Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2018; 7(6): 528-533
Received: 28-09-2018
Accepted: 30-10-2018

Amita Singh
Department of Biotechnology, RAMA University Kanpur, Uttar Pradesh, India

Dr. Ajay Kumar
Department of Biotechnology, RAMA University Kanpur, Uttar Pradesh, India

Dr. Vivek Srivastava
Department of Biotechnology, RAMA University Kanpur, Uttar Pradesh, India

Correspondence
Amita Singh
Department of Biotechnology, RAMA University Kanpur, Uttar Pradesh, India

Extraction of bioactive compound from different medicinal plants and rhizospheric soil bacteria and their comparative study against human pathogens

Amita Singh, Dr. Ajay Kumar and Dr. Vivek Srivastava

Abstract
*Murraya Koenigii (*Meethi Neem*), Curcuma longa (*Turmeric*), Achyranthes aspera (*Latjira*), Withania Somnifera (*Ashwagandha*) and Syzygium cumini (*Jamun*)* are being widely used in ayurvedic/traditional systems of medicines and source of several bioactive compounds. These plants are also used as culinary items. The present study was conducted to investigate the anti-bacterial effect of these plants against *E. coli* and *P. aeruginosa* are seem to cause many diseases in humans. We also considered the rhizospheric soil bacteria of these medicinal plants. In this project, we have done comparative studies of the metabolite extracted from five different medicinal plants on human pathogen i.e *E. coli* and *P. aeruginosa*. This was done by preparing the methanolic and water extract from dried leaves of these plants through soxhlet extractor and test them against the test micro-organisms through performing Disc assay. Two plants Murraya Koenigii (Meethi Neem) and Curcuma longa (Turmeric) showed good results and their both extracts i.e water and methanol gave no susceptible results against test micro-organisms The second finding was the effect of secondary metabolite extracted from the rhizospheric soil bacteria of same five plants on the test organism i.e *P. aeruginosa* and E. coli. During this total 20 colonies were isolated and out of 20 only 10 gave good result based on well diffusion test. Thus, this whole study revealed the antimicrobial effect of the metabolite and rhizospheric soil bacteria from these plants and so can be used to effectively to cure the disease caused by *E. coli* and *P. aeruginosa*.

Keywords: *Murraya koenigii*, *Curcuma longa*, *Achyranthes aspera*, *withania somnifera Syzygium cumini*, rhizospheric soil

Introduction

Medicinal Plants

Plants produce a diverse range of bioactive molecules, making them rich sources of different types of medicine. Most of the drugs today are obtained from natural sources or semi synthetic derivatives of natural products and used in the traditional systems of medicine. Thus it is a logical approach in drug discovery to screen traditional natural products. Approximately 20% of the plants found in the world have been submitted to pharmaceutical or biological test and a sustainable number of new antibiotics introduced on the market are obtained from natural or semi synthetic resources (Shariff N 2006).

Antibiotics are (Greek anti-against, and Bios life) microbial products or their derivatives that can kill susceptible microorganisms or inhibit their growth. Antibiotics are special kind of chemotherapeutic agent usually obtained from living organisms. Chemical agents, chemotherapeutic that are used to treat disease drug that disrupt a microbial function. The pharmaceutical industry has become increasingly interested in screening fungi for novel antibiotics and other secondary metabolites. Historical discovery novel compounds from fungi usually resulted from accidental finds or screening of ubiquitous fungi.

Considering the number of novel bioactive compounds that have been isolated from endophytic fungi and the fact that the number of the compound is increasing rapidly. (Rajasekar and Balaji, *et al* 2012). Secondary metabolites is a prerequisite for the development of novel pharmaceuticals, and this is an especially urgent task in the case of antibiotics due to the rapid spreading of bacterial resistance and the emergence of multi resistant pathogenic strains, which severe clinical problems in the treatment of infectious disease. The thematic series of on the biosynthesis and function of secondary metabolites deals with the discovery of new biologically active compounds from all kinds of source, including plants, bacteria and fungi and also with their biogenesis. Biosynthetic aspects are closely related to functional investigation, because the deep understanding of metabolic pathway of natural products. New secondary metabolites available from microorganisms may be used to optimize their

availability by fermentation for further research and also for production in the pharmaceutical industry

Material and Method

In this study we have used five medicinal plants and two test organisms that are *E. coli* and *P. aeruginosa*.

