IDENTIFYING ANTIMICROBIAL POTENTIAL OF PHYTOMOLECULES ISOLATED FROM *MORINGA OLEIFERA* LAM. (MORINGACEAE)

A THESIS

Submitted for the Award of the Degree of

DOCTOR OF PHILOSOPHY

in

BOTANY

By

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Under the Supervision

of

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CERTIFICATE

This is to certify that the thesis entitled **"Identifying Antimicrobial Potential of Phytomolecules Isolated from *Moringa oleifera* Lam. (Moringaceae)"** submitted to Department of Botany, Maharishi University of information Technology, Lucknow, Uttar Pradesh, in the fulfillment of the requirement for the award of degree of Doctor of Philosophy in Botany, embodies the original research work carried out by **Mr. Anoop Kumar Tiwari**, under my supervision and has not been submitted in part or full for any degree or diploma of this or any other University. It is further certified that scholar fulfills all the requirements as per the ordinance of the University for the purpose of submission of Ph.D. thesis.

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DECLARATION

I hereby, declare that the work presented in this thesis entitled **"Identifying Antimicrobial Potential of Phytomolecules Isolated from *Moringa oleifera* Lam. (Moringaceae)"** in fulfillment of the requirements for the award of **'Degree of Doctor of Philosophy'** of Maharishi University of Information Technology, Lucknow, Uttar Pradesh, is an authentic record of my own research work carried out under the supervision of **Dr. Madhu Prakash Srivastava**, Department of Botany, Maharishi University of Information Technology, Lucknow.

I also declare that the work embodied in the present thesis is my original work and has not been submitted by me for any other Degree or Diploma of any University or Institution.

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Place:

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MAHARISHI UNIVERSITY OF INFORMATION TECHNOLOGY, LUCKNOW
(Established Vide Uttar Pradesh Act No.: 31 of 2001)

Ref No. .....

Date : .....

VIVA VOCE CERTIFICATE

This is to certify that the research work embodied in this thesis entitled **"Identifying antimicrobial potential of phytomolecules isolated from *Moringaoleifera* Lam. (Moringaceae)"** in fulfillment of ther requirements for the award of Degree of Doctor of Philosophy of Maharishi University of Information Technology Lucknow Uttar Pradesh has been approved after an oral examination of the same in collaboration with an external examiner.

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ACKNOWLEDGEMENT

First of all, I would like to express my profound sense of gratitude to my respected supervisor **Dr. Madhu Prakash Srivastava** for their valuable guidance, keen interest, constructive counseling and critical appreciation throughout the course of present investigation. I am thankful to Almighty for providing me a mentor like them.

I convey my deepest gratitude to **Prof. Bhanu pratap Singh,** Vice-Chancellor, Maharishi University of Information Technology, Lucknow for providing the necessary Laboratory facilities to work, Sir! I am very thankful to you for your constant support, encouragement and supreme guidance.

I would also extend my deepest gratitude towards **Prof. Akhand Pratap Singh,** Maharishi University of Information Technology Lucknow, for being an excellent administrator and teacher.

I also express my gratitude to **Mr. Girish Chhimwal**, Deputy Registrar, Maharishi University of Information Technology, Lucknow, Whose affectionate behavior has supported me through the course of this investigation.

Words are less to extend my highest gratitude of deep sense of thanks to **Dr. Sandhya Sinha,** Dean Research, Maharishi University of Information Technology Lucknow, for always extending her unconditional support and encouragement during my journey of doctoral research

My special thanks to **Mr. Rohit Dixit**, Agra; **Dr. Rajeev Sharma**, Assist. Prof., Govt. College, Banmore (MP) and **Dr. Mahesh Chandra**, Assist. Prof., P.C. Bagla PG College, Hathras (UP) for their moral support, pensive suggestions, inspiration and help throughout the work.

Most importantly none of this would have been possible without the love and patience of my family. I can't imagine my current position without the

love and support from my family. I very much thank my Father **Dr. Ram Prakash Tiwari** for striving hard to provide a good education for me. I must sincerely thanks **Mrs. Anuradha Tiwari** my betterhalf for her patience, understanding and magnificent devotion to our family and my sweet ever smiling daughters **Tanya, Riddhi** and **Siddhi** and son **Tejas** for making everything worthwhile.

Last, but not the least, I thank to Invincible **God Almighty** by whose grace and blessings I find myself in a position of putting forth this presentation.

(Anoop Kumar Tiwari)

CONTENTS

Introduction

Chapter- 1 INTRODUCTION

Microbial infections are widespread throughout the world making situation is worse, especially in the third world countries. Many of the currently used antimicrobial drugs are show adverse effects such as hypersensitivity, toxicity and immuno-suppression creating public health hazard. Beside this, the newer broad spectrum antibiotics are costly therefore not reachable poor. The existence of potent antibiotic and antifungal agent resistant or multi-resistant strains are continuously appearing, imposing the need for a permanent search and development of new drugs (Silver, 1993). Above disadvantages reduces the therapeutic utility of the currently used antimicrobial drugs therefore necessitating the need for finding alternative treatment remedies for diseases.

There has been an exponential growth in the field of herbal medicine during last few decades. It is getting popularized in developing and developed countries owing to its natural origin and lesser side effects (Brahmachari, 2001). Herbal drugs plays vital role in recognized health systems of in India that are Ayurveda, Yoga, Unani, Siddha, Homeopathy and Naturopathy. More than 70% of India's 1.1 billion populations still use these non-allopathic systems of medicine (Vaidya, 2007)., large proportion of the population depends on traditional practitioners and their knowledge of medicinal plants in order to meet health care needs in many

developing countries. Traditional practice of herbal medicines has often maintained their popularity for historical and cultural reasons even in the presence of modern medicines. Use of herbal medicines in developed countries has expanded sharply and demand of such products have raised, especially in developed countries in the latter half of the twentieth century. In India, herbal drugs are an integral part of the Indian System of Medicine (Ayurveda) which is an ancient and mainstream system (Rai, 2005).

