *K. reticulata*.

H. influenzae is highly inhibited to methanol extract *B. ceiba* moderately inhibited to leaf methanol extract of *C. gynandra* and it is resistant to stem methanol extract of *C. pareira*.

E. coli is highly sensitive to the stem extract methanol of (*A. indica*) moderately sensitive *H. influenzae* and *S. flexneri* are less sensitivity to the stem extract methanol and distilled water.

S. flexneri is highly sensitive to the stem extract methanol of (*A. aspera*) moderately sensitive *H. influenzae* and *B. bronchiseptica* are less sensitive to the stem and flower extract and distilled water.

E. coli is highly sensitive to the stem extract methanol of *Z. glabrata* moderately *H. influenzae* less sensitive to the stem extract methanol and distilled water.

E. coli is highly sensitive to the stem extract methanol of *Z. oenoplia* moderately *S. flexneri* less sensitive to the stem extract methanol and distilled water.

E. coli is highly sensitive to the stem extract distilled water and methanol of *C. dactylon* moderately to *H. influenzae*, *S. flexneri*, *S. aureus*, *B. bronchiseptica* are Less Sensitivity to the stem extract methanol and distilled Water.

It is observed that *E. coli* is highly sensitive to the leaf methanol extract of *M. concanensis* (22mm) and moderately sensitive to leaf methanol extract of *S. cumini* (11mm) and resistant to leaf methanol extract of *Tridax procumbens*.

Sensitivity of *S. aureus* is highest to the leaf methanol extract of *Tridax procumbens* (30mm) and moderately sensitive to leaf methanol and distilled water extract of *M. concanensis* (14mm) and resistant to leaf methanol extract of *B. tomentosa*.

B. bronchiseptica is observed to be highly sensitive to the leaf methanol extract of *S. cumini* (11mm) and moderately sensitive to methanol stem extract of *S. cumini* (9mm) and not resistant to any plant extract in terms of micro organism sensitivity.

Sensitivity of *S. flexneri* is highest to the leaf methanol extract of *M. concanensis* (26mm) and moderately sensitivity to leaf methanol extract of *J. curcus* (13mm) and not resistant to any plant extract.

In terms of micro organism sensitivity it is observed it is observed that *H. influenzae* is highly sensitive to the leaf methanol extract of *Tridax procumbens* (22mm) and moderately sensitive to leaf distilled water extract of *B. tomentosa* (11mm) and leaf and stem extract of *S. cumini* (11mm) and resistant to methanol extract of *Tridax procumbens*.

Result in terms of solvent used

While reporting the result in terms of the solvent employed for the extraction, it can be said that the D/W extract is observed to produce greatest activity for leaf extract of *A. indica* towards *S. aureus* and lower activity on *B. bronchiseptica*.

The aqua extract is observed to produce greatest activity for leaf extract of *A. salvifolium* towards *H. influenzae* and lower activity on *S. aureus*.

The methanol extract is observed to produce greatest activity for leaf extract of *B. ceiba* towards *E. coli*, *B. bronchiseptica*, *S. flexneri* and lower activity on *H. influenzae*.

The methanol extract is observed to produce greatest activity for leaf extract of *B. ceiba* towards *E. coli*, *B. bronchiseptica*, *S. flexneri* and lower activity on *H. influenzae*.

The methanol extract is observed to produce greatest activity for leaf extract of *C. pareira* towards *S. flexneri* and lower activity on *E. coli*.

While reporting the result towards on the extract are, it can be said that the methanol extract is observed to produce greatest activity for leaf extract of *C. gynandra* *E. coli* towards tower activity on *S. aureus*.

The methanol extract is observed to produce greatest activity for leaf extract of *K. reticulata* towards *S. aureus* and lower activity on *B. bronchiseptica*.

It can be said that methanol extract is observed to produce greater activity for stem extract of *A. indica* towards *E. coli*.

The methanol extract is observed to produce greater activity for stem extract of *A. aspera* of *A. aspera* towards *S. flexneri*.

The methanol extract is observed to produce greater activity for leaf extract of *Z. oenoplia* towards *E. coli*.

The methanol and distilled water was observed to produce greater activity for Leaf extract of *C. dactylon* towards *E. coli*.

The methanol extract is observed to produce grater activity (22mm) for leaf extract of medicinal plant *M. concanensis* with *E. coli*.

The methanol extract is observe to produce greater activity (30mm) for leaf extract towards *Tridex procumbens* with *S. aureus*.

It can be said that the methanol extract is observe to produce grater activity (11mm) for leaf extract of *S. cumini* with *B. bronchiseptica*.

The methanol extract is observed to produce grater activity (26mm) for leaf extract of both the plant *M. concanensis* and *T. procumbens* with *S. flexneri*.

The result based on the extract use it can be said that the methanol extract is observe to produce grater activity (22mm) for leaf extract of medicinal plant *T. procumbens* with *H. influenzae*.

DISCUSSION

The present study reports the antipathogenic activity of *Acalypha indica* on *S. aureus*. This work is line with the research carried out by other scientist viz. (Komathi, S. et al.2013) where in it the Antimicrobial activity of the leaf extract of the same plant is reported to the work on the *S. aureus*. It is inferred that ethanolic extract exhibited strong antibacterial activity with a maximum activity recorded against *S. aureus* (20mm).

The present study reports the anti-pathogenic activity of *Alangium salvifolium* on *S. aureus*. This work is line with the research carried out by other scientist viz. (Uday Prakash NK et al. 2013) where in it the antimicrobial activity of the leaf extract of the *A. salvifolium* is reported to the chloroform extract showed maximum zone of inhibition against the bacteria *P. aeruginosa*, *S. aureus*, and *S. typhi*.

The present study reports the anti-pathogenic activity of *Bombax ceiba* on *E. coli*. This work is line with the research carried out by other scientist viz. (Islam MK. et al. 2010) where in it the antimicrobial activity of the leaf extract of the same plant is reported to the work on the *E. coli*.

The present study reports the anti-pathogenic activity of *C. pareira* on *S. aureus* and *E. coli*. This work is line with the research carried out by other scientist viz. (Chaudhary, p. et al. 2012) *Cissampalous pareira* showed better activity against *B. subtilis* which is comparable with that of the standard drug ofloxacin. It was clearly observed that methanolic extract of both the herbs exhibit impact antimicrobial activity against *B. subtilis*.

Antimicrobial activity of *C. gynadra* against *S. aureus* and *E. coli*. This work is line with the research carried out by other scientist viz. (Ajaiyeoba, E.O., 2000). The antifungal assay, the little plants have again displayed high antifungal activities.

The present study reports the anti-pathogenic activity of *K. reticulata* on *S. aureus*. This work is line with the research carried out by other scientist viz. (Shruthi, SD., 2010) where in it the Antimicrobial activity of the leaf extract of the *K. reticulata* the inhibition zone for *P. aeruginosa*, was much high which was followed by *S. typhi* and *S. aureus*.

With regard to the study of *Anchranthes aspera* employing *B. subtilis* and *Klebsiella* the ethanol and chloroform extract provided a wider zone against *Klebsiella* and extract was large effective for *Klebsiella* (Mohinder.K 2004), whereas our present study on *Anchranthes aspera* employing methanol leaf extract provided highest activity against *E. coli*.

With regard to the study of *Zizyphus oenoplia* employing *B. subtilis*, *Streptococcus pyogenes*, *S. aureus*, *E. coli*, *S. typhi*, the ethanol and chloroform extract provided a wider zone against *B. subtilis* and extract was large effective for *B. subtilis* (Shoeb M) Whereas our present study on *Zizyphus oenoplia* a employing methanol leaf extract provided highest activity against *E. coli*.

With regard to the study of *Anisomeles indica* employing *Bacillus anthracis*, *Proteus vulgaris*, *Streptococcus pyogenes*, *S. aureus*, *S. typhi*, *Klebsiella* the ethanol and distilled water extract provided a wider Zone against *Bacillus anthracis*, and extract was large effective for *Bacillus anthracis* (Yadava.R.N 1997) Whereas our present study on *Anisomeles indica* a employing methanol stem extract provided highest activity against *E. coli*.

With regard to the study of *Cynodon dactylon* employing *B. subtilis*, *Streptococcus pyogenes*, *S. aureus*, *E. coli*, *K. pneumoniae*, *P. aeruginosa*, *P. mirabilis* *Klebsiella* the ethanol, Chloroform, ethyl acetate extract provided a wider zone against, *S. aureus*, *E. coli* and extract was largely effective for *S. aureus*, *E. coli*

(Chaudhri Y. 2011) Whereas our present study on *Cynodon dactylon* employing methanol leaf extract provided highest activity against *E. coli*.

Antibacterial activity of the aqueous and methanol extracts of the leaves of *Bauhinia tomentosa* are listed in the table. (5) Methanol extract exhibits higher antibacterial effect than that of the aqueous extract. Both the extracts showed Antimicrobial activity against the tested strains but The methanol extract showed the maximum inhibitory effect against *H. influenzae* and lowest inhibitory effect on *S. aureus* if we compare this present work with reference (Anju J et al 2011) the methanol extract showed the maximum inhibitory effect against *Streptococcus faecalis* and it is minimum for *Enterobacter aerogenes*. All the tested pathogens showed significant inhibitory effects in reference.

Aqueous extract showed the highest inhibitory action against *E. coli* as that of methanol extract. It was minimum for *S. aureus*. *S. aureus* showed no inhibitory effects. Overall the leaf extracts of *Bauhinia tomentosa* showed significant antimicrobial activity against the tested pathogens, when comparing with the standards. The selection of this plant for the present study was based on its medicinal properties and its use in traditional system.

Antibacterial activity of the aqueous and methanol extracts of the leaves and stem of *Jatropha curcus* are listed in the table. (5) Here stem methanol extract exhibits higher antibacterial effect than that of the leaf extract. Both the leaf and stem extracts showed Antimicrobial activity against the tested strains but the methanol stem extract showed the maximum inhibitory effect against *S.aureas* and lowest inhibitory effect on *E. coli* leaf distilled water extract. if we compare this present work with reference (Kalimuthu K et al. 2010) the methanol extract (inhibition zone 8-20mm) was found to be more effective than the ethanol extract (inhibition zone 5- 12mm) against all the organisms. The water extract showed low antibacterial activity with inhibition zones ranging between 0 and 8 mm for different bacteria tested.

Antibacterial activity of the aqueous and methanol extracts of the leaves and stem of *Moringa concanensis* are listed in the table. (5) Methanol extract exhibits higher antibacterial effect than that of the aqueous extract. Both the extracts showed antimicrobial activity against the tested strains but the methanol leaf extract showed the maximum inhibitory effect against *S. aureus* and lowest inhibitory effect on leaf methanol extract on *S. aureus* when we compare the present work with reference (Balamurugan V et al. 2013) than present work not support the reference because in present work methanol extract observe higher Antimicrobial activity against all tested organism.

Aqueous extract showed the highest inhibitory action against *S. flexneri* as that of the methanol extract. It was minimum for *E. coli*. Overall the leaf methanol extracts of *Moringa concanensis* showed significant antimicrobial activity against the tested pathogens.

Antibacterial activity of the aqueous and methanol extracts of the leaves and stem of *Syzygium cumini* are listed in the table. (6) Methanol extract of leaf exhibits higher antibacterial effect than that of the stem extract. Both the extracts showed Antimicrobial activity against the tested strains but the methanol leaf extract showed the maximum inhibitory effect against *H. influenzae* and lowest inhibitory effect on *B. bronchiseptica* with leaf and methanol extract. If we compare this present work with

(Sharma S et al. 2012) the methanol leaf extract showed the maximum inhibitory effect against *S. aureus* and it is minimum for *S. flexneri*. All the tested pathogens showed significant inhibitory effects as in present work.