1. Murraya Koenigii (Meethi Neem) plant.
2. Curcuma longa (Turmeric)
3. *Achyranthes* Aspera9Latjeera) plant
4. Withania Somnifera (Ashwagandha) plant
5. *Syzygium cumini* (Jammu) plant

Extraction of bioactive compound from plants sample:

Sample collection and processing

Plant leaves were collected from different places. Leaves are kept in sun for drying. 2gm of these dried leaves were taken and extract was prepared in soxhlet extractor. Water and methanol was used as solvents thus total five water and five methanol extract were prepared from the collected five plant samples i.e. Murraya Koenigii (Meethi Neem) plant, Curcuma longa (Turmeric), *Achyranthes* Aspera (Latjira) plant, Withania Somnifera (Ashwagandha) plant, *Syzygium cumini* (Jammu) plant.

Fig 1: Dried and crushed leaves of turmeric

Antimicrobial extract against test organism

Antimicrobial properties of each extract (water and methanol) from the sample plants are tested on the test pathogens i.e. *E. coli* and *P. aeruginosa* by Disc Diffusion method [3].

Disc diffusion Method

Kirby–Bauer antibiotic testing (KB testing or disc diffusion antibiotic sensitivity testing) is a test which uses antibiotic-impregnated wafers to test whether bacteria are affected by antibiotic [4].

Extraction of secondary metabolite from rhizospheric soil sample

Well diffusion Method

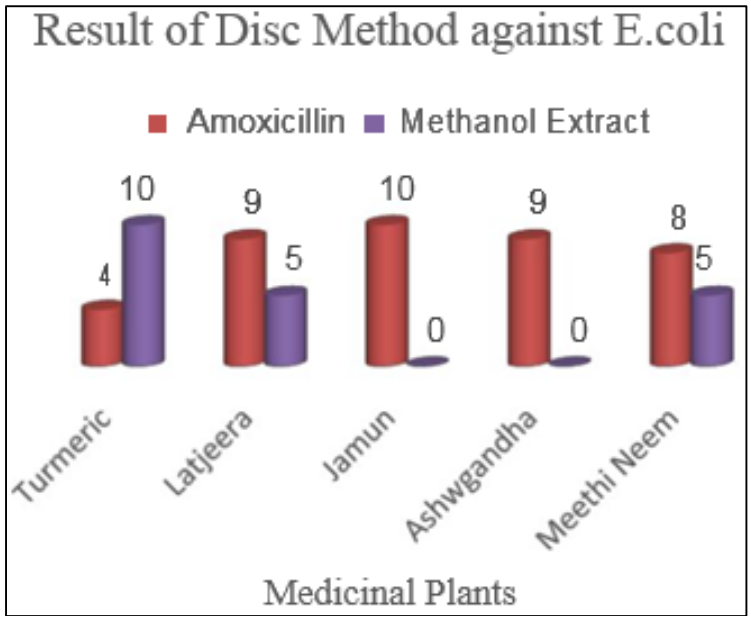
This diffusion was the basis of the agar diffusion assay devised in 1944 [11]. Effectiveness of each metabolite are tested against the test organism i.e. *P. aeruginosa* and E.coli. The well diffusion method consist of Agar medium that is prepared and solidified in a petri plates. Test organism suspension is spread onto the surface of the agar. Then, extracted metabolite is applied to a number of wells in the plate.