A number of higher plants have been used for centuries as remedies for human diseases. This has encouraged scientists to screen higher plants for various biological activities including antibacterial and antifungal effects (Eilert *et al.,* 1980; 1981; Omer and Elnima, 2003; Saadabi, 2006; Saadabi *et al.,* 2006; 2007; 2009). The acceptance of traditional medicine as an alternate form of health care and the development of microbial resistance to the available antibiotics has led researches to investigate the antimicrobial activity of medicinal plants (Hemmer *et al.,* 1999).

Plants plays vital source of natural products for human health for a long time. The antimicrobial properties of plants have been investigated by number of studies world-wide and many of them have been used in the therapeutic alternative because of their antimicrobial properties (Adriana *et al.,* 2007). The practice of complementary and alternative medicine is

now on the increasing in developing countries in response to World Health Organization directives culminating in several pre-clinical and clinical studies that had provided the scientific basis for the efficiency of many plants used in folk medicine to treat infections (Vijaya and Ananthan, 1997; Dilhuydy and Patients, 2003).

Plants having Medicinal potential are heritage for local populations with global importance. Our earth is endowed with a rich source of plant with medicinal potential. In India principal form of medicine used is Herbs and their popularity is rising throughout the developing world. As people in the present world want to stay healthy to face the chronic stress and pollution and to treat illness with medicines that work in concern with the body's own defenses (Prajapati *et al.*, 2003).

Many antimicrobial agents have been obtained from medicinal plants. Plants are used medicinally in different countries as a source of many potent and powerful drugs (Srivastava *et al*., 1996). As they contain components of therapeutic values so they have been used for centuries as remedy for human disease because. Extract of fruit and vegetables are used for the treatment of diseases and for maintain health. Through numerous studies, many components have been identified with in herbal plants that are effective antibiotic. Plants are rich source of biochemical agents since time immemorial. An impressive number of drugs have been

isolated from natural products of plants based upon their use in traditional medicines (Hemmer *et al.,* 1999).

Till now, more than lac of biologically active phytomolecules have been isolated from higher plants, with most of these phytomolecules are classified in four main classes that are phenolics (flavonoids, Phenols, tannins, lignins and quinines), nitrogen compounds (alkaloids, amines, cyanogenic glycosides and non-protein amino acids), sulphur compounds and triterpanoids (monoterpenes, lactones, diterpenes, sesquiterpene, saponins and others). In the present time, research into the effect of local medicinal plants against diseases caused by the pathogens is required. Moreover, most of these plants used in folk medicine have not been screened for their antimicrobial activity (Kubmarawa *et al.,* 2007). Phytochemical studies have attracted the attention of plant scientists clue to the development of new and sophisticated techniques. In the search for additional resources of raw material for pharmaceutical industry these experiment played a significant role in providing solution to systematic problems and On the other hand, plant synthesizes a wide variety of chemical compounds, which can be sorted out by their chemical class, biosynthetic origin and functional groups into primary and secondary metabolites (Mukherjee and Laloraya, 1977).

By analyzing different plant products used traditionally for treating disease and having medicinal value based on their therapeutic efficacy

can act as source for discovery of newer and recent drugs for treating various ailments. Above idea forms the basis concept for the discovery of new drugs from various plant sources. Plants are cheapest and safe alternative source of antimicrobials (Pretorious and Watt, 2001; Sharif and Banik, 2006; Doughari *et al.,* 2007).

The antimicrobial research based on ethno-pharmacological information is generally considered an effective approach in the discovery of new anti-infective agents from higher plants (Deshmarchelier and Schans, 2000). Routine experiments are performed in laboratories to screen phtomolecules having antimicrobial potential. The search for components with antimicrobial activity has gained increasing importance in recent times due to growing worldwide concern about the alarming increase in the rate of infection by antibiotic resistant micro-organisms (Davies, 1994). Attentions have been given to the medicinal value of herbal remedies for safety, efficacy and economy (Mahabir and Gulliford, 1997).

Moringa olifera commonly known as 'Shajna' in Hindi and Horse radish in English is one of such plants having medicinal value. It belongs to family Moringaceae. It is a fast growing deciduous or evergreen tree that reaches height 10 to 12 m. It is distributed among Sub Himalayan tracts, Assam, Bengal and Peninsular India (Gupta, 2010). Various

properties are attributed to it like antispasmodic, diuretic, expectorant and abortifacient (Nadkarni, 2009).

In historical review *M. oleifera* tree is described as a 'Miracle tree' because every part of this tree has products useful for human. Plant parts like pods and leaves are eaten. The plant is also reported to be medicinally important and almost all parts of the *M. oleifera*tree are considered to possess medicinal properties and are used in the treatment of ascites, rheumatism and venomous bites and as cardiac and circulatory stimulant (Shindano and Kasase, 2009). Leaves are also known to have anti-oxidant properties and are known to cures hallucinations, dry tumors, hiccups and asthma (Mehta and Agrawal, 2008). For the treatment eye diseases, dyspepsia, inflammation, heart complaints, and enlargement of spleen root and bark is used. For curing cure inflammations and muscle diseases flowers are used. In treatment of leprous ulcers seed oil is known to be useful (Fahey 2005).