Antibacterial activity of the aqueous and methanol extracts of the leaves *Tridax procumbens* are listed in the table. (6) Methanol extract exhibits higher antibacterial effect than that of the aqueous extract. In present work methanol extract showed effect on *E. coli* showed no inhibitory effects. When we compare this present work with reference (Kumar A et al. 2009) the methanol extract showed the maximum inhibitory effect against *S. aureus* and it is minimum for *E. coli*.

Aqueous extract showed the highest inhibitory action against *S. aureus* and *S. flexneri* as that of the methanol extract. It was minimum for *H. influenzae* showed no inhibitory effects. Overall the leaf extracts of *Tridax procumbens* showed significant Antimicrobial activity against the tested pathogens, when comparing with the standards. The selection of this plant for the present study was based on its medicinal properties and its use in traditional system.

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CHAPTER NO: 6

**Invitro antibacterial activity of few
ethno-medicinal plants of Danta forest
on some common pathogens.**

Invitro antibacterial activity of few ethno-medicinal plants of Danta forest on some common pathogens.

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INTRODUCTION

Lawsonia inermis

Root of this plant is considered as a potent medicine for gonorrhoea and herpes infection. Leaves, flowers, seeds, stem bark and roots are used in traditional medicine to treat a variety of ailments such as rheumatoid arthritis, headache, ulcers, diarrhoea, leprosy fever, leucorrhea, diabetes, cardiac disease, hepatoprotective, and colouring agent. Leaf is used for alleviating jaundice, skin diseases, venereal diseases, small pox and spermatorrhoea. Bark is applied in the form of a decoction to cure burns and scalds. It is given internally in a variety of affections such as, jaundice, enlargement of the spleen, calculus, as an alternative in leprosy and obstinate skin infections.

Tecomella undulata

This plant is extensively used in ayurvedic system of medicine for the treatment of leucorrhea and leucoderma, enlargement of spleen, also used for treatment of urinary discharge due to kapha and pitta. In Bolan, it is extensively employed in the treatment of liver diseases. The bark has been used in treatment of syphilis, painful swellings and cancer traditionally. Antibacterial activity has been reported in stem extract as well. This plant has been extensively screened for wide range of pharmacological activities.

Salvadora persica

It has great medicinal use in treatment of nose troubles, piles, scabies, leucoderma, scurvy, gomorrhea, boils and toothache, to treat hook worm, venereal diseases, teeth cleaning, in rheumatism, cough and asthma, to lower cholesterol plasma levels, reestablishment of the components of gastric mucosa, and as a laxative.

Limonia acidissima

The fruits are edible and considered to be a stomachic, astringent, diuretic, and tonic for liver and lungs. The leaves are aromatic and carminative and are used for the treatment of indigestion and minor bowel infections in children. The roots are also sometimes given as antidote to snake bites.

Aegle marmelos

The fresh ripe pulp of this plant is taken as a mild laxative, tonic, and digestive aid. A decoction of unripe fruit, with fennel and ginger, is prescribed in cases of hemorrhoids. It has been observed that the pulp increases tolerance to sunlight and aids in maintaining normal skin colour. It is employed in the treatment of leucoderma.

Marmelosin derived from the pulp is given as a laxative and diuretic. In large doses, it lowers the rate of respiration, depresses heart action and causes sleepiness. The fruits, roots and leaves have anti-infective activity. The root leaves and bark are used in treating snake bites. A decoction of the flowers is used as eye lotion and given as an antiemetic. Decoctions of the root are taken to relieve palpitation of the heart, indigestion and bowel inflammation. The bark decoction is administered in cases of malaria. A hot poultice of the leaves is considered as an effective treatment for ophthalmia, and various inflammations, also febrile delirium, and acute bronchitis.

Ficus carica

The fruit juice with honey is used to check hemorrhagia. In unani medicine anjeer is used as a mild laxative, expectorant and diuretic. It is also used for the diseases in liver and spleen. The paste of fruit is applied in swellings, tumours and inflammation for relieving pain. The figs are astringent and carminative, they are given for relieving menorrhagia, hepatitis and dysentery. Due to the high iron content, it is ideal to include it in one's diet in anaemic condition. The burnt ash of the fig fruit is highly basic in nature and can be consumed before meals to counter hyperacidity.

Carissa carandus

It has been used as a traditional medicinal plant for over thousands of years in the Ayurvedic system of medicine. The leaf decoction is useful in cases of intermittent fever, diarrhea, oral inflammation and ear ache. The root is employed as a bitter stomachic and vermin fuge and it is an ingredient of a remedy for itches.

Cocculus hirsutus

Traditionally, the plant is patronized for its unique property of healing of all types of cuts, wounds and boils in a short time and less pain. It is also used in the treatment of gonorrhoea, spermatorrhoea, urinary tract infection, diarrhoea and hyperglycemia. The leaves of the plant have been evaluated for anti hyperglycemic and diuretic effects. Folk medicine claims that it may be used in jaundice.

Materials and Methods:

Solvent:

Distilled water, Acetone and methanol were used for the extraction method.

Extraction method:

The methods are same as those mentioned in the previous manuscripts.

Microorganism and media:

Bacteria causing infectious disease in humans were used in present study. They were of both the gram type viz., gram positive and gram negative. The stock cultures of *Staphylococcus citreus*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Staphylococcus albus*, *Escherichia coli* and, *Proteus vulgaris* isolates were collected from A.M.C.M.E.T College, L.G. Hospital compound, Ahmedabad.

Preparation of inoculum::

The methods are same as those mentioned in the previous manuscripts.

Preparation of the McFarland standard:

As previously described in the earlier manuscripts (page no:22) (Jennifer M. Andrews, 2001).

Preparation of dilutions:

The dilutions were prepared in following manner:

1:2 (high): 1ml solvent + 1ml extract

1:5 (medium): 4ml solvent + 1ml extract

1:8 (low): 7ml solvent + 1ml extract.

Results and Discussion:

			<i>Salvadora persica</i>		<i>Limonia acidissima</i>		<i>Aegle marmelos</i>	
Pathogen	Solvent and Dilution		Leaf	Stem	Leaf	Stem	Leaf	Stem
<i>S. citreus</i>	Acetone	1:2	12	14	9	10	11	7
		1:5	8	9	4	8	8	3
		1:8	7	6	3	5	6	2
	Methanol	1:2	11	15	10	13	6	10
		1:5	9	10	9	10	4	9
		1:8	7	9	6	6	3	6
	Water	1:2	0	0	0	5	0	7
		1:5	0	0	0	2	0	3
		1:8	0	0	0	0	0	2
<i>S. aureus</i>	Acetone	1:2	4	10	6	12	11	14
		1:5	0	6	4	7	6	10
		1:8	0	3	3	4	2	8
	Methanol	1:2	6	14	10	11	8	15
		1:5	0	6	4	7	6	10
		1:8	0	3	3	4	2	8
	Water	1:2	4	0	6	0	0	0
		1:5	0	0	4	0	0	0
		1:8	0	0		0	0	0
<i>Klebsiella sp.</i>	Acetone	1:2	6	14	9	14	8	11
		1:5	4	10	7	12	5	8
		1:8	5	9	4	10	3	6
	Methanol	1:2	7	12	13	20	12	10
		1:5	6	10	10	13	10	6
		1:8	3	6	9	10	6	3
	Water	1:2	0	0	0	0	0	0
		1:5	0	0	0	0	0	0
		1:8	0	0	0	0	0	0
<i>S. albus</i>	Acetone	1:2	7	13	10	17	9	10
		1:5	5	9	9	13	8	6

	Methanol	1:8	0	6	6	10	10	4
		1:2	10	17	8	10	9	10
		1:5	9	14	6	8	7	10
	Water	1:8	7	11	3	7	6	7
		1:2	0	0	0	0	7	9
		1:5	0	0	0	0	6	7
		1:8	0	0	0	0	2	4
		1:2	7	12	15	12	14	17
		1:5	6	10	11	10	11	15
<i>E. coli</i>	Acetone	1:8	3	5	12	9	7	12
		1:2	15	13	10	15	18	14
		1:5	13	11	9	12	14	12
	Methanol	1:8	7	10	5	9	9	9
		1:2	0	0	0	0	0	0
		1:5	0	0	0	0	0	0
	Water	1:8	0	0	0	0	0	0
		1:2	0	0	0	0	0	0
		1:5	0	0	0	0	0	0
<i>P. vulgaris</i>	Acetone	1:2	6	13	9	14	11	15
		1:5	4	12	5	11	8	13
		1:8	5	10	3	9	6	10
	Methanol	1:2	12	20	12	13	18	15
		1:5	11	17	10	10	14	12
		1:8	8	12	9	9	12	10
	Water	1:2	0	0	0	0	0	0
		1:5	0	0	0	0	0	0
		1:8	0	0	0	0	0	0
<i>P. aeruginosa</i>	Acetone	1:2	9	13	12	15	12	16
		1:5	3	12	10	13	8	12
		1:8	6	9	9	9	6	10
	Methanol	1:2	12	17	14	17	14	19
		1:5	11	14	12	15	10	17
		1:8	9	13	10	12	9	13
	Water	1:2	0	0	0	0	0	0
		1:5	0	0	0	0	0	0
		1:8	0	0	0	0	0	0
			<i>Ficus carica</i>		<i>Carissa carandas</i>		<i>Cocculus hirsutus</i>	
<i>S. citreus</i>	Acetone	1:2	13	10	7	15	12	13
		1:5	10	9	6	10	8	10
		1:8	8	6	4	6	6	9
	Methanol	1:2	9	12	11	14	10	14
		1:5	8	9	6	10	5	10
		1:8	5	6	3	8	3	9
	Water	1:2	0	0	6	0	4	0
		1:5	0	0	3	0	0	0
		1:8	0	0	0	0	0	0
<i>S. aureus</i>	Acetone	1:2	7	10	9	15	10	12
		1:5	6	8	7	11	7	11

		1:8	3	4	6	7	5	7
	Methanol	1:2	10	6	8	11	10	13
		1:5	9	4	5	9	4	10
		1:8	6	0	2	7	0	6
	Water	1:2	4	0	0	0	7	5
		1:5	2	0	0	0	6	3
		1:8	0	0	0	0	3	0
<i>Klebsiella</i> <i>sp.</i>	Acetone	1:2	12	14	10	9	18	14
		1:5	6	10	8	7	14	10
		1:8	3	9	6	5	9	8
	Methanol	1:2	0	4	13	7	14	8
		1:5	0	0	10	3	9	4
		1:8	0	0	7	0	7	3
	Water	1:2	6	7	0	0	4	7
		1:5	3	3	0	0	2	5
		1:8	2	0	0	0	0	3
<i>S. albus</i>	Acetone	1:2	6	13	0	0	6	10
		1:5	7	9	0	0	5	7
		1:8	3	7	0	0	0	5
	Methanol	1:2	7	10	4	12	6	15
		1:5	0	7	5	11	4	11
		1:8	0	6	3	7	3	7
	Water	1:2	0	0	6	9	0	0
		1:5	0	0	3	7	0	0
		1:8	0	0	0	5	0	0
<i>E. coli</i>	Acetone	1:2	11	13	12	13	9	10
		1:5	9	9	8	10	7	8
		1:8	8	6	6	9	5	5
	Methanol	1:2	17	14	15	13	10	15
		1:5	15	13	10	8	8	13
		1:8	11	9	9	6	5	10
	Water	1:2	10	12	0	0	0	0
		1:5	7	8	0	0	0	0
		1:8	3	6	0	0	0	0
<i>P. vulgaris</i>	Acetone	1:2	0	9	9	14	0	0
		1:5	0	6	7	13	0	0
		1:8	0	4	6	10	0	0
	Methanol	1:2	22	14	12	20	13	20
		1:5	17	10	11	13	10	17
		1:8	16	9	10	10	8	0
	Water	1:2	6	0	0	0	0	0
		1:5	5	0	0	0	0	0
		1:8	2	0	0	0	0	0
<i>P. aeruginosa</i>	Acetone	1:2	12	14	12	15	11	18
		1:5	7	11	9	14	10	16
		1:8	6	9	7	10	7	13
	Methanol	1:2	12	16	13	19	15	20
		1:5	10	13	10	17	12	13