Plant Extract

Two types of plant extracts were prepared and tested against test micro-organisms [9]. The results of both plant extracts are given below:

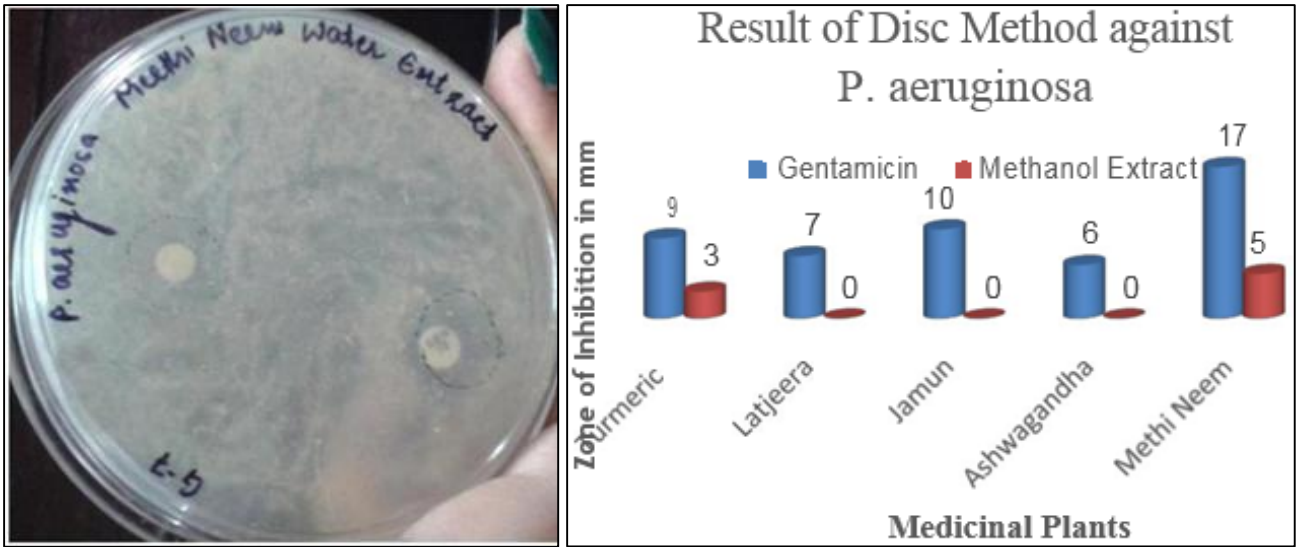
Methanol Extract

Result



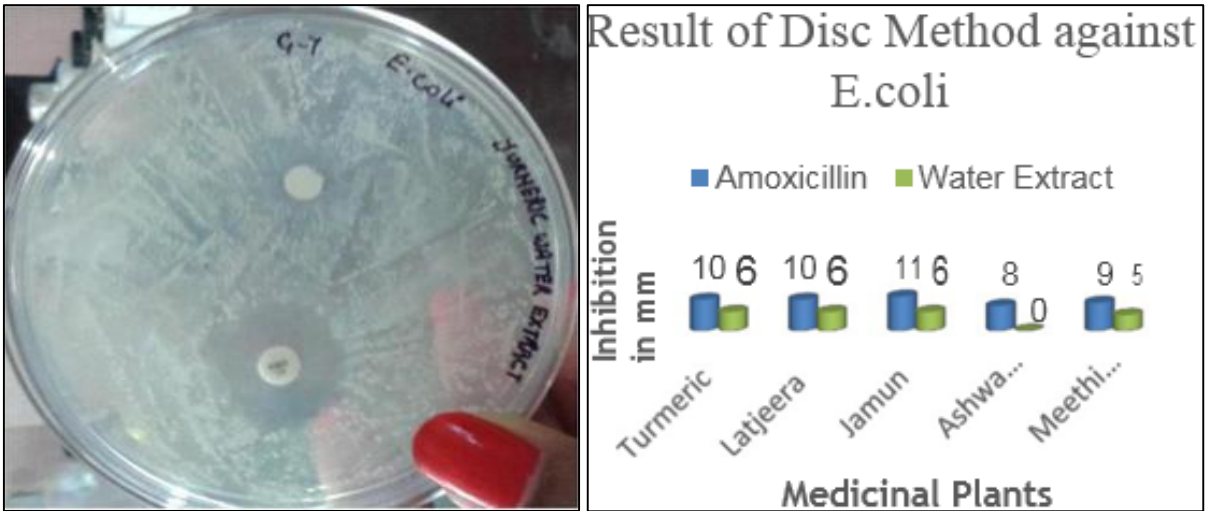
Disc method of Turmeric methanol extract