Moringa oleifera (Drumstick) is one such plant which is reported to possess several medicinal properties. The different parts of this plant viz., leaves, bark (stem, root) flowers, fruits and seeds are used in the indigenous systems of medicine for the treatment of variety of human ailments and some parts are also eaten as vegetable (Chopra *et al.,* 1956; Nadkarni *et al*., 1976). In last few years lot of have been done to explore pharmacological potential of the leaves and seeds of *Moringa oleifera* on

scientific lines. But limited data is available about antimicrobial potential of *Moringa oleifera* leaves and bark. Therefore, it was considered worthy to investigate the antimicrobial potential of *Moringa oleifera* leaves & bark.

Objectives

Chapter- 2

OBJECTIVES

On analysis of literature *Moringa oleifera* Lam is selected to search active phytomolecule. Following experimental design used for their effective application in traditional medicine with the following objectives to analysis antimicrobial potential of its leaves and bark extracts:

1. Isolation and identification of phytomolecules present different extracts of *Moringaoleifera Lam. (Moringaceae)*
2. To investigate antibacterial activity of different extracts of *Moringaoleifera Lam. (Moringaceae)* using disc diffusion method.
3. To investigate antifungal activity of different extracts of *Moringaoleifera Lam. (Moringaceae)* using disc diffusion method.
4. To determine the MIC (Minimum Inhibitory Concentration) of different extracts *Moringaoleifera Lam. (Moringaceae)* against different microorganism by broth dilution method.

Review of Literature

Chapter- 3 REVIEW OF LITERATURE

The present literature is divided into four parts. The first part deals with the development of antibiotic resistance in microbes. Second parts deals with herbal extracts as an alternate regime to combat resistance microbes. Third deals with the literature on antimicrobial activity of *Moringa oleifera*. While fourth part deals with phytochemical analysis of *M. oleifera* extracts.

DEVELOPMENT OF ANTIBIOTIC RESISTANTANCE IN MICROBES OF CLINICAL IMPORTANCE:

According to their view point different microbiologist define the term antimicrobial resistance in different ways. *Sensitive* organisms are those that do not show any resistance against conferring factor, while in clinical words we can say that bacteria that can be controlled by therapeutically achievable levels of the agent. *Resistance* level is that which affects therapeutic used. Therefore an organism is described as *sensitive* if the pharmacologically suited level of antibiotic is adequate to inhibit or destroy the pathogen. Clinically, the sensitivity of bacterium is described in terms of the minimum inhibitory concentration (MIC), a term that was given during pioneering studies in chemotherapy. This methodology has withstood the test of time (Struelens, 2003).

At present many antibacterial agents are used in treating infections caused by bacterial. However, widespread and indiscriminate use of antibacterial agents resulted in development of drug resistance among many virulently pathogenic bacteria species (Berkowitz, 1995).

The development of bacteria is listed at first position among the microorganisms causing opportunistic diseases (Kone *et al.,* 2004). The antibiotic was highly successful in the initial lag phase which is followed by steady, often rapid, rise in resistance levels plateauing to equilibrium depending on the organism, its ability to circulate and antibiotic pressure (Seppala *et al.,* 1997; Austin and Anderson, 1999). Newer antibiotics come with a higher cost, implementing hospital practices to control spread of resistant bacteria and investigation of outbreaks add to the cost of health care (Plowman *et al.,* 2001).

The emergence of multi-drug resistant bacterial strains throughout the global limits the effectiveness of current drugs and significantly limits treatment, leading to prolonged infections (Hancock, 2005). The problem is even more evident in bacterial infections which contribute most of the global infectious disease burden such as diarrheal, respiratory tract, meningitis, sexually transmitted infections and tuberculosis (WHO, 2002). Infectious diseases continue to be a leading cause of mortality the world over and more in developing countries which have poorly accessed health services (World Health Report, 2007). From the studies it was

observed that the bacteria causing *sexually transmitted diseases for* example *Neisseria gonorrhoeae* there is a change in the antibiotic susceptibility showing Penicillin resistance and fluoroquinolone resistance. Alternate drugs like azithromycin and the third generation cephalosporins have to be used (Ray *et al.,* 2006; Khaki *et al.,* 2007). In recent years there has been a rapid rise in multidrug resistance by *S. typhi* all over the world (Chin *et al.,* 2002; Benoit *et al*., 2003; Abdullah *et al.,* 2005).

At present, most clinical isolates of *S. aureus* are multiple drug resistant (resistant to three or more of agents such as ciprofloxacin, erythromycin, clindamycin) (Styers *et al.*, 2006; Adegoke and Komolafe, 2008a; 2008b; 2009). *Eschericha coli, Pseudomonas Spp.* and *Klebsiella Spp.* were implicated in drug resistant bacterial septicaemia in Ile-Ife, Nigeria (Komolafe and Adegoke, 2008). The two probable Likewise coagulase negative staphylococci have acquired multiple resistances and become important nosocomial pathogens (Singhal *et al.,* 2006).

Antibacterial resistance in *S. pneumoniae* has now become a global phenomenon, particularly in India's neighbourhood (JaeHoonSong *et al.,* 2004). Except for a high degree of resistance to cotrimoxazole, the Indian and Nepalese strains have retained their sensitivity to the penicillins, macrolides and fluoroquinolones (Lalitha, 2008).