	Water	1:8	5	11	9	15	8	10
		1:2	0	10	0	0	5	9
		1:5	0	6	0	0	3	4
		1:8	0	5	0	0	0	0

			<i>Tecomella undulata</i>		<i>Lawsonia inermis</i>	
<i>S. citreus</i>	Acetone	1:2	9	10	0	17
		1:5	6	6	0	12
		1:8	3	0	0	8
	Methanol	1:2	10	19	10	13
		1:5	7	15	9	10
		1:8	3	0	5	9
	Water	1:2	10	0	0	0
		1:5	6	0	0	0
		1:8	0	0	0	0
<i>S. aureus</i>	Acetone	1:2	0	12	20	13
		1:5	0	11	13	10
		1:8	0	10	10	0
	Methanol	1:2	18	14	17	18
		1:5	14	12	16	15
		1:8	12	10	13	14
	Water	1:2	9	6	0	0
		1:5	8	4	0	0
		1:8	5	2	0	0
<i>Klebsiella</i>	Acetone	1:2	0	18	17	11
		1:5	0	15	14	9
		1:8	0	10	12	7
	Methanol	1:2	10	10	0	0
		1:5	9	9	0	0
		1:8	8	7	0	0
	Water	1:2	0	0	0	0
		1:5	0	0	0	0
		1:8	0	0	0	0
<i>S. albus</i>	Acetone	1:2	15	12	10	12
		1:5	9	8	7	9
		1:8	7	6	3	7
	Methanol	1:2	11	11	9	14
		1:5	6	7	8	11
		1:8	0	0	7	9
	Water	1:2	0	0	15	22
		1:5	0	0	12	20
		1:8	0	0	0	15
<i>E. coli</i>	Acetone	1:2	18	12	0	15
		1:5	10	11	0	12
		1:8	10	10	0	10
	Methanol	1:2	10	15	15	15
		1:5	9	10	10	10

		1:8	7	8	9	10
	Water	1:2	0	7	9	0
		1:5	0	5	0	0
		1:8	0	0	0	0
<i>P. vulgaris</i>	Acetone	1:2	10	20	0	20
		1:5	0	15	0	10
		1:8	0	0	0	9
	Methanol	1:2	9	18	17	18
		1:5	7	16	13	16
		1:8	6	14	7	14
	Water	1:2	0	0	0	0
		1:5	0	0	0	0
		1:8	0	0	0	0
<i>S. aeruginosa</i>	Acetone	1:2	12	18	9	17
		1:5	10	10	7	10
		1:8	8	7	0	8
	Methanol	1:2	12	21	11	13
		1:5	11	19	10	9
		1:8	10	17	9	7
	Water	1:2	0	0	0	11
		1:5	0	0	0	10
		1:8	0	0	0	8

Discussion

Among the many human microbe interactions, the one taking its toll on human health is the human-pathogen interaction, which is a never ending battle in which human develops newer drugs and its combinations to combat pathogen and the latter develops drug resistance to keep inflicting injuries to human.

It is in the light of these event, newer drugs and therapies for combating diseases are discovered.

Urinary tract infection is one of the most important causes of morbidity in the human population and is the second most common reason of hospital visits. UTI is an old problem that continues to present new challenges due to the change in etiology and antimicrobial susceptibility of urinary pathogens over the years. Factors such as changes in patient population and extensive use and abuse of anti-microbial agents could contribute to changes in the microbial profile of urinary tract isolates.

Seven microorganisms were studied for their susceptibility-resistance pattern. Antibiotic susceptibility tests were performed to isolate various organisms like *E. coli*, *S. citreus*, *P. aeruginosa*, *S. aureus*, and *K. pneumoniae* associated with urinary tract infections

Extracts of eight plants were taken for the study, two plant extracts showed highest antimicrobial effect against two pathogens.

The methanol extract of *Tecomella undulata* (stem) and *Ficus carica* (leaf) were the most effective against *Pseudomonas aeruginosa* and *Proteus vulgaris*.

Limonia acidissima stem (methanol) extract inhibited *S. citreus*, *K. pneumoniae*, *E. coli*, *S. albus* (acetone).

S. persica stem (methanol) extract was effective in inhibiting *S. albus*, *P. vulgaris*, and *P. aeruginosa*.

A. marmelos stem (methanol and acetone) extract was inhibitory to *E. coli*, *P. vulgaris*, and *P. aeruginosa*.

Carissa carandas stem (acetone) extract showed good activity against *S. aureus*, *P. vulgaris*, and *P. aeruginosa*.

Cocculus hirsutus (methanol) extract was inhibitory to *P. aeruginosa*, *E. coli*, *S. albus*, (acetone) *K. pneumoniae*, and *S. albus*.

Lawsonia inermis extracts inhibited *S. citreus* (acetone), *S. aureus* (methanol), and *S. albus* (water).

Thus this study provides an exhaustive insight into the effective *in vitro* inhibition of urinary tract pathogens by ethno medicinal plants.

Further study on the ADMET properties of the plant products can help in designing a compound for its *in vivo* therapeutic application.

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CHAPTER NO: 7

Antibacterial efficacy of some ethno-medicinal plants used by local medicine men of Danta forest on some common pathogens.

Antibacterial efficacy of some ethno-medicinal plants used by local medicine men of Danta forest on some common pathogens.

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INTRODUCTION

The plants are indispensable to man for his life. The three important necessities of life – food, clothing and shelter – and a host of other useful products are supplied to him by the plant kingdom. Nature has provided a complete store-house of remedies to cure all ailments of mankind. The knowledge of drugs has accumulated over thousands of years as a result of man's inquisitive nature so that day we possess many effective means of ensuring health care.

The human being appears to be afflicted with more diseases than any other animal species. There can be little doubt then that he, very early, sought to alleviate his sufferings from injury and disease by taking advantage of plants growing around him. In this past, almost all the medicines used were from the plants, the plant being man's only chemist for ages. Today, a vast store of knowledge concerning therapeutic properties of different plants has accumulated.

The history of herbal medicines is as old as human civilization. The documents, many of which are of great antiquity, revealed that plants were used medicinally in China, India, Egypt and Greece long before the beginning of the Christian era.

Plant-chemistry has undergone significant development in recent years as a distinct discipline. It is concerned with the enormous variety of substances that are synthesised and accumulated by plants and the structural elucidation of these substances. The technology involving extraction, purification and characterisation of pharmaceuticals from natural sources is a significant contribution to the advancement of natural and physical sciences.

Ayurveda-Ancient Science of life is believed to be prevalent for last 5000 years in India, it is one of the most noted systems of medicine in the world. Ayurveda is based on the hypothesis that everything in the universe is composed of five basic elements viz. space, air, energy, liquid and solid.

Naturopathy is not merely a system of treatment, but also a way of life, which is based on laws of nature. The attention is particularly paid to eating and living habits, adoption of purificatory measures, use of hydrotherapy, mud packs, baths, massage, etc.

Justicia adhatoda

The leaves, the roots and flowers of *Justicia adhatoda* are extensively used in indigenous medicine as remedy for cold, cough, bronchitis and asthma. The medicine was considered so useful in tuberculosis that it was said that no man suffering from the disease need despair as long a vasica plant exists in this world. The juice of the

leaves is used in diarrhoea and dysentery and powdered leaves in malaria in southern India. (Sampath KP. 2010)

Alangium salvifolium

Root bark is emetic, febrifuge, purgative, anthelmintic, diaphoretic, antipyretic; useful in fever and piles. It is also in leprosy, syphilitic and other skin diseases. Leaves are useful as poultice in rheumatic pains. Fruits are laxative, expectorant, carminative, anthelmintic, alexiteric; useful in inflammation, burning of the body, spermatorrhoea, gleet, acute fever and lumbago. EtOH(50%) extract of the leaves is hypoglycaemic, spasmolytic and antiprotozoal (Asolkar et al., 1992).

Madhuca longifolia

It is cultivated in warm and humid regions for its oleaginous seeds (producing between 20 and 200 kg of seeds annually per tree, depending on maturity), flowers and wood. *Madhuca indica* has several pharmacological activity, and potential to provide health to the society. It is used as Anti diabetic, antiulcer, hepato protective, anti-pyretic, anti-fertility, analgesic, anti-oxidant, swelling, inflammation, piles, emetic, dermatological, laxative, tonic, anti-burn, anti-earthworm, wound healing headache and many more problems.

Arbus precatorius

A tea is made from the leaves and used to treat fevers, coughs and colds. It is very effective in treating leucoderma. In abdominal pain it is applied it over the abdomen. The oil extracted from the leaves of Gundumani has great medicinal properties in stimulating the growth of hairs. (Rajani A. 2012).

Alianthus excelsa

The leaf and stem bark has infertility activity. Excelsin was found to inhibit the growth of malarial parasites even at a concentration of 0.2 µg. A single administration of leaves or stem bark extracts of A. excels lowered the blood glucose tolerance test. Canthinh-6-one alkaloid from ailanthus was found to be active against these protozoans. (Ravi kumar 2013, M. F. Roberts).

Amaranthus spinosus

The seed is used as a poultice for broken bones. The plant is astringent, diaphoretic, diuretic, emollient, febrifuge and galactagogue. Externally, it is used to treat ulcerated mouths, vaginal discharges, nose bleeds and wounds. The juice of the root is used in Nepal to treat fevers, urinary troubles, diarrhoea and dysentery.

Delonix elata

The leaf and bark extracts of *D. elata* are anti-inflammatory agents; a root decoction is drunk for abdominal pains. Leaves are reported to be used by traditional practitioners in cases of inflammatory joint disorders as a folklore remedy. A psychosomatic medicinal use relating to scorpion bite treatment is reported from India. Leaf and seed

extracts have antimalarial and antiovicidal activity; hence these extracts are used by traditional practitioners to treat malaria.

Martynia annua

The plant has medicinal values. In tribal pockets of Chhindwara and Betul Districts, Madhya Pradesh, root decoction is administered for snake bite. In Marudhamalai hills, tribes use the juice of leaf for epilepsy, tuberculosis and sore throat. Besides these, the stem of the plant is used by Tantriks in some parts of India.

Oroxylum indicum

The tree is often grown as an ornamental for its strange appearance. Materials used include the wood, tannins and dyestuffs. The *Oroxylum indicum* seed is used in the traditional Indian ayurvedic medicine. The root bark is also used, administered as astringent, bitter tonic, stomachic and anodyne. It is included in famous tonic formulations, such as Chyawanprash.