Result for *P. aeruginosa*



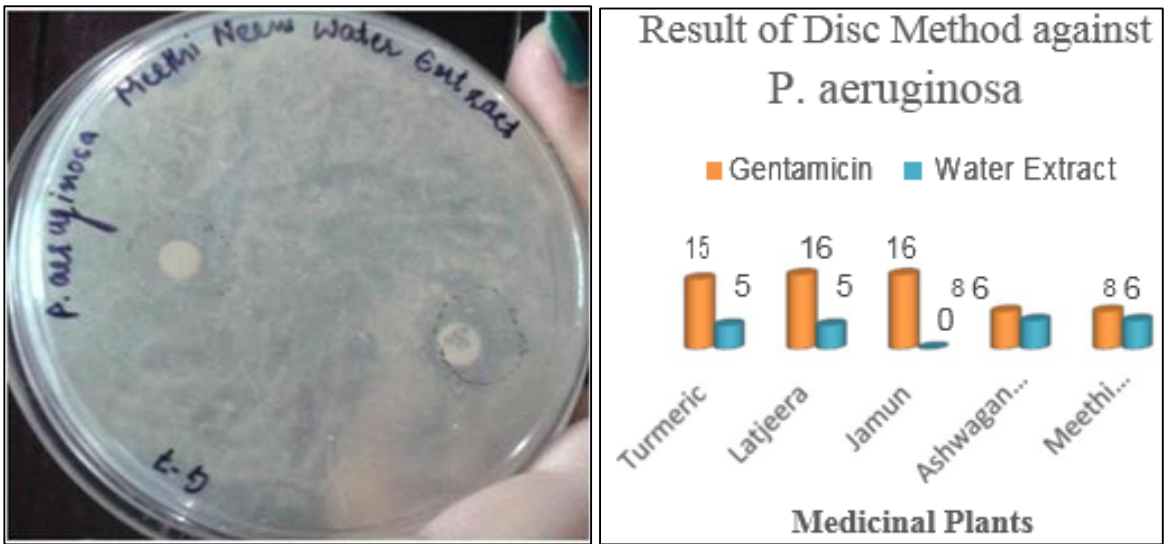
Disc method of Meethi Neem methanol extract

Water Extract:
Result for *E. coli*



Disc method of Turmeric water extract

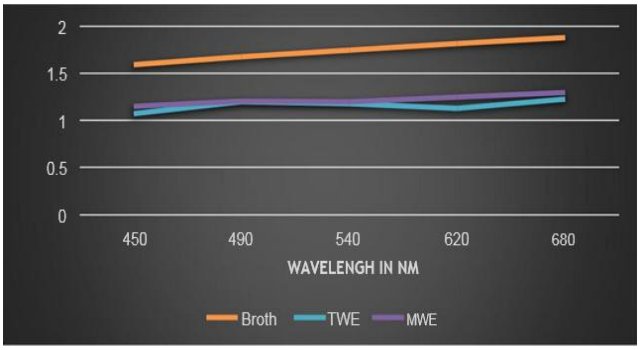
Result for *P. aeruginosa*



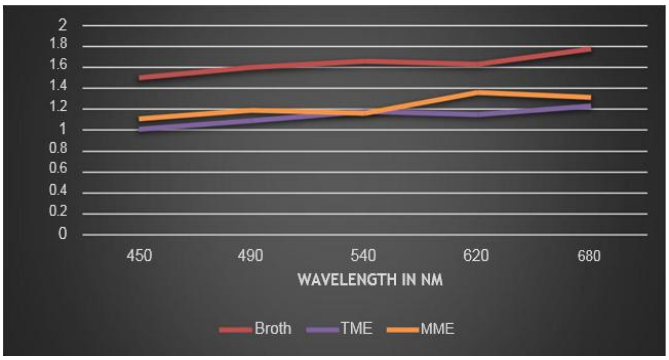
Disc method of Meethi Neem water extract