The rapid emergence of resistance to antibiotics amongst pathogens generates visions of the 'potential post-antibiotic era threatening present and future medical advances (Wise, 2008). Beside the medical problems of antibiotic resistance, it has bigger social impact. In the absence of a Central Monitoring Agency, the national scenes in exception are *M. tuberculosis* and *Leishmania donovani*. The former has been studied consistently by the Tuberculosis Research Centre, Chennai, National Tuberculosis Institute, Bangalore and National JALMA Research Institute, Agra (Paramasivam, 1998). *L. donovani* has reemerged in a limited geographic area and the intense interest has documented the evolution of drug resistance in the pathogen (Jha, 2006). Spreading of resistance factors into environmental gram negative bacteria leads in emergence of multidrug resistant bacteria. Only the recently introduced artimesine derived drugs are uniformly effective (Mohanty *et al.,* 2006).

V. cholerae shows resistance to many of antimicrobials used and its spectrum of resistance varies in differently in local population. Due to the the reason it is compulsory to understand the local pattern of antimicrobials to be used in treatment. (Sharma *et al.,* 2007) in his study surveyed the area around Delhi he noticed extensive resistance to furazolidone, cotrimoxazole and nalidixic acid while tetracycline remained effective. Most tertiary care hospitals are faced with extensive resistance problems in *E. coli* and *Klebsiellae* (Rodrigues *et al.,* 2004;

Shukla *et al.,* 2004; Kumar *et al*., 2006; Gupta *et al.,* 2007; Jain, 2007; Wattal, 2008).

Development of resistant in *M. tuberculosis* to successively introduced antibacterial agents and combined resistance to isoniazid and rifampicin (multi-drug resistance) strongly impacts to control its spread in population. This reason there is a steady rise in such strains observed during treatment of patients (Jesudasan *et al.,* 2003; Ramachandran and Narayanan, 2008).

HERBAL EXTRACT AS AN ALERNATIVE REGIME TO COMBAT RESISTANT MICROBES:

Due to the increase in the resistance of bacterial isolates it is important to search alternatives to available antibiotics for managing control of disease in population. This has necessitated has the requirement of second and third line drug (Williams, 1998).

Many of the antibacterial drugs currently used are associated with side effects such as toxicity, immuno-suppression and hypersensitivity leading in hazardous impact on public health. Beside this the new broad spectrum antibiotics are costly and are not reachable to poor patients. These disadvantages undermine the therapeutic utility of the currently available antibacterial and thus necessitating the need for finding alternative remedies for treatment of bacterial diseases (Chopra *et al.*, 1956).

As the global scenario is now changing towards the use of non-toxic and eco-friendly products, development of modern drugs from traditional medicinal plants should be emphasized for the control of various human and animal diseases (Nadkarni and Nadkarni, 1976).

Last few decades observed an exponential growth in use of herbal medicine. It is getting popularized in developing and developed countries owing to its natural origin and lesser side effects (Brahmachari, 2001).

Due to the increased resistance of many microorganisms towards the currently available commercial antibiotics, investigation of the chemical compounds in medicinal plants has become desirable (Yasunaka *et al*., 2005). Drug discovery from medicinal plants continues to provide new and important leads against various pharmacological targets including cancer, HIV/AIDS, Alzheimer's, malaria, infections and pain (Balunas and Kinghorn, 2005).

Plants are cheapest and safe alternatives source of antimicrobials (Pretorious and Watt, 2001; Sharif and Banik, 2006; Doughari *et al.,* 2007). As conventional antityphoid agents became ineffective in treatment of typhoid infections this is the reason that herbal formulations are gaining popularity among both rural and urban populations for curing the disease. Besides, the medicinal plants are said to have minor side effects compared to the chemical agents (Maghrani *et al.,* 2005). From officially recognized systems of health in India it was observed that

Herbal drugs constitute a major share of all forms of treatment used such Ayurveda, Yoga, Unani, Siddha, Homeopathy and Naturopathy, except Allopathy. More than 70% of India's 1.1 billion populations still use these non-allopathic systems of medicine (Vaidya and Devasagay, 2007). The reasons behind above fact are reduction discovery of new antibacterial molecules, an increase in antimicrobial resistance against exist antimicrobial molecules and there is demand of antimicrobials for treating emerging pathogens. Thousands of plant species are explored in search of new antimicrobials and are evaluated for their antimicrobial potential against thousands of bacterial strains. In vitro experiments gives promising results and many medicinal plants are found active against a range of gram negative and gram positive bacteria. However, very few of these medicinal plant extracts have been tested in animal or human studies to determine safety and efficacy (Mahady, 2005).

In the latter half of the twentieth century Use of herbal medicines expanded sharply in developed countries. In India, herbal drugs are an integral part of The Indian System of Medicine (Ayurveda) which is an ancient and mainstream system (Rai, 2005).

Natural products of higher plants may possess a new source of antimicrobial agents with possibly novel mechanisms of action (Barbour *et al.,* 2008; Ahmad and Aqil, 2007). Out of more than 2,00,000 plants species of known higher plants approximately 1500-2000 are said to

possess medicinal properties in Indian system of medicine (Tiwari *et al.*, 1998). They are effective in the treatment of infectious diseases while simultaneously mitigating many of the side effects that are often associated with synthetic antimicrobials (Iwu *et al*., 1999).

Within the recent years, infections have increased to a great extent and antibiotics resistance effects become an ever-increasing therapeutic problem (Mahesh and Satish, 2008).

Above facts created t interest to evaluate these plants in order to confirm their use as traditional medicine and to screen their active principle by isolating and characterization of their phytomolecules. Systematic screening of them may result in the discovery of novel active compounds (Tomoko *et al*., 2002).