Pedaliium murex

Leaves are antibilious. Seeds are demulcent, diuretic, tonic, muscilaginous and aphrodisiac. Used in male impotence, gonorrhoea and incontinence. Nervine weakness, Pains, Inflammation, Indigestion, Piles, Constipation, Heart related problems, Cough, Asthma, Epitasis, Frigidity, Impotence, Renal calculi, Dysurea, Infections.

Amaranthus viridis.

The plant is cooling, alexiteric, laxative, stomachic, appetizer and antipyretic; used in burning sensation, hallucination, leprosy, bronchitis, piles, leucorrhoea and constipation. The leaves are used as an emollient. The root is heating and expectorant; lessens the menstrual flow; useful in leucorrhoea and leprosy. Petroleum ether extract of the plant possesses juvenomimetic activity (Asolkar et al., 1992).

Ficus religiosa

An aqueous extract of the bark has an antibacterial activity against *Staphylococcus aureus* and *Escherichia coli*. It is used in the treatment of gonorrhoea, diarrhoea, dysentery, haemorrhoids and gastrophelcosis. Leaves and tender shoots have purgative properties and are also recommended for wounds and skin diseases. Fruits are laxative and digestive. Bark-astringent, antiseptic, alterative, laxative, haemostatic, vaginal disinfectant (used in diabetes, diarrhoea, leucorrhoea, menorrhagia, nervous disorders; also in skin diseases.)

Gmelina arborea

The root and bark of *Gmelina arborea* are claimed to be stomachic, galactagogue laxative and anthelmintic; improve appetite, useful in hallucination, piles, abdominal pains, burning sensations, fevers, 'tridosha' and urinary discharge. Leaf paste is applied to relieve headache and juice is used as wash for ulcers.

Materials and Methods:

Collection of Plant samples:

Plant specimens were collected from Danta forest region, identified with the help of Dr. K.C.Patel, Dept. of Botany.

Fresh, disease free, leaves, stems, flowers were collected, washed, dried, and powdered with a mixer for use.

Solvent:

Distilled water and methanol were used for the extraction method.

Extraction method:

The methods are same as those mentioned in the previous manuscripts.

Following bacterial strains are taken for this study:

E. coli, *S. aureus*, *B. bronchiseptica*, *S. flexneri*, *H. influenzae*

Results and Discussion:

Table No. 01

Zone of inhibition (mm)							
Organism	Solvent	Dilution µg/ml	<i>A. precatorious</i>	<i>J. adhatoda</i>	<i>M. longifera</i>	<i>A. salvifolium</i>	<i>A. excellsa</i>
			Leaf	Leaf	Leaf	Leaf	Leaf
<i>E. coli</i>	Methanol	100	11	10	10	8	7
		200	13	12	12	9	6
		300	12	12	15	11	7
		400 µg	17	15	16	12	8
	Distilled water	100	11	9	14	0	0
		200	13	11	10	0	0

		300	12	13	15	0	0
		400	14	13	16	0	0
<i>S. aureus</i>	Methanol	100	9	14	11	0	6
		200	13	16	12	10	6
		300	14	17	15	12	7
		400	14	18	16	0	8
	Distilled water	100	10	9	12	9	0
		200	12	9	14	8	0
		300	14	10	16	0	0
		400	16	11	11	0	0
<i>B. bronchiseptica</i>	Methanol	100	10	12	11	10	7
		200	14	14	13	11	8
		300	14	13	14	14	7
		400	16	13	16	12	8
	Distilled water	100	0	10	11	0	0
		200	0	9	13	0	0
		300	10	11	14	0	0
		400	11	12	16	0	0
<i>S. flexneri</i>	Methanol	100	8	11	9	8	8
		200	7	14	13	10	9
		300	14	16	14	12	9
		400	14	17	15	14	10
	Distilled water	100	9	10	0	7	0

<i>H. influenzae</i>		200	10	12	12	9	0
		300	12	13	12	9	0
		400	12	15	14	10	0
	Methanol	100	7	13	9	10	9
		200	11	14	12	12	9
		300	13	17	14	13	10
		400	15	18	18	13	12
	Distilled water	100	10	11	0	6	0
		200	10	13	10	9	0
		300	12	11	14	10	0
		400	14	14	11	8	0

Table No. 02

Zone of inhibition (mm)									
Organism	Solvent	Dilution	<i>A. spinosus</i>		<i>D. elata</i>		<i>O. indicum</i>		
			Leaf	Stem	Leaf	Stem	Leaf	Stem	Flower
<i>E. coli</i>	Methanol	100	20	0	0	0	9	0	0
		200	30	0	20	0	10	0	0
		300	31	0	22	0	13	0	0
		400	33	25	23	0	15	0	0
	Distilled water	100	0	0	0	0	0	30	0
		200	0	0	0	0	0	30	0
		300	0	0	0	0	0	32	0
		400	0	0	0	0	9	33	0

<i>S. aureus</i>	Methanol	100	20	15	22	0	13	0	0
		200	22	32	23	0	15	0	0
		300	25	40	30	0	20	0	0
		400	30	40	32	0	22	0	0
	Distilled water	100	0	0	0	0	16	29	0
		200	0	0	0	0	18	30	0
		300	0	0	0	0	15	31	0
		400	0	0	0	0	20	35	0
<i>B. bronchiseptica</i>	Methanol	100	0	0	0	0	10	0	0
		200	0	0	0	0	11	0	0
		300	0	0	20	0	13	0	0
		400	20	15	22	0	15	0	0
	Distilled water	100	0	0	0	0	0	0	0
		200	0	0	0	0	0	0	0
		300	0	0	0	00	0	0	0
		400	0	0	0	0	10	0	0
<i>S. flexneri</i>	Methanol	100	25	10	0	0	12	0	0
		200	25	12	0	0	13	0	0
		300	26	15	0	0	15	0	0
		400	28	20	0	0	16	0	0
	Distilled water	100	0	0	0	0	0	5	0
		200	0	0	0	0	0	6	0
		300	0	0	0	0	9	10	0

		400	0	0	0	0	32	12	0
<i>H. influenzae</i>	Methanol	100	0	0	0	0	11	0	0
		200	0	0	0	0	12	0	0
		300	0	12	10	0	16	0	0
		400	20	15	20	0	17	0	0
	Distilled water	100	0	0	0	0	0	0	0
		200	0	0	0	0	0	0	0
		300	0	0	0	0	9	0	0
		400	0	0	0	0	10	0	0

Table No. 03

Organism	Solvent	Dilution	<i>M. annua</i>		<i>Annona squamosa</i>		<i>P.murex</i>	
			Leaf	Stem	Leaf	Stem	Fruit	Stem
<i>E. coli</i>	Methanol	100		0	10	0	40	0
		200		0	8	0	42	0
		300		10	11	0	42	0
		400		15	13	0	43	0
	Distilled water	100		18	9	7	40	0
		200		20	12	11	40	0
		300		20	14	10	40	0
		400		26	13	12	40	0
<i>S. aureus</i>	Methanol	100		0	15	0	0	0
		200		0	10	0	0	0

		300		11	12	0	15	0
		400		13	9	0	20	0
	Distilled water	100		0	11	9	30	0
		200		0	7	11	30	0
		300		10	14	10	40	0
		400		14	13	12	40	0
<i>B. bronchiseptica</i>	Methanol	100		0	16	0	0	0
		200		0	12	0	0	0
		300		0	14	0	0	0
		400		10	13	0	10	0
	Distilled water	100		8	10	12	0	0
		200		9	8	8	0	0
		300		11	11	11	9	0
		400		13	13	10	10	0
<i>S. flexneri</i>	Methanol	100		0	13	0	0	0
		200		0	12	0	0	0
		300		10	10	0	10	0
		400		10	9	0	30	0
	Distilled water	100		9	7	12	0	0
		200		10	10	14	0	0
		300		10	8	16	0	0
		400		11	9	10	0	0
<i>H. influenzae</i>	Methanol	100		25	12	0	30	0

		200		26	15	0	33	0
		300		28	14	0	35	0
		400		30	11	0	37	0
	Distilled water	100		0	10	9	0	0
		200		0	11	11	0	0
		300		10	11	10	10	0
		400		12	13	10	15	0

Table No. 04

Zone of inhibition (mm)									
Organism	Solvent	Dilution	<i>A. viridis</i>		<i>F. religiosa</i>		<i>G. arborea</i>		
			Leaf	Stem	Leaf	Stem	Leaf	Stem	Flower
<i>E. coli</i>	Methanol	100	11		10	8	7	0	0
		200	12		9	10	9	0	0
		300	10		12	9	10	0	0
		400	15		13	11	12	0	0
	Distilled water	100	12		15	13	8	0	0
		200	11		10	12	0	0	0
		300	10		9	10	0	0	0
		400	13		11	9	6	0	0
<i>S. aureus</i>	Methanol	100	9		12	10	10	0	0
		200	14		15	16	0	0	0
		300	14		17	11	0	0	0
		400	11		16	13	9	0	0

	Distilled water	100	10		10	14	10	0	0
		200	13		10	12	8	0	0
		300	15		9	10	0	0	0
		400	14		11	11	11	0	0
<i>B. bronchiseptica</i>	Methanol	100	10		10	7	10	0	0
		200	13		13	10	12	0	0
		300	14		12	12	14	0	0
		400	15		14	13	15	0	0
	Distilled water	100	9		9	10	0	0	0
		200	0		9	9	0	0	0
		300	11		10	11	9	0	0
		400	10		12	13	11	0	0
	Methanol	100	7		11	15	8	0	0
		200	9		13	14	10	0	0
		300	13		15	16	12	0	0
		400	12		17	13	14	0	0
	Distilled water	100	10		10	10	8	0	0
		200	9		13	12	10	0	0
		300	11		12	9	9	0	0
		400	11		15	8	10	0	0
	Methanol	100	8		12	13	10	0	0
		200	10		11	13	12	0	0
		300	12		17	11	12	0	0

		400	14		15	9	11	0	0
	Distilled water	100	9		10	10	7	0	0
		200	9		14	15	6	0	0
		300	10		9	10	9	0	0
		400	13		14	13	8	0	0

Plants showing highest zone size:

The antimicrobial activity of leaf extract of *Arbus precoterious* is observed to be higher on *E.coli* with 17mm zone size in comparison to test organism and Moderate antimicrobial activity of leaf extract of *Arbus pracoterious* is observed in *S. flexneri* Methanol extract with zone size 8 mm. lowest on *B.bronchiseptica* water extract with no zone inhibition

The activity of leaf extract of *Adhatoda vasica* is observed to be higher on *S. aureus* with 18mm zone size in comparison to test organism and. Moderate antimicrobial activity of leaf extract of *Adhatoda vasica* is observed in Methanol extract with zone size 14 mm against *S. aureus*, *B. bronchiseptica*, *S. flexneri* and *H. influenzae* Methanol extract and *H. influenzae* water extract with zone size 14mm. *S. flexneri* lowest on *E. coli*, *S. aureus* and *B. bronchiseptica* water extract with 9 mm zone size.

The activity of leaf extract of *Alangium salvifolium* is observed to be higher on *S. flexneri* and *B. bronchiseptica* with 14mm zone size in comparison to test organism Moderate antimicrobial activity of leaf extract of *Alangium salvifolium* is observed in water extract with zone size 7 mm against *S. flexneri* water extract and lowest on *B. bronchiseptica* and *E. coli* water extract in which zone of inhibition is not seen.

The antimicrobial activity of leaf extract of *Alianthus excelsa* is observed to be higher on *H. influenzae* with 12 mm zone size in comparison to the test organism Moderate antimicrobial activity of leaf extract of *Alianthus excelsa* is observed in Methanol extract with zone size 9 mm against *S. flexneri* and *H. influenzae* with zone size 9mm. and lowest on *S. aureus* Methanol with 6mm zone size and 6 mm zone size on *E. coli* water extract.