Effectiveness of Plant Extracts

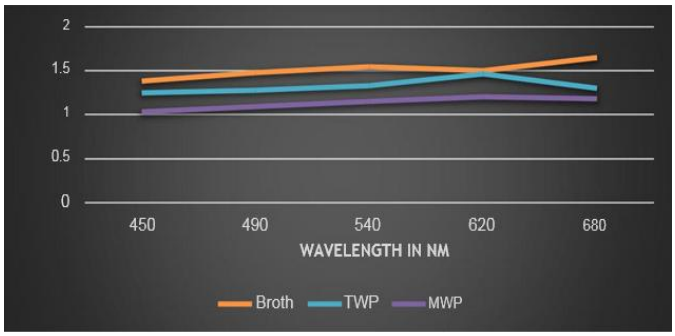
After analyzing the above result we find that extracts of turmeric and Meethi neem doesn't show any susceptible result, so we took their extracts further for secondary screening. The results are given below:



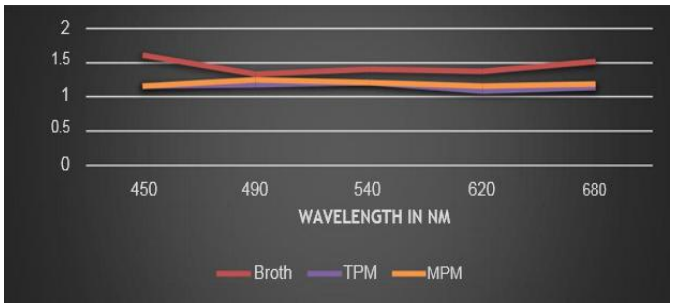
Graph 1: Effectiveness of water extract against *E. coli*



Graph 2: Effectiveness of methanol extract against *E. coli*



Graph 3: Effectiveness of water extract against *P. aeruginosa*



Graph 4: Effectiveness of methanol extract against *P. aeruginosa*

Rhizospheric Soil Bacteria

20 colonies were obtained from all the 5 plants. Then well diffusion test was conducted against test micro-organisms for all 20 colonies. Tables showing results of well diffusion test are given below:

Result for *E. coli*

Colonies name	Zone of inhibition in mm (Supernatant)	Zone of inhibition in mm (Pellet)
Latjeera-1	No Visible Zone	15
Latjeera-2	14	No Visible Zone
Latjeera-3	No Visible Zone	11
MeethiNeem-1	16	9
MeethiNeem-2	No Visible Zone	No Visible Zone
MeethiNeem-3	11	10
MeethiNeem-4	6	8
MeethiNeem-5	7	No Visible Zone
MeethiNeem-6	9	10
Ashwagandha-1	No Visible Zone	No Visible Zone
Ashwagandha-2	No Visible Zone	No Visible Zone
Ashwagandha-3	No Visible Zone	No Visible Zone
Turmeric-1	No Visible Zone	10
Turmeric-2	No Visible Zone	7
Turmeric-3	No Visible Zone	6
Turmeric-4	No Visible Zone	10
Jamun-1	6	7
Jamun-2	No Visible Zone	5
Jamun-3	8	7
Jamun-4	5	11