For successful treatment regime it is necessary to search new drugs to combat the challenge created by resistant microorganism. A number of plants parts have been used for centuries for treating human diseases. This has encouraged scientists to screen higher plants for various biological activities including antibacterial and antifungal effects (Eilert *et al*., 1980-1981; Omer and Elnima, 2003; Saadabi, 2006; Saadabi *et al*., 2006-2007, 20009) studies on Phytochemistry of plant extracts and development of modern experimental tools played important role in problems and for additional resources of raw material for pharmaceutical industry. On the other hand, plant synthesizes a wide variety of chemical

compounds which can be sorted out by their chemical class, biosynthetic origin and functional groups into primary and secondary metabolites (Mukherjee and Laloraya, 1977).

EXRACTS OF *MORINGA OLEIFERA AS* ACTIVE ANTIMICROBIAL AGENTS

Recently, focus on plant research has increased and a large body of evidence has been collected to show immense potential of medicinal plants used in various traditional systems (Ernest *et al.,* 2007).

One such plant which is known to possess several medicinal properties is *Moringa oleifera* (Drumstick). The different parts of this plant viz., leaves, stem bark, root bark, flowers, fruits and seeds are used in the indigenous systems of medicine for the treatment of variety of human ailments and some parts are also eaten as vegetable (Chopra *et al.*, 1956; Nadkarni and Nadkarni, 1976).

From ancient times parts of plant *M. oleifera* such as the flower, leaf, bark, and fruit have been used as herbal medicines for treating paralysis, hypertension and inflammatory disorders. Moreover, many reports indicate that *M. oleifera* has highly potent anti-inflammatory (Ezeamuzle *et al*., 1996) hepatoprotective (Pari and Kumar, 2002), antihypertensive (Faizi *et al.,* 1995) and antitumor properties in humans (Murakami *et al.,* 1998). Also, its seed has strong coagulative and anti-microbial properties (Eilert *et al.*, 1981). The seed oil has physical and

chemical properties equivalent to that of olive oil and contains a large quantity of tocopherols (Tsaknis *et al*., 1999).

ANTIMICROBIAL ACTIVITY OF *M. OLEIFERA*

From in vitro experiments against bacteria, yeast, dermatophytes and helminths by a disk-diffusion method Caceres *et al.,* (1991) concluded that *Moringa oleifera* leaves, roots, bark and seeds show antimicrobial activities. Growth *Pseudomonas aeruginosa* and *Staphylococcus aureus* is inhibited by Extracts of leaves and seeds. No activity was demonstrated against other pathogenic Gram-negative and Gram-positive bacteria.

Antifungal activity of *M. oleifera* against seven pathogenic fungi using the broth dilution and agar plate methods was analyzed by Nwosu and Okafor (1995). It was observed that plant extracts from M. oleifera were active against *T. mentagraphytes* and *Trichophyton rubrum*.

Dahot (1998) investigated antimicrobial activity from three fractions of *Moringa oleifera* leaves against *E. coli, K. aerogenes*, *K. pneumoniae, S. aureus, B. subtilis* and *Aspergillus niger* from the study he noted that all three fractions showed strong inhibitory activity against *E. coli*, *S. aureu* and *B. subtilis* but clear zone of inhibition was also noted against *K. aerogenes* while Fraction 2 showed significant zone of inhibition against *Aspergillus niger*.

Nikkon *et al.,* (2003) reported antimicrobial activity of aglycone of Deoxy-Niazimicine using chloroform extract of *M. oleifera* roots barks. antibacterial and antifungal activities of the compound was observed against *Shigella boydii*, *S. aureus* and *S. dysenteriae*. Antibacterial activity from the methanolic crude extract and purified dichloromethane extract of *M. oleifera* capsules was analyzed by Nantachit (2006). It was observed that the crude methanolic extracts haven't had any activity against *K. pneumoniae, E. coli, P. aeruginosa* and *S. aureus.* Extract purified by column chromatography of dichloromethane showed antibacterial activity against selected bacteria.

Doughari *et al.,* (2007) investigate antibacterial activity of aqueous, acetone and ethanolic extracts of the leaves of *M. oleifera*. From the three solvents used, aqueous extract showed the least activity at 100 mg/ml, while the ethanolic extract of the plant demonstrated the highest activity. Almost similar Zone of inhibition obtained by in-vitro analysis of activities of the plant extracts when compared to the antibiotics chloramphenicol, cotrimoxazole and ciprofloxacin.

Jamil *et al.,* (2008) evaluated antimicrobial activity from the seeds of *M. oleifera* against bacterial (*Pasturella multocida, E. coli, B. subtilis* and *S. aureus*) and fungal (*Fusarium solani* and *Rhizopus solani*) strains. One is comparing zones of inhibition bacterial strains showed greater sensitivity in comparison of the fungal strains. Results of MIC extracts

showed that *B. subtilis* and *P. multocida* were most sensitive strains. Abdulmoneim *et al.*, (2011) investigate antimicrobial activity of *M. oleifera* seed extracts against four types of bacteria viz., *S. aureus, B. subtilis, E. coli* and *P. aeruginosa* and two species of fungi viz., *A. niger and C. albicans* for their response. Different concentrations All of the seed extracts shows varying degrees inhibition against all microbes to. In comparison of methanol or petroleum ether aqueous extract showed strong antibacterial activity against all selected strains of bacteria especially with gram positive bacteria (*B. subtilis* and *S. aureus*) while no or less activity was observed against *C. albicans* and *A. niger.* Atieno *et al., (*2011) shown that the seed extracts of *M. oleifera* and *M. stenopetala* bear antimicrobial properties against *S. typhii, V. cholerae* and *E. coli.* As these extracts have potential in controlling bacteria that cause water borne diseases so they can utilized as promising natural antimicrobial agents. *M. oleifera* and *M. stenopetala* methanol extract showed highest inhibitions at dilution of 20% and 40% against *E. coli, S. typhi* and *V. cholerae* respectively. While *M. oleifera* and *M. stenopetala* extracts of n-hexane showed high inhibition against *S typhi* in comparison of *V.cholerae and E.coli*.