The activity of leaf extract of *Madhuca longifolium* is observed to be higher on *H. influenzae* with 18mm zone size in comparison to other test organism and. Moderate antimicrobial activity of leaf extract of *M. longifolium* is observed on *S. flexneri* with 7mm zone size in water extract, lowest on *H. influenzae* water extract with no zone inhibition.

The antimicrobial activity of leaf Methanol extract of *Oroxylum indicum* is observed to be in the highest on *S. aureus* in comparison to the test organism and lower on *E. coli*.

The antimicrobial activity of fruit Methanol extract of *Amaranthus spinosus* is observed to be in the highest on *S. aureus* in comparison to the test organism and lower on *B. septica*.

The activity of leaf Methanol extract of *Delonix elata* is observed to be in the highest on *S. aureus* in comparison to the test organism and lower on *H. influenzae*.

The activity of fruit Methanol extract of *Pedaliium murex* is observed to be in the highest on *E. coli* in comparison to the test organism and lower on *B. septica*.

The activity of stem Methanol extract of *Martynia annua* is observed in the higher on *H. influenzae* in comparison to the test organism and lower on *B. septica*.

The antimicrobial activity of leaf extract of *Amaranthus viridis* is observed to be highest on *E. coli* methanol extract with 15mm zone size and *S. aureus* DW extract with 15mm zone size in comparison to the test organism and moderate on *E. coli* (Methanol extract and DW), *S. aureus* (Methanol extract) *S. flexneri* (DW extract) with 11mm zone size and *B. bronchiseptica* is resistant to *Amaranthus viridis* leaf DW extract

Medicinal plant *Ficus religiosa* leaf Methanol extract show the highest antimicrobial activity on *H. influenzae* and *S. aureus* with 17mm zone size and the moderate on *S. flexneri*, *E. coli*, *B. bronchiseptica* with the 13mm zone size and the lowest antimicrobial on *E. coli* with the 9mm zone size inhibition

Ficus religiosa steam Methanol extract show the highest antimicrobial activity on *S. flexneri* with 16mm zone size inhibition in comparison to organism. Moderate on *S. flexneri*, *S. aureus*, *E. coli* with the 12mm size zone and the lowest *E. coli* and *S. flexneri* with 8mm of zone size inhibition in comparison on the test organism

The antimicrobial activity of *Gmelina arborea* leaf extract is observe to maximum on Methanol extract of *B. bronchiseptica* with 15mm of zone size in comparison to other organism and the moderate on *E. coli* DW extract, *S. aureus* DW extract, *S. flexneri* Methanol extract and *H. influenzae* with 8mm of zone size and the resistant on *E. coli* and *S. aureus* leaf DW extract

The antimicrobial activity of *Annona squamosa* of leaf extract show the highest antimicrobial activity *B. bronchiseptica* with 16mm zone size inhibition in comparison to other organism, moderate on *E. coli* (Methanol extract), *S. aureus* (DW extract), *H. influenzae* (Methanol and DW) with 11 mm of size zone and the lowest *S. aureus* with 8mm of zone size inhibition.

Medicinal plant *Annona squamosa* of steam extract show the highest antimicrobial activity on *S. flexneri* with 16mm zone size inhibition in comparison to all the test organism. Moderate on. *E. coli* (DW extract), *S. aureus* (DW extract) , *H. influenzae* (DW extract) with 11mm of zone size and The lowest *E. coli* with the 7mm of zone size inhibition

Result of Bacterial inhibition by plants:

In terms of microbial sensitivity, it is observed that *E. coli* is highly sensitive to the Methanol and water leaf extract of *Madhuca longifolium* with zone size 16mm *E. coli* is moderately sensitive to the Methanol extract of *Alangium salvifolium* and *Alianthus excelsa* with zone size 8mm and *E. coli* is resistant to the water extracts of *Alangium salvifolium* and *Alianthus excelsa*.

S. aureus is highly sensitive to the methanol leaf extract of *Adhatoda vasica* with zone size 18mm. *S. aureus* is moderately sensitive to the Methanol extract of *Arbus precatorius*, water extract *Adhatoda vasica* and water extract of *Alangium salvifolium* with zone size 9mm. and *S. aureus* is resistance toward the water extract of *Alianthus excelsa*.

B. bronchiseptica is highly sensitive to the methanol leaf extract of *Arbus precatorius* and *Justicia adhatoda* with zone size 16mm. *B. bronchiseptica* is moderately sensitive to the Methanol extract of *Alanthus excelsa* with zone size 8mm and *B. bronchiseptica* is resistant to the water extracts of *Alangium salvifolium* and *Alanthus excelsa* water extract.

S. flexneri is highly sensitive to the methanol leaf extract of *Justicia adhatoda* with zone size 17mm. *S. flexneri* is moderately sensitive to the methanol extract of *Alanthus excelsa* and *Madhuca longifolium* Methanol extract and also *Arbus precatorius* and *Alangium salvifolium* water extract with zone size 9mm & *S. flexneri* is resistance toward the water extract of *Alanthus excelsa* water extract.

H. influenzae is highly sensitive to the Methanol leaf extract of *Justicia adhatoda* and *M. longifolium* with zone size 18mm. *H. influenzae* is moderately sensitive to the Methanol extract of *Alanthus excelsa* and Methanol extract *H. influenzae* is resistance toward the water extract of *Alanthus excelsa* water extract.

S. aureus is highly sensitive to methanol leaf extract of *Oroxylum indicum* moderately sensitive to *E. coli* and resistant to *B. septica*.

E. coli is highly sensitive to methanol fruit extract of *Amaranthus spinosus* moderately sensitive to *S. aureus* and resistant to *H. influenzae*.

S. aureus is highly sensitive to methanol leaf extract of *Delonix elata* moderately sensitive to *E. coli* and resistant to *S. flexneri*.

H. influenzae is highly sensitive to methanol stem extract of *Martynia annua*, moderately sensitive to *E. coli* and resistant to *B. septica*.

E. coli is highly sensitive to the methanol fruit extract of *Pedaliium murex* moderately sensitive to *S. aureus* and resistant to *B. septica*.

E. coli is highly sensitive to the leaf methanol extract of *A. viridis*, leaf distilled water extract of *F. religiosa* (15mm) and moderately sensitive to leaf Methanol extract of *G. arborea*, stem Distilled water extract of *A. squamosa* (7mm) and not resistant to any plant extract.

S. aureus is highly sensitive of the leaf Methanol extract of *F. religiosa* (17mm) and moderately sensitive to leaf Distilled water extract of *G. arborea* (8mm) and not resistant to any plant extract.

It is observed that *B. bronchiseptica* is highly sensitive to the leaf extract of *A. squamosa* (16mm) and moderately sensitive to leaf and steam extract of *A. squamosa* (8mm) and not resistant to any plant extract.

S. flexneri is highly sensitive to leaf extract of *F. religiosa* (17mm) and moderately sensitive to steam Distilled water extract of *F. religiosa* (4mm), leaf Distilled water extract of *G. arborea* (8mm) and *A. squamosa* (8mm) and not resistant to any plant extract.

H. influenzae is highly sensitive to leaf Methanol extract of *F. religiosa* (17mm) and moderately sensitive to leaf Methanol extract of *A. viridis* (8mm) and leaf Distilled water extract of *G. arborea* (8mm) and not resistant to any plant extract.

Solvent wise result:

The methanol extract produces greater activity (17mm) for leaf extract of plant *Arbus precatorius* with *E. coli*

While reporting the result based on the extract used it can be said that the methanol extract is observed to produce the greater activity (18mm) for leaf extract of plant *Justicia adhatoda* with *S. aureus*.

Result based on the extract used, it can be said that the methanol and water extract is observed to produce greater activity (16mm) for leaf extract of plant *Arbus precatorious* and *Madhuca longifolium* with *B. bronchiseptica*.

It can be said that the methanol extract produces the greater activity (17mm) for leaf extract of *Justicia adhatoda* with *S. flexneri*

While reporting the result based on the extract used it can be said that the methanol extract is observed to produce greater activity (18mm) for leaf extract of plant *Justicia adhatoda* and *Madhuca longifolium* with *H. influenzae*,

While reporting the result towards on the extract are, it can be said that the methanol extract is observed to produce greatest activity for leaf extract of *Oroxylum indicum* towards *S. aureus* and lower activity on *E. coll*.

The methanol extract is observed to produce greatest activity for fruit extract of *Amaranthus spinosus* towards *E. coll* and lower activity on *H. influenzae*.

Methanol extract is observed to produce greatest activity for leaf extract of *Delonix etala* towards *E. coli* and lower activity on *S. flexneri*.

Methanol extract is observed to produce greatest activity for leaf extract of *Martynia annua* towards *H. influenzae* and lower activity on *B. septica*.

The methanol extract is observed to produce greatest activity for leaf extract of *Pedaliium murex* towards *E. coli* and lower activity on *B. septica*.

It can be said the methanol extract is observed to produce grater activity (15mm) for leaf extract of plant *A. viridis* and leaf Distilled water extract of *F. religiosa* with *E. coli*.

Methanol extract is observed to produce grater activity (17mm) for leaf extract of *F. religiosa* with *S. aureus*

The methanol extract is observe to produce grater activity (16mm) for leaf extract of *A. squomosa* with *B. bronchiseptica*

Methanol extract is observe to produce grater activity (17mm) for leaf extract of *F. religiosa* with *S. flexneri* and *H. influenzae*.

DISCUSSION

Antibacterial activity of the aqueous and methanol extracts of the leaves of *Arbus precatorius* are listed in the table.(I) methanol extract exhibits higher antibacterial effect than that of the aqueous extract. Both the extracts showed antimicrobial activity against the tested strains but the methanol extract showed the maximum inhibitory effect against *E. coli* and lowest inhibitory effect on *S. flexneri*, if we compare this present work with reference the methanol extract showed the maximum inhibitory effect against *Enterobacter aerogenes* and it is minimum for *Klebsiella pneumoniae*.

Aqueous extract showed the highest inhibitory action against *S. aureus* as that of the Methanol extract. It was minimum for *H. influenzae*. *B. bronchiseptica* showed no inhibitory effects. Overall the leaf extracts of *Arbus precatorius* showed significant

antimicrobial activity against the tested pathogens, when comparing with the standards.

Antibacterial activity of the aqueous and methanol extracts of the leaves of *Justicia adhatoda* are listed in the table. (I) Methanol extract exhibits higher antibacterial effect than that of the aqueous extract. Both the extracts showed antimicrobial activity against the tested strains but The Methanol extract showed the maximum inhibitory effect against *S. aureus* and *H. influenzae* and lowest inhibitory effect on *E. coli*. If we compare this present work with reference the Methanol extract showed the maximum inhibitory effect against *Bacillus subtilis* and it is minimum for *Klebsiella pneumoniae*.

Aqueous extract showed the highest inhibitory action against *H. influenzae* as that of the Methanol extract. It was minimum for *S. aureus*, *B. bronchiseptica*. Overall the leaf extracts of *Justicia adhatoda* showed significant antimicrobial activity against the tested pathogens, when comparing with the standards.

Antibacterial activity of the aqueous and Methanol extracts of the leaves of *Alangium salvifolium* are listed in the table. (1) Methanol extract exhibits higher antibacterial effect than that of the aqueous extract. Both the extracts showed antimicrobial activity against the tested strains but The Methanol extract showed the maximum inhibitory effect against *B. bronchiseptica* and *S. flexneri* and lowest inhibitory effect on *E. coli* and *B. bronchiseptica* if we compare this present work with reference the ethanol extract showed the maximum inhibitory effect against *Staphylococcus aureus* and it is minimum for *pseudomonas aeruginosa*.