Fig 2: Result of well diffusion method against *E. coli*

Result for *P. aeruginosa*

Colonies name	Zone of inhibition in mm (Supernatant)	Zone of inhibition in mm (Pellet)
Latjeera-1	No Visible Zone	No Visible Zone
Latjeera-2	No Visible Zone	No Visible Zone
Latjeera-3	No Visible Zone	No Visible Zone
MeethiNeem-1	No Visible Zone	12
MeethiNeem-2	No Visible Zone	14
MeethiNeem-3	No Visible Zone	No Visible Zone
MeethiNeem-4	No Visible Zone	14
MeethiNeem-5	No Visible Zone	No Visible Zone
MeethiNeem-6	No Visible Zone	No Visible Zone
Ashwagandha-1	No Visible Zone	No Visible Zone
Ashwagandha-2	No Visible Zone	No Visible Zone
Ashwagandha-3	No Visible Zone	11
Turmeric-1	8	No Visible Zone
Turmeric-2	7	No Visible Zone
Turmeric-3	4	5
Turmeric-4	No Visible Zone	5
Jamun-1	No Visible Zone	No Visible Zone
Jamun-2	No Visible Zone	No Visible Zone
Jamun-3	No Visible Zone	No Visible Zone
Jamun-4	No Visible Zone	No Visible Zone

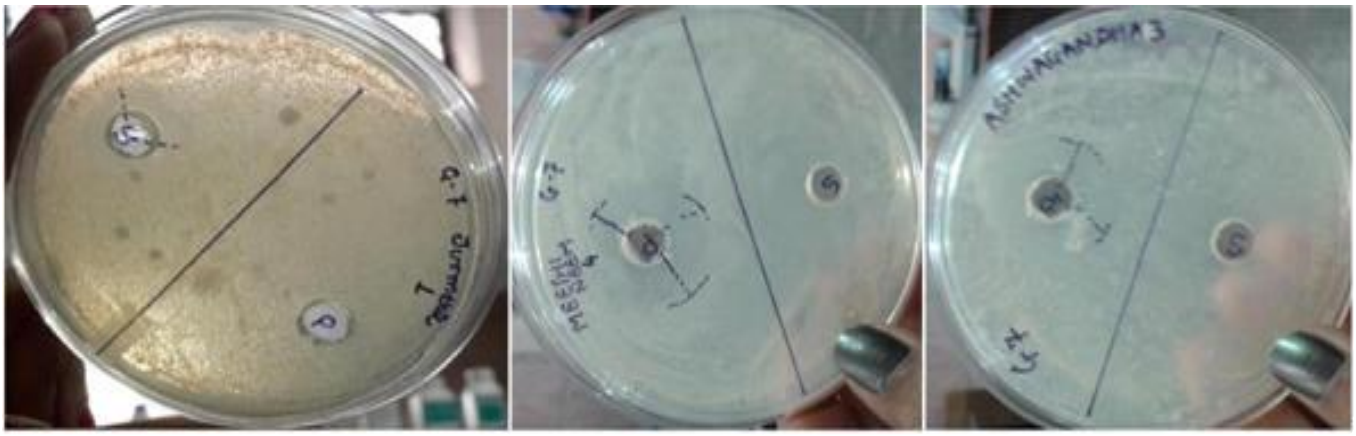


Fig 3: Result of well diffusion test against *P. aeruginosa*

Conclusion

Positive results were given by water extract of all plants and among the methanol extract except ashwagandha and jamun all gave positive result. Extract of turmeric and Meethi neem showed good result against test-organism i.e. *E. coli* and *P. aeruginosa* so they could be possibly used as a source of new drug or they can be used as a nutraceuticals to confer resistance against *E. coli* and *P. aeruginosa*. Rhizospheric soil samples from these five plants were taken and after spreading twenty different colonies were identified and slants were prepared. Secondary metabolites were extracted from these rhizospheric soil samples. All of them showed positive result against test organism E. coli, *P. aeruginosa* and thus they can be used to produce antibiotics.

Based on the findings of this study the following recommendations are suggested:

- The extracts of these plants should be further analyzed to isolate the specific antibacterial principles in them.
- Research on the effectiveness of other parts of the plant as the roots or flowers, etc.
- Toxicity studies of the effective plants should also be done to determine the safety indices of the extracts.

Clinical trials should be carried out to explore the potential of these plant extracts in the treatment of these infectious diseases.