The antibacterial activity of leaf juice and extracts of *M. oleifera* was studied by Alam *et al.,* (2009) using disc diffusion and MIC determination method against human pathogenic bacteria. Extract of fresh

leaves showed antibacterial potential at disc concentration of 1175 g /disc against all the tested four six Gram-positive bacteria (*S. aureus, B. cereus, Streptococcus-B- haemolytica, B. subtilis, Sarcina lutea and B. megaterium*) and Gram negative bacteria (*S. shinga, S. sonnei, P. aeruginosa* and *P. spp*). However, ethanol extract (1175 g) of fresh leaves showed inhibitory potential against all the Gram-positive bacteria except in *S. aureus* and *Streptococcus-B- haemolytica* while all Gram-negative bacteria are inhibited.

Antibacterial and antifungal efficacy of steam distillate of *M. oleifera* was studied by Kekuda *et al.,* (2010). He noted that better inhibitory activity was observed in case of *E. coli* in comparison to *S. aureus, K. pneumoniae, B. subtilis and P. aeruginosa.*antifungal activity was observed using *plates* poisoned with distillate from the results it was observed as less colony diameter in as comparison to control plates.inhibitory effect is maximum in *A. niger* followed by *A. oryzae, A. terreus* and *A. nidulans*

By disc diffusion study, to different extracts of *M. oleifera* showed variable sensitivity against Gram +ve and Gram -ve organisms. The extracts obtained using solvent acetone and ethyl acetate showed maximum antibacterial activity against *S. gallinarum, P. aeruginosa, E. coli* and *S. aureus*. The inhibitory effect of methanol and chloroform extracts against these bacteria ranges between the inhibitory effect

conferred by aqueous and ethyl acetate/ acetone extracts. Among the extracts tested the aqueous extract had minimum antibacterial activity against all selected bacteria. However, ciprofloxacin showed highest antibacterial activity against all the test organisms (Dewangan *et al*., 2010).

Thilza *et al., (*2010) investigate *in vitro* antibacterial activity of the water extract of *M. oleifera* leaf stalk by using paper disc diffusion method. Observations reveled mild activity against *E. coli* and *E. aerogenes* at dilution of 1000mg/ml, 700mg/ml, 400mg/ml and 200mg/ml while *S. pyogenus, P. aerogenosa, S. aureus* and *S. albus* were found resistant at these concentrations. The maximum activity was observed in case of *Escherichia coli* at dilution 1000mg/l which is less effective when compared to standard drug tetracycline (250mg/ml). Observations reveled that at higher dilutions water extracts of *M. oleifera* showed some degree of antimicrobial potential.

Talreja (2010) analyzed antimicrobial activity of crude extracts prepared from the flowers and callus of *M. oleifera* using ethanol extract against bacterial and fungal pathogen by paper disc method. For the study gram positive *B. subtilis* and gram negative *S. aureus E.coli, K. pneumoniae* bacterial strains were selected and *C. albicans* is selected as fungal pathogen. Among the extracts of flowers and unorganized tissue

tested, the ethanol extract of callus showed better antimicrobial potential in comparison to the extract floral parts.

The phytomolecules obtained from *M. oleifera* act as rich source of to fight against infections caused by susceptible bacteria. The anti bacterial activity of methanolic extract of *M. oleifera* arial parts were evaluated against oral bacteria using well diffusion technique by Rao *et al.*, (2011). The extract inhibited the *Steptococcus salvarius*, *Steptococcus mitis, Lactobacillus acidophilus and S. aureus*. The most significant effect was seen against *S. aureus*, which is important oral pathogen. A bioguided extraction and fractionation of the methanol extract of the leaf of *M. oleifera* possess following phytomolecules Niazinin A, Quercetin-3-o-β-D- glucopyranoside, Stigmasterol, Kaempferol-3-o-β-D-glucopyranoside and β-sitostirol. All the above isolated phytomolecules from *M. oleifera* were found active against selected test microorganism *S. mutans, S. salivarius*, *L. fermentum, S. anginosus, S. gordonil* and *L. acidophilus*.

Devi *et al.*, (2011) investigate the antibacterial activity of different solvent extracts of *M. oleifera* plant by agar well diffusion method against various bacterial species that frequently cause enteric infections in humans. The extracts of *M. oleifera* leaves, bark, seed and flesh prepared using solvent benzene, methanol and water have shown strong antibacterial potential against all the selected microorganisms such as *S.*

aureus, K. pneumonia, S. dysentriae, E. coli and *B. subtilis*. The inhibitory zone ranges between 7mm to 23mm.

PHYTOCHEMISTRY OF *M.OLEIFERA*

From higher plants almost one lac biologically active phytomolecules having antimicrobial potential have been isolated. These secondary compounds fall in four main chemical classes viz., Phenolics (Phenols, flavonoids, quinines, tanins and lignins), Triterpanoids (monoterpenes, sesquiterpene lactones, diterpenes, saponins and others), Sulphur compounds and Nitrogen compounds (alkaloids, amines, non-protein amino acids and cyanogenic glycosides) (Kubmarawa *et al.,* 2007).