Aqueous extract showed the highest inhibitory action against *E. coli* as that of the Methanol extract. It was minimum for *S. aureus* and showed no inhibitory effects. Overall the leaf extracts of *Alangium salvifolium* showed significant antimicrobial activity against the tested pathogens, when comparing with the standards.

Antibacterial activity of the aqueous and Methanol extracts of the leaves of *Alanthus excelsa* are listed in the table. (I) Methanol extract exhibits higher antibacterial effect than that of the aqueous extract. Both the extracts showed antimicrobial activity against the tested strains but the methanol extract showed the maximum inhibitory effect against *H. influenzae* and lowest inhibitory effect on *E. coli* if we compare this present work with reference the Methanol extract showed the maximum inhibitory effect against *Streptococcus*, *Salmonella typhimurium* and it is minimum for *Shigella flexneri*.

Aqueous extract showed no inhibitory effects on test organisms. Overall the leaf extracts of *Alanthus excelsa* showed significant antimicrobial activity against the tested pathogens, when comparing with the standards.

Antibacterial activity of the aqueous and Methanol extracts of the leaves of *Madhuca longifolia* are listed in the table. (1) Methanol extract exhibits higher antibacterial effect than that of the aqueous extract. Both the extracts showed antimicrobial activity against the tested strains but The Methanol extract showed the maximum inhibitory effect against *H. influenzae* and lowest inhibitory effect on *E. coli* if we compare this present work with reference the Methanol extract showed the maximum inhibitory effect against *Streptococcus mutant* and it is minimum for *streptococcus salivarius*.

Aqueous extract showed the highest inhibitory action against *S. aureus* and *E. coli* as that of the Methanol extract. It was minimum for *E. coli* and *H. influenza*, *S. flexneri* showed no inhibitory effects. Overall the leaf extracts of *Madhuca longifolia* showed

significant antimicrobial activity against the tested pathogens, when comparing with the standards.

With regard to the present study of *Pedaliium murex*, employing *Bacillus subtilis* & *A. niger*, the ethanol extract provide a wider zone against *B. subtilis* & aqueous extract has lower effective for *A. niger* (Shelke TT et al.), whereas on present study support a higher efficiency of *Pedaliium murex* with a methanol extract showing greater inhibition towards *E. coli*. Study of *Pedaliium murex* employing *Streptococcus faecalis*, *S. pyogenes*, *Enterococcus Faecalis*, *Bacillus subtilis*, *B.thuringinesis*, *Klebsiella pneumonia*, *Proteus mirabilis*, *Proteus vulgaris*, *Salmonella paratyphi*, the ethanol extract provide a wider zone against gram positive bacteria (Sermakkani M et al. 2011) and whereas on present study supports a higher efficiency of *Pedaliium murex* with an Methanol extract showing greater inhibition towards *E. coli*, *S. aureus*, *H. influenzae*.

In present study it is observed that *O. indicum* successfully inhibits the *S. aureus*, *H. influenzae*, *E. coli*. Whereas the study of Acharya R et al. (2011) has reported that root bark and stem bark provides an effective anti-inflammatory activity when used in albino rat. Veereshan C et al (2013) have also worked on antiarthritic activity of root bark of *O.indicum* against adjuvant induced arthritis. This supports and enhances our study as to potential application of this plant for further study and detailing at *in vivo* level to establish the plant for therapeutic application.

Our study of *Amaranthus spinosus* suggests that it inhibits *E. coli*, *S. flexneri*. Whereas it has been reported by Chaudhari M A (2012) that *A. spinosus* provides laxative property by gut modulation and agonistic activity as bronchodilator on rabbits and guinea pigs. Girija K et al. (2011) reported that the Methanol extract of *A. spinosus* has anti-diabetic and anticholesterolemic activity.

The present study on *Martynia annua* suggests that it inhibits *H. influenzae* and *E. coli*. It has been reported by Singhal AK et al. (2011) that ethanol extract of *M. annua* is found most effective in wound healing as observed by better angiogenesis, matured collagen fibers and fibroblast cells. Sermakkani et al. (2010) study on phytochemical and antibacterial activity of *M. annua* against the different pathogen bacteria.

Our study on *Delonix elata* suggest that it inhibits *S. aureus* and *E. coli*. It has been reported by Vasugi C et al (2013) that *D.elata* has been reported to provide biological activity on various stage of *Aedes aegypti*. The mortality rate shown & to be 100%, in first imbrute grab. Shanmugavadivu R et al (2008) also study on antimicrobial activity of leaf extract of *D. elata* higher inhibitory effect on bacteria and fungi.

Antibacterial activity of the aqueous and methanol extracts of the leaves of *Amaranthus viridis* are listed in the table.(I) methanol extract exhibits higher antibacterial effect than that of the aqueous extract. Both the extracts showed antimicrobial activity against the tested strains but the methanol extract showed the maximum inhibitory effect against *E. coli*, *S. aureus* and lowest inhibitory effect on *S. flexneri* if we compare this present work with reference the methanol extract showed the maximum inhibitory effect against *Salmonella typhi* and it is minimum for *S. aureus*.

Aqueous extract showed the highest inhibitory action against *S. aureus* as that of the Methanol extract. It was minimum for *B. bronchiseptica*. Overall the leaf extracts of *Amaranthus viridis* showed significant antimicrobial activity against the tested pathogens, when comparing with the standards.

Antibacterial activity of the aqueous and methanol extracts of the leaves and stem of *Ficus religiosa* are listed in the table. (I) Methanol extract exhibits higher antibacterial

effect than that of the aqueous extract. Both the extracts showed antimicrobial activity against the tested strains but the methanol extract showed the maximum inhibitory effect against *S. aureus* and lowest inhibitory effect on *B. bronchiseptica* if we compare this present work with reference the methanol extract showed the maximum inhibitory effect against *Pseudomonas aeruginosa* and it is minimum for *Klebsiella pneumoniae*. All the tested pathogens showed significant inhibitory effects.

Aqueous extract showed the highest inhibitory action against *S. flexneri* as that of the Methanol extract. It was minimum for *S. flexneri*. Overall the leaf extracts of *Ficus religiosa* showed significant antimicrobial activity against the tested pathogens, when comparing with the standards. The selection of this plant for the present study was based on its medicinal properties and its use in traditional system.

Activity of the aqueous and Methanol extracts of the leaves of *Gmelina arborea* are listed in the table. (1) Methanol extract exhibits higher antibacterial effect than that of the aqueous extract. Both the extracts showed antimicrobial activity against the tested strains but The Methanol extract showed the maximum inhibitory effect against *B. bronchiseptica* and lowest inhibitory effect on *E. coli* if we compare this present work with reference the Methanol extract showed the maximum inhibitory effect against *Streptococcus faecalis* and it is minimum for *Enterobacter aerogenes*. All the tested pathogens showed significant inhibitory effects in reference.

Aqueous extract showed the highest inhibitory action against *E. coli* as that of the Methanol extract. It was minimum for *S. aureus*. Overall the leaf extracts of *Gmelina arborea* showed significant antimicrobial activity against the tested pathogens, when comparing with the standards. The selection of this plant for the present study was based on its medicinal properties and its use in traditional system.

Activity of the aqueous and Methanol extracts of the leaves and stem of *Annona squamosa* are listed in the table. (2) Methanol extract exhibits higher antibacterial effect than that of the aqueous extract. Both the extracts showed antimicrobial activity against the tested strains but The Methanol extract showed the maximum inhibitory effect against *B. bronchiseptica* and lowest inhibitory effect on *E. coli* if we compare this present work with reference the Methanol extract showed the maximum inhibitory effect against *S. aureus* and it is minimum for *E. coli*. All the tested pathogens showed significant inhibitory effects in reference.

Aqueous extract showed the highest inhibitory action against *E. coli* as that of the Methanol extract. It was minimum for *S. aureus*. Overall the leaf and stem extracts of *Annona squamosa* showed significant antimicrobial activity against the tested pathogens, when comparing with the standards. The selection of this plant for the present study was based on its medicinal properties and its use in traditional system.

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CHAPTER NO: 8

Study of invitro inhibitory activity of some ethno medicinal plants of Danta forest against bacterial pathogens.

Study of invitro inhibitory activity of some ethno medicinal plants of Danta forest against bacterial pathogens.

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Introduction:

Forest ecosystems are rich habitats for living kind. The flora, fauna of this ecosystem is the one of the richest among all. Danta forest serves as a habitat for a diverse range of flora and fauna. The human population residing in an around this forest reaps the benefits form it, which includes not only food, shelter and clothing, but the flora provides to cure them of their diseases and discomforts. Local medicine men provide them with herbal medicine prepared in a very crude but effective manner. This study attempts to understand the relation between the treatments provided by them with the actual antibacterial activity of the plant products. Following is the list of plants and bacterial pathogens employed in the study.

Materials and methods:

The methods and materials for the study are same as those mentioned in the previous manuscripts.

No:	Name of the plants
1.	<i>Acacia nilotica</i>
2.	<i>Vitex negundo</i>
3.	<i>Holarrhena antidysentrica</i>
4.	<i>Butea monosperma</i>
5.	<i>Melilotus indica</i>
6.	<i>Embllica officinalis</i>
7.	<i>Ficus virens</i>
8.	<i>Annona squamosa</i>
9.	<i>Hemigraphis latebrosa</i>
10.	<i>Cuscuta reflexa</i>
11.	<i>Euphorbia nivulia</i>
12.	<i>Lannea coromandelica</i>
13.	<i>Crataeva nurvala</i>
14.	<i>Solanum surattense</i>
15.	<i>Triticum aestivum</i>

No:	Name of the bacterial pathogen
1.	<i>E. coli</i>
2.	<i>B. bronchiseptica</i>
3.	<i>S. flexneri</i>
4.	<i>S. aureus</i>
5.	<i>H. influenzae</i>

Results and Discussion

Zone of inhibition (mm)

Organism	Solvent	Dilution µg/ml	<i>Acacia nilotica</i>		<i>Vitex negundo</i>		<i>Holarrhena antidysenterica</i>	
			Leaf	Stem	Leaf	Stem	Leaf	Stem
<i>E. coli</i>	Methanol	25	0	0	0	0	0	0
		50	0	0	0	0	0	0
		75	0	0	0	0	0	0
		100	0	0	0	6	12	0
		125	10	0	15	18	14	14
	D/water	25	0	0	0	0	0	0
		50	0	0	0	0	0	0
		75	0	0	0	0	0	0
		100	12	0	8	0	0	0
		125	19	11	13	0	0	13
<i>S. aureus</i>	Methanol	25	15	10	12	10	16	12
		50	19	14	17	15	18	16
		75	25	17	21	19	18	17
		100	27	28	21	14	20	19
		125	31	28	28	23	24	25
	D/water	25	15	0	18	0	13	11
		50	21	0	20	14	18	14
		75	21	18	24	18	21	19
		100	29	22	28	21	29	20
		125	38	27	31	26	33	26
<i>B. bronchiseptica</i>	Methanol	25	14	9	10	9	0	0
		50	17	14	12	13	0	0
		75	23	18	17	16	10	7
		100	23	18	20	16	13	11
		125	26	24	27	24	18	16
	D/water	25	8	0	7	0	8	0