- Determine the activity of these plant extracts on the types of human pathogens, in addition to the synergistic activity of these medicinal plants with antibiotics.
- Strains of isolated microbes can be studied to know there characteristics and morphology and hence can help in future research.
- The strains of microbes can be studied and improved through molecular biology and bioinformatics and thus they can prove to be a better source for new drug.

References

1. Mulligen ME, Murry-Leisure KA, Ribner BS, Standiford HC, John JF, Karvick JA, Kauffman CA, *et al.* Methicillin resistant Staphylococcus aureus. American Journal of Medicine. 1993; 94:313-328.
2. Nagenawa R, Twata N, Ishikawa K, Suzuki A. Inhibition of microbial growth by ajoene, a sulfur compound derived from garlic. Applied and Environmental Microbiology. 1996; 62(11):4238-4242.
3. Nimri LF, Meqdam MM, Alkofahi A. Antibacterial activity of Jordanian medicinal plants. Pharmaceutical Biology. 1999; 37(3):196-201.
4. Odebiyi A, Sofowora AE. Phytochemical screening of Nigerian medicinal plants. Part III. Lloydia. 1990; 41:234-246.
5. Perez C, Pauli M, Bazerque P. An antibiotic assay by the well agar method. Acta Biologiaeet Medicine Experimentalis. 1990; 15:113-115.
6. Piddock KJV, Wise R. Mechanisms of resistance to quinolones and clinical perspective. Journal of Antimicrobial Chemotherapy. 1989; 23:475-483.
7. Ratnakar P, Murthy PS. Purification and mechanisms of action of antitubercular principle from garlic (*Allium sati*6*um*) active against isoniazid susceptible and resistant Mycobacterium tuberculaeH37RV. Indian Journal of Clinical Biochemistry. 1995; 10:14-18.
8. Rajalakshmi S, Mahesh N. Production and Characterization of Bioactive Metabolites Isolated from Aspergillusterreus in Rhizosphere Soil of Medicinal Plants Department of Chemistry and Biosciences, SASTRA University, Kumbakonam Campus, Tamil Nadu, India.
9. Umesh Kumar, Hindumathy CK. Study of antibacterial activity of *Rosa indica* against gram positive and gram negative bacteria Department of Biotechnology, VMKV Engineering College, Vinayaka.
10. Iqbal Ahmad, Arina Z. Antimicrobial and phytochemical studies on 45 Indian medicinal plants against multi-drug resistant human pathogens. Beg Department of Agricultural Microbiology, RAK Institute of Agricultural Sciences, Aligarh Muslim University, Aligarh 202002, India Received. February 2000; received in revised form 10 August, 2000.
11. Bishnu Joshi, Govind Prasad Sah, Buddha Bahadur Basnet, Megh Raj Bhatt, Dinita Sharma, Krishna Subedi, Janardhan Pandey, *et al.* Phytochemical extraction and antimicrobial properties of different medicinal plants*: Ocimum sanctum* (Tulsi), *Eugenia caryophyllata* (Clove), *Achyranthes bidentata* (Datiwan) and *Azadirachta indica* (Neem) Central Department of Biotechnology, Tribhuvan University, Nepal. Accepted 22 October, 2010
12. Fernandes Junior A. Biological properties of medicinal plants: a review of their antimicrobial activity. Silva NCC (1), (1) Department of Microbiology and Immunology, Botucatu Biosciences Institute, São Paulo State University (UNESP – Univ Estadual Paulista), Botucatu, São Paulo State, Brazil.
13. Ibtisam Mohammed Ababutain. Antimicrobial Activity of Ethanolic Extracts From Some Medicinal Plant Department of Biology, Faculty of Science University of Dammam, P.O. Box 838, Dammam-31113, Saudi Arabia.
14. Mahesh, Satish. Antimicrobial Activity of Some Important Medicinal Plant Against Plant and Human Pathogen 1Department of Studies in Applied Botany and Biotechnology, University of Mysore, Manasagangotri, Mysore-570 0062. Herbal Drug Technology Laboratory, Department of Studies in Microbiology, University of Mysore, Manasagangotri, Mysore-570