Phytochemicals are chemicals which are produced by plants and may have potential to impact health beside this they impart flavor, texture, smell, or color to plants, sometimes they may not be required as essential nutrients by humans. Phytochemical analysis of *Moringa* species gives an insight to excess range of unique compounds it possesses. In particular, this plant family is rich in compounds containing the simple sugar, rhamnose and it is rich in a fairly unique group of compounds called glucosinolates and isothiocyanates (Bennett *et al.,* 2003; Fahney *et al.,* 2001). Various phytochemical have been isolated from *M. oleifera* plant such as cyanogenic glycosides viz., 4-(α-L-Rhamnosyloxy) benzyl isothiocyanate (Eilert *et al.,* 1981), 4(-L-

rhamnosyloxy)-phenylacetonitrile, 4-hydroxyphenylacetonitrile, and 4-hydroxyphenyl acetamide (Villasenor *et al.,* 1989), O-[2'-hydroxy-3'-(2''-heptenyloxy)]-propyl undecanoate, O-ethyl-4- [(alpha-L-rhamnosyloxy)-benzyl] carbamate, methyl phydroxybenzoate, niaziminin (Murakami *et al.,* 1998; Guevara *et al.,* 1999; Shanker *et al*., 2007), Niazimicine (Nikkon *et al*., 2003), O-ethyl-4-(alpha-L-rhamnosyloxy) benzylisothiocyanate, niazirin (Shanker *et al*., 2007; Guevara *et al.,*1999) aurantiamide acetate 4, 1, 3-dibenzyl urea (Sashidhara *et al.,* 2009).

Also various flavonoids are also found in *M. oleifera* such as quercetin, kaempferol (Siddhuraju and Becker, 2003; Ndong *et al.,* 2007; Verma *et al.,* 2009), Vanillin (Verma *et al.*, 2009). Sugars such as L-rhamnose, D-galacturonic acid, 6-O-Me-D-galactose, D-galactose and l-arabinose (Roy *et al.,* 2007), glycerol-1-(9-octadecanoate) (Guevara *et al.* 1999).

Besides this, sterols such as stigmasterol (Anwar and Rashid 2007), β-sitosterol (Faizi *et al.,* 1998; Guevara *et al.,* 1999), 3-O-(6'-Ooleoyl-beta-D-glucopyranosyl)-β-sitosterol and β-sitosterol-3-O-beta-D-glucopyranoside (Guevara *et al.,* 1999; Yammuenart *et al*., 2008), linoleic sitosteroate, linoleic acid, 1,2,3-triolein, 1,3-dilinoleoyl-2-olein, 1,3-dioleoyl-2-linolein and 1,2,3-trilinolein isothiocyanato- methylbenzene (Yammuenart *et al*., 2008), tocopherols such as α - tocopherol and fatty

acids (Anwar and Rashid, 2007) Monoterpenoids such as α-phellandrene and p-cymene are also present in *M. oleifera* (Ogunbinu *et al.,* 2009).

Presence of phenolic acids in *M. oleifera* like gallic acid, chlorogenic acid, rutin (Ndong *et al.,* 2007; Verma *et al.,* 2009), ellagic acid, ferulic acid (Singh *et al*., 2009; Verma *et al.,* 2009).

Kwaghe (2009) invesigate preliminary phytochemical screening of the crude aqueous extract of fresh and dried *Moringa oleifera* leaves, of the organic solvent and residual fractions of the extract according to the standard methods. On examination of chemical constituents of the extract of the fresh leaves prepared using water as solvent showed the presence of flavonoids, tannins, carbohydrates, saponins, alkaloids, cardiac glycosides, terpenes and steroids. While on analyzing extracts prepared using organic solvent, extracts prepared using chloroform contains carbohydrate, saponins, alkaloids, cardiac glycosides and flavonoid on other hand extracts prepared using solvent ethyl acetate contains similar chemical constituents to chloroform extract and in addition to tannins. In the same way extracts prepared using solvent n-butanol tannins, saponin, carbohydrate, alkaloid and flavonoid. While the residual aqueous fraction showed presence of phlobatannin, carbohydrate, flavonoid, and tannins from the analysis of leaf extracts of *Moringa oleifera* it was observed that Anthraquinones were completely absent. Faizi *et al.,* (1995) isolated six new and three synthetically known glycosides from the leaves *of*

Moringa oleifera, Most of these compounds, bearing thiocarbamate, carbamate or nitrile groups, are fully acetylated glycosides, which are very rare in nature.

Materials and Methods

Chapter- 4 MATERIALS AND METHODS

(A) MATERIALS:

Moringa oleifera belonging to family *Moringaceae,* is one of the best known and most widely cultivated species of a monogenic family. It is native to the sub-Himalayan tracts of India, Pakistan, Bangladesh and Afghanistan.

(1) PLANT MATERIAL

Taxonomic Classification

Kingdom	-	Plantae
Sub kingdom	-	Tracheobionta
Super Division	-	Spermatophyta
Division	-	Magnoliophyta
Class	-	Magnoliopsida
Subclass	-	Dilleniidae
Order	-	Capparales
Family	-	Moringaceae
Genus	-	*Moringa*
Species	-	*Oleifera*

Geographical Source

The tree is wild in the Sub-Himalayan tracts from Chenab to Oudh. It grows at elevations from sea level to 1400 m. It is very commonly cultivated near houses in Assam, Bengal and peninsular India. It is a prolific coppicer Gupta (2009). It is also cultivated in north-eastern

Pakistan, north-eastern Bangladesh, Sri Lanka, West Asia, the Arabian Peninsula, East and West Africa, throughout the West Indies and southern Florida, in Central and South America from Mexico to Peru, as well as in Brazil and Paraguay Roloff (2009).