<i>S. flexneri</i>		50	10	6	9	0	11	0
		75	16	13	13	10	15	9
		100	16	15	15	13	17	15
		125	25	15	19	14	20	15
	Methanol	25	14	10	18	17	15	11
		50	18	16	19	20	17	16
		75	23	21	24	21	26	24
		100	25	21	26	23	29	28
		125	29	26	30	29	35	33
	D/water	25	11	8	21	20	16	14
		50	14	15	28	24	20	19
		75	19	15	30	28	25	21
		100	24	19	35	30	29	28
		125	30	23	35	32	34	33

Zone of inhibition (mm)

Organism	Solvent	Dilution µg/ml	<i>Acacia nilotica</i>		<i>Vitex negundo</i>		<i>Holarrhena antidysenterica</i>	
			Leaf	Stem	Leaf	Stem	Leaf	Stem
<i>H. influenzae</i>	Methanol	25	11	10	13	10	7	8
		50	14	12	16	13	11	10
		75	18	15	18	17	14	10
		100	19	18	21	19	16	13
		125	23	20	22	23	18	15
	D/water	25	7	0	0	0	10	11
		50	10	0	8	0	14	12
		75	13	12	14	10	14	12
		100	16	15	14	14	16	14
		125	21	18	16	17	17	15

Zone of inhibition (mm)

Organism	Solvent	Dilution µg/ml	<i>Butea monosperm a</i>		<i>Melilotus indica</i>		<i>Emblica officinalis</i>	
			Leaf	Stem	Leaf	Stem	Leaf	Stem
<i>E. coli</i>	Methano l	25	0	0	0	0	0	
		50	0	0	0	0	0	0
		75	0	0	8	0	0	0
		100	7	0	10	0	0	0
		125	10	8	14	12	10	0
	D/water	25	0	0	0	0	0	0
		50	0	0	7	0	0	0
		75	0	0	9	0	0	0
		100	0	0	10	11	0	0
		125	13	0	10	15	14	13
<i>S. aureus</i>	Methano l	25	16	14	21	20	16	15
		50	18	15	21	21	19	21
		75	18	20	25	24	23	25
		100	23	21	27	26	26	29
		125	29	28	24	29	30	14
	D/water	25	24	21	26	21	27	18
		50	27	27	29	24	30	25
		75	29	29	32	26	32	29
		100	29	30	35	29	35	32
		125	34	32	19	32	35	12
<i>B. bronchisepti ca</i>	Methano l	25	10	9	20	0	13	13
		50	12	12	22	0	14	15
		75	14	17	24	0	17	17
		100	14	19	10	18	19	22
		125	18	21	12	24	23	10
	D/water	25	10	0	16	8	11	13
		50	14	0	20	11	15	15

<i>S. flexneri</i>	Methanol	75	17	0	23	14	18	18
		100	21	9	26	14	20	21
		125	24	14	28	19	23	21
		25	12	10	28	24	23	24
		50	16	15	30	25	25	25
		75	19	17	30	26	27	27
		100	25	24	32	29	29	29
		125	30	29	24	31	31	22
	D/water	25	14	12	29	21	29	24
		50	17	15	29	25	32	26
		75	21	20	35	27	35	28
		100	26	28	32	31	38	31
		125	34	32	35	31	40	7

Zone of inhibition (mm)

Organism	Solvent	Dilution µg/ml	<i>Butea monosperma</i>		<i>Melilotus indica</i>		<i>Emblica officinalis</i>	
			Leaf	Stem	Leaf	Stem	Leaf	Stem
<i>H. influenzae</i>	Methanol	25	10	8	19	0	11	12
		50	13	11	20	0	13	14
		75	17	15	24	19	16	16
		100	20	19	25	21	18	20
		125	24	23	27	25	21	10
	D/water	25	15	14	15	12	13	14
		50	19	16	18	14	16	17
		75	21	20	21	19	19	22
		100	24	23	23	22	24	26
		125	25	24	28	26	28	26

Zone of inhibition (mm)

Organism	Solvent		<i>Ficus virens</i>	<i>Annona squamosa</i>	<i>Hemigraphis latebrosa</i>
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		Dilution µg/ml	Leaf	Stem	Leaf	Stem	Leaf	Stem
<i>E. coli</i>	Methanol	25	0	0	0	0	0	0
		50	0	0	0	0	0	0
		75	9	0	0	0	0	0
		100	11	0	0	0	8	0
		125	13	0	10	0	10	0
	D/water	25	0	12	0	0	0	0
		50	0	0	0	0	0	0
		75	0	0	0	0	10	0
		100	12	0	0	0	13	9
		125	14	11	0	0	13	17
<i>S. aureus</i>	Methanol	25	23	21	14	11	19	20
		50	25	23	17	16	21	21
		75	27	25	20	19	25	28
		100	31	25	24	21	29	28
		125	34	30	29	26	30	21
	D/water	25	25	22	24	22	25	24
		50	28	26	27	26	28	26
		75	29	27	29	26	29	29
		100	35	34	31	29	31	32
		125	39	37	34	32	33	0
<i>B. bronchiseptica</i>	Methanol	25	12	10	0	0	10	9
		50	11	10	0	0	14	15
		75	13	11	0	9	17	16
		100	16	14	9	12	19	19
		125	19	16	14	16	20	8
	D/water	25	14	12	12	10	12	14
		50	16	14	14	11	15	17
		75	18	14	19	16	18	21
		100	20	16	21	20	25	25

		125	22	20	25	24	28	25
<i>S. flexneri</i>	Methanol	25	19	17	23	21	24	21
		50	23	21	26	24	25	24
		75	27	25	29	27	28	26
		100	30	27	32	30	30	29
		125	32	30	36	32	31	29
	D/water	25	22	21	25	21	25	21
		50	26	25	28	25	27	24
		75	29	27	30	29	29	26
		100	32	30	35	32	31	29
		125	35	33	40	39	34	32

Zone of inhibition (mm)

Organism	Solvent	Dilution µg/ml	<i>Ficus virens</i>		<i>Annona squamosa</i>		<i>Hemigraphis latebrosa</i>	
			Leaf	Stem	Leaf	Stem	Leaf	Stem
<i>H. influenzae</i>	Methanol	25	10	9	9	0	8	0
		50	13	11	12	8	10	11
		75	16	14	16	12	10	12
		100	18	17	19	12	15	14
		125	20	17	19	15	18	16
	D/water	25	14	13	14	11	11	0
		50	17	15	16	14	14	0
		75	19	17	16	15	17	9
		100	21	20	18	17	19	14
		125	24	23	21	20	24	18

Zone of inhibition (mm)					
Organism	Solvent	Dilution µg/ml	<i>Cuscuta reflexa</i>	<i>Euphorbia nivulia</i>	<i>Lansea coromandelica</i>
			Stem	Stem	Stem
<i>E. coli</i>	Methanol	25	0	0	0

		50	0	0	0
		75	0	0	15
		100	10	0	16
		125	14	16	16
	D/water	25	0	0	0
		50	0	0	0
		75	14	14	15
		100	17	18	17
		125	19	21	20
<i>S. aureus</i>	Methanol	25	19	29	20
		50	21	32	24
		75	27	33	26
		100	29	37	29
		125	31	40	29
	D/water	25	24	29	24
		50	28	32	24
		75	29	35	27
		100	32	39	29
		125	32	40	32
<i>B. bronchiseptica</i>	Methanol	25	11	0	11
		50	13	0	13
		75	15	9	14
		100	15	17	16
		125	19	23	17
	D/water	25	14	9	15
		50	19	12	18
		75	21	15	19
		100	25	19	24
		125	20	24	25
<i>S. flexneri</i>	Methanol	25	25	21	19
		50	32	24	22

		75	34	26	24
		100	25	29	26
		125	29	29	29
	D/water	25	29	23	24
		50	34	25	26
		75	39	28	28
		100	15	28	32
		125	19	31	34
Organism	Solvent	Dilution μg/ml	<i>Cuscuta reflexa</i>	<i>Euphorbia nivulia</i>	<i>Lansea coromandelica</i>
			Stem	Stem	Stem
<i>H. influenzae</i>	Methanol	25	15	12	15
		50	19	13	17
		75	20	24	18
		100	23	24	20
		125	25	29	22
	D/water	25	17	22	16
		50	19	24	18
		75	24	25	20
		100	26	29	22
		125	29	30	24

Zone of inhibition (mm)							
Organism	Solvent	Dilution μg/ml	<i>Crataeva nurvala</i>	<i>Solanum surattense</i>		<i>Triticum aestivum</i>	
			Stem	Leaf	Stem	Leaf	Stem
<i>E. coli</i>	Methanol	25	0	0	0	0	0
		50	0	0	0	0	0
		75	0	8	0	30	0
		100	0	18	9	32	27
		125	0	21	19	33	31
	D/water	25	0	0	0	21	19

		50	0	0	0	24	22
		75	8	0	0	29	26
		100	10	10	0	31	29
		125	10	14	12	32	30
<i>S. aureus</i>	Methanol	25	15	15	13	21	20
		50	19	17	15	26	24
		75	20	17	18	27	24
		100	25	19	18	29	26
		125	27	24	20	33	31
	D/water	25	27	0	0	23	20
		50	29	0	0	25	22
		75	32	0	0	29	26
		100	34	0	0	32	30
		125	34	15	0	32	31
<i>B. bronchiseptica</i>	Methanol	25	36	0	0	12	10
		50	0	0	0	15	13
		75	10	0	0	21	17
		100	12	0	9	25	22
		125	13	0	15	29	25
	D/water	25	16	0	12	15	11
		50	12	0	14	19	14
		75	13	0	14	25	17
		100	14	12	16	25	19
		125	16	16	16	23	21
<i>S. flexneri</i>	Methanol	25	17	0	0	25	23
		50	18	0	0	27	25
		75	23	0	9	28	26
		100	25	0	10	30	28
		125	29	10	16	17	15
	D/water	25	31	13	10	22	17
		50	20	14	12	24	22

		75	25	14	14	29	26
		100	29	16	14	32	29
		125	31	18	16	15	13

Organism	Solvent	Dilution µg/ml	<i>Crateva nurvala</i>	<i>Solenum surattense</i>		<i>Triticum aestivum</i>	
			Stem	Leaf	Stem	Leaf	Stem
<i>H. influenzae</i>	Methanol	25	34	14	15	19	17
		50	15	17	16	20	18
		75	17	18	18	21	20
		100	17	19	20	28	26
		125	19	21	25	16	14
	D/water	25	24	24	21	24	21
		50	12	25	23	23	22
		75	16	25	22	25	23
		100	19	28	26	27	26
		125	24	31	29	27	28

Acacia nilotica

The leaf methanol extract inhibited *E. coli* (10mm), *S. aureus* (31mm), *B. bronchiseptica* (26mm), *S. flexneri* (29mm), *H. influenzae* (23mm).

Leaf aqua extract inhibited *E. coli* (19mm), *S. aureus* (38mm), *B. bronchiseptica* (25mm), *S. flexneri* (30mm), *H. influenzae* (21mm).

Stem methanol extract inhibited *S. aureus* (27mm), *B. bronchiseptica* (15mm), *S. flexneri* (23mm), *H. influenzae* (18mm).

Stem aqua extract inhibited *E. coli* (11mm), *S. aureus* (27mm), *B. bronchiseptica* (15mm), *S. flexneri* (23mm), *H. influenzae* (18mm).

Vitex negundo

The leaf methanol extract inhibited *E. coli* (15mm), *S. aureus* (28mm), *B. bronchiseptica* (27mm), *S. flexneri* (30mm), *H. influenzae* (22mm).

Leaf aqua extract inhibited *S. aureus* (26mm), *B. bronchiseptica* (19mm), *S. flexneri* (35mm), *H. influenzae* (16mm).

Stem methanol extract inhibited *E. coli* (18mm), *S. aureus* (23mm), *B. bronchiseptica* (24mm), *S. flexneri* (29mm), *H. influenzae* (23mm).

Stem aqua extract inhibited *S. aureus* (33mm), *B. bronchiseptica* (14mm), *S. flexneri* (32mm), *H. influenzae* (17mm).

Holarrhena antidysentrica

The leaf methanol extract inhibited *E. coli* (14mm), *S. aureus* (24mm), *B. bronchiseptica* (18mm), *S. flexneri* (35mm), *H. influenzae* (23mm).

Leaf aqua extract inhibited *S. aureus*(33mm), *B. bronchiseptica*(20mm), *S. flexneri* (34mm), *H. influenzae* (17mm).

Stem methanol extract inhibited *E. coli* (14mm), *S. aureus* (25mm), *B. bronchiseptica* (16mm), *S. flexneri* (33mm), *H. influenzae* (18mm).

Stem aqua extract inhibited *E. coli* (13mm), *S. aureus* (26mm), *B. bronchiseptica* (15mm), *S. flexneri* (33mm), *H. influenzae* (15mm).

Butea Monosperma

The Leaf methanol extract inhibited *E. coli* (10mm), *S. aureus* (19mm), *B. bronchiseptica* (18mm), *S. flexneri* (30mm), *H. influenzae* (24mm).

Leaf aqua extract inhibited *E. coli* (13mm), *S. aureus* (34mm), *B. bronchiseptica* (24mm), *S. flexneri* (34mm), *H. influenzae* (25mm).

Stem methanol extract inhibited *E. coli* (08mm), *S. aureus* (28mm), *B. bronchiseptica* (21mm), *S. flexneri* (29mm), *H. influenzae* (23mm).

Stem aqua extract inhibited *S. aureus* (32mm), *B. bronchiseptica* (14mm), *S. flexneri* (32mm), *H. influenzae* (24mm)

Melilotus indica

The leaf methanol extract inhibited *E. coli* (14mm), *S. aureus* (30mm), *B. bronchiseptica* (26mm), *S. flexneri* (32mm), *H. influenzae* (27mm).

Leaf aqua extract inhibited *E. coli* (10mm), *S. aureus*(35mm), *B. bronchiseptica* (23mm), *S. flexneri* (35mm), *H. influenzae* (28mm).

Stem methanol extract inhibited *E. coli* (12mm), *S. aureus* (30mm), *B. bronchiseptica* (23mm), *S. flexneri* (35mm), *H. influenzae* (28mm).

Stem aqua extract inhibited *E. coli* (15mm), *S. aureus* (32mm), *B. bronchiseptica* (19mm), *S. flexneri* (31mm), *H. influenzae* (26mm).

Emblica officinalis

The leaf methanol extract inhibited *E. coli* (10mm), *S. aureus* (30mm), *B. bronchiseptica* (23mm), *S. flexneri* (31mm), *H. influenzae* (21mm).

Leaf aqua extract inhibited *E. coli* (14mm), *S. aureus* (35mm), *B. bronchiseptica* (23mm), *S. flexneri* (40mm), *H. influenzae* (28mm).

Stem methanol extract inhibited *S. aureus* (29mm), *B. bronchiseptica* (22mm), *S. flexneri* (29mm), *H. influenzae* (20mm).

Stem aqua extract inhibited *S. aureus* (32mm), *B. bronchiseptica* (21mm), *S. flexneri* (31mm), *H. influenzae* (26mm).

Ficus virens

The leaf methanol extract inhibited *E. coli* (11mm), *S. aureus* (34mm), *B. bronchiseptica* (29mm), *S. flexneri* (32mm), *H. influenzae* (20mm).

Leaf aqua extract inhibited *E. coli*(14mm), *S. aureus* (39mm), *B. bronchiseptica* (22mm), *S. flexneri* (35mm), *H. influenzae* (24mm).

Stem methanol extract inhibited *S. aureus* (30mm), *B. bronchiseptica* (16mm), *S. flexneri* (30mm), *H. influenzae* (17mm).

Stem aqua extract inhibited *E. coli* (11mm), *S. aureus* (37mm), *B. bronchiseptica* (20mm), *S. flexneri* (33mm), *H. influenzae* (23mm).

Annona squamosa

The leaf methanol extract inhibited *E. coli* (10mm), *S. aureus* (29mm), *B. bronchiseptica* (14mm), *S. flexneri* (36mm), *H. influenzae* (19mm).

Leaf aqua extract inhibited *S. aureus* (34mm), *B. bronchiseptica* (25mm), *S. flexneri* (40mm), *H. influenzae* (21mm).

Stem methanol extract inhibited *S. aureus* (26mm), *B. bronchiseptica* (16mm), *S. flexneri* (32mm), *H. influenzae* (15mm).

Stem aqua extract inhibited *S. aureus* (32mm), *B. bronchiseptica* (24mm), *S. flexneri* (39mm), *H. influenzae* (20mm).

Hemigraphis latebrosa

The leaf aqua extract inhibited *E. coli* (13mm), *S. aureus* (33mm), *B. bronchiseptica* (28mm), *S. flexneri* (34mm), *H. influenzae* (24mm).

Leaf methanol extract inhibited *E. coli* (10mm), *S. aureus* (30mm), *B. bronchiseptica* (20mm), *S. flexneri* (31mm), *H. influenzae* (18mm).

Stem methanol extract inhibited *S. aureus* (28mm), *B. bronchiseptica* (19mm), *S. flexneri* (29mm), *H. influenzae* (14mm).

Stem aqua extract inhibited *E. coli* (09mm), *S. aureus* (32mm), *B. bronchiseptica* (25mm), *S. flexneri* (32mm), *H. influenzae* (18mm).

Cuscuta reflexa

The stem aqua extract inhibited *E. coli* (19mm), *S. aureus* (32mm), *B. bronchiseptica* (25mm), *S. flexneri* (39mm), *H. influenzae* (29mm).

Stem methanol extract inhibited *E. coli* (14mm), *S. aureus* (31mm), *B. bronchiseptica* (19mm), *S. flexneri* (34mm), *H. influenzae* (25mm).

Euphorbia nivulia

The stem methanol extract inhibited *E. coli* (16mm), *S. aureus* (40mm), *B. bronchiseptica* (23mm), *S. flexneri* (29mm), *H. influenzae* (29mm).

Stem aqua extract inhibited *E. coli* (21mm), *S. aureus* (40mm), *B. bronchiseptica* (24mm), *S. flexneri* (31mm), *H. influenzae* (30mm).

Lannea coromandelica

The stem methanol extract inhibited *E. coli* (16mm), *S. aureus* (29mm), *B. bronchiseptica* (17mm), *S. flexneri* (29mm), *H. influenzae* (22mm).

Stem aqua extract inhibited *E. coli* (20mm), *S. aureus* (32mm), *B. bronchiseptica* (25mm), *S. flexneri* (34mm), *H. influenzae* (24mm).

Crataeva nurvala

The stem methanol extract inhibited *S. aureus* (27mm), *B. bronchiseptica* (16mm), *S. flexneri* (31mm), *H. influenzae* (24mm).

Stem aqua extract inhibited *E. coli* (10mm), *S. aureus* (36mm), *B. bronchiseptica* (17mm), *S. flexneri* (34mm), *H. influenzae* (27mm).

Solanum surattense

The leaf methanol extract inhibited *E. coli* (21mm), *S. aureus* (24mm), *S. flexneri* (24mm), *H. influenzae* (21mm).

Leaf aqua extract inhibited *E. coli* (14mm), *S. aureus* (15mm), *B. bronchiseptica* (16mm), *S. flexneri* (18mm), *H. influenzae* (31mm).

Stem methanol extract inhibited *E. coli* (19mm), *S. aureus* (20mm), *B. bronchiseptica* (15mm), *S. flexneri* (16mm), *H. influenzae* (25mm).

Stem aqua extract inhibited *E. coli* (12mm), *B. bronchiseptica* (16mm), *S. flexneri* (16mm), *H. influenzae* (29mm).

Triticum aestivum

The fruit methanol extract inhibited *E. coli* (31mm), *S. aureus* (31mm), *B. bronchiseptica* (25mm), *S. flexneri* (15mm), *H. influenzae* (14mm).

Fruit aqua extract inhibited *E. coli* (30mm), *S. aureus* (31mm), *B. bronchiseptica* (21mm), *S. flexneri* (13mm), *H. influenzae* (28mm).

Leaf methanol extract inhibited *E. coli* (33mm), *S. aureus* (33mm), *B. bronchiseptica* (29mm), *S. flexneri* (17mm), *H. influenzae* (16mm).

Leaf aqua extract inhibited *E. coli* (32mm), *S. aureus* (32mm), *B. bronchiseptica* (23mm), *S. flexneri* (15mm), *H. influenzae* (27mm).

Bacteria inhibited by plant extract:

Among the bacteria studied, *H. influenzae* was the most sensitive being inhibited by all plants taken up for study whereas *E. coli* was the most resistant as it could be inhibited by 13 plants from the total study of 15 plants.

S. aureus and *S. flexneri* were highly sensitive in terms of zone of inhibition among the plant extract taken up for the study.

B. bronchiseptica was highly sensitive to *Triticum aestivum*, *Melilotus indica*, and *Embllica officinalis*.

Results of antibiotics assay:

Among the Eight antibiotic disc used, *S. flexneri* was inhibited by Tetracycline while *H. influenzae* resisted all antibiotic discs. Whereas *E. coli* inhibited by Erythromycin, Tobramycin, and Kanamycin. *B. bronchiseptica* inhibited by Tobramycin and Tetracycline. *S. aureus* also inhibited Tobramycin and Tetracycline.

None of the antibiotics used could inhibit all of the Bacteria whereas *H. influenzae* was most resistant among all Bacteria.

Plants	Bacteria	Zone of inhibition (mm)
<i>A. nilotica</i>	<i>S. aureus</i>	31
<i>V. negundo</i>	<i>S. flexneri</i>	35
<i>L. acidissima</i>	<i>S. flexneri</i>	35
<i>M. indica</i>	<i>S. aureus</i>	35
<i>B. monosperma</i>	<i>S. aureus</i>	34
<i>E. officinalis</i>	<i>S. flexneri</i>	40
<i>F. virens</i>	<i>S. aureus</i>	39

<i>A. squamosa</i>	<i>S flexneri</i>	40
<i>H. latebrosa</i>	<i>S flexneri</i>	34
<i>C. reflexa</i>	<i>S flexneri</i>	39
<i>E. nivulia</i>	<i>S. aureus</i>	40
<i>L. coromandelica</i>	<i>S flexneri</i>	34
<i>C. nurvala</i>	<i>S. aureus</i>	36
<i>S. surattense</i>	<i>H influenzae</i>	31
<i>T. aestivum</i>	<i>E coli</i>	33

CONCLUSION

Mankind is infected with diseases through-out its origin & history on earth. Science has come of age where in *in silico* designing & development of drug is done with successful results. Newer drugs replace the ones against which pathogens have developed resistance. On the other hand newer pathogens charge that need to be & controlled. In this never ending quest, has always found ways to control diseases. But with the technological advancement all the study has been taken up at a higher level. Nevertheless we are in search of primary data of plants that possess antimicrobial properties or further explore the existing herbs for the control of newer diseases.

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