Morphology

Moringa oleifera is a small, fast-growing evergreen or deciduous tree that usually grows as high as 9 m, with a soft and white wood and corky and gummy bark. Roots have the taste of horseradish. Leaves are longitudinally cracked leaves, 30-75 cm long main axis and its branch jointed, glandular at joints, leaflets are glabrous and entire. The leaflets are finely hairy, green and almost hairless on the upper surface, paler and hairless beneath, with red-tinged mid-veins, with entire (not toothed) margins, and are rounded or blunt-pointed at the apex and short-pointed at the base. The twigs are finely hairy and green. Flowers are white, scented in large axillary down panicles, pods are pendulous, ribbed, seeds are 3-angled (Gupta, 2009, Roloff, 2009).

(a) Media for bacterial culture

Nutrient Agar Medium

Peptone	-	5 gm
Beef extract	-	3 gm
Agar-agar	-	16 gm
Sodium chloride	-	5 gm
Distilled water	-	1000 ml (pH = 7.2)

Fig. 1: *M. oleifera* (Bark)

Fig. 2: *M. oleifera* (leaves)

Fig. 3: Soxhlet extractor

Fig. 4: Rotary evaporator

Preparation of Nutrient Agar medium

- 3 gm beef extract and 5 gm peptone is dissolved in 200ml of distilled water.
- Solution mixture is heated to dissolve the constituents.
- For making up the volume of More 2 liter distilled water was added.
- pH meter is used to examine the pH and to maintain pH at 7.2.
- Nutrient Agar medium was distributed to conical flasks.
- Conical flask was plugged with cotton and wrapped by aluminum foil.
- Prepared medium is sterilized using autoclaved maintaining temperature at 121°C, pressure at 15 Ibs for 15 minutes.
- In sterilized conditions of laminar the medium was poured into already sterilized petri plates and then allow solidifying.

(b) Nutrient Broth medium

Peptone	-	5 gm
Beef extract	-	3 gm
Sodium chloride	-	5 gm
Distilled water	-	1000 ml (pH = 7.2)

Preparation of Nutrient Broth Medium

- 3 gm beef extract and 5 gm peptone is dissolved in 200ml of distilled water.
- Solution mixture is heated to dissolve the constituents.
- For making up the volume of More 2 liter distilled water was added.
- pH meter is used to examine the pH and to maintained at pH at 7.2.
- 10 ml broth prepared is poured per tube was.

- Cotton plugs were applied.
- Prepared medium is sterilized using autoclaved maintaining temperature at 121°C, pressure at 15 Ibs for 15 minutes.
- The nutrient broth tubes were removed and store at room temperature (covered with butter paper) for further use.

(c) Mac-Conkey's Agar medium:

Peptone	-	20g
Nacl	-	5.0g
Bile salt	-	1.5g
Lactose	-	10.0g
Neutral red solution	-	10.0g
Crystal violet	-	0.001g
Agar	-	13.5g
Distilled water	-	100.0ml

(d) Muller-Hinton Ager medium:

Beef infusion	-	300.og
Casaminoacida	-	17.5g
Starch	-	1.5g
Agar	-	15.0g
Distilled water	-	1000.0ml

(e) Media for fungal Culture

PDA

Potato	-	200g
Dextrose	-	20g
Agar	-	20g
Distilled water	-	1000ml

Preparation of PDA

- Boiled potatoes were filterated through muslin cloth and every one liquid squeezed out 500 ml of water were taken in another beaker and

heated, 20 gm Agar was added into it bit by bit to dissolve, followed by addition of 20 gm of Dextrose.

- Potato extract was mixed with agar solution and water was added to form volume upto 1 litre.
- PDA medium was distributed to five conical flasks. The conical flasks were plugged with cotton and wrapped by aluminum foil.
- Conical flasks containing PDA media were autoclave at 121° C at 15 P.S.I pressure for quarter-hour.
- Then flasks containing medium were allowed to chill until the flasks are often holded by hand. Then medium was poured into already sterilized petriplates under sterilized conditions in laminar air flow then allowed to solidify.

(f) SOBOURAUD`S DEXTROSE AGAR MEDIUM

Peptone	-	10g
Dextrose	-	40g
Agar-agar	-	20g
Distilled water	-	1000ml

Preparation of SDA Medium

1000ml of warm distilled water was taken in one liter beaker and 10g of peptone. 40g of dextrose and 20g of other was added one by one until they dissolve.

- The pH of the medium was adjusted to 5-6 with the help of pH meter.

- Medium was transferred into 500ml conical flasks and then tightly plugged with cotton and wrapped by aluminum foil.
- Medium was autoclaved at 115°C at 15 P.S.I. pressure for 20min
- The low pH and high dextrose content of this medium is particularly selective for fungi contamination.

(B) METHODS

COLLECTION AND STORAGE OF PLANT: (PLANT MATERIAL)

Place of Collection:

For conducting the present study, the plant material was collected from different locations of Agra and Plant parts (leaves and bark) devoid of contaminant parts were carefully collected and kept in polythene bags which were then subsequently sealed. The stored specimens were thoroughly washed with tap water. They were shade dried and ground with grinder to obtain course particle.

Extraction of Plant materials:

a. **Aqueous Extract:** For aqueous extract leaf powder was separately homogenized with sterile water at 1:8 w/v ratio during a pestle and mortar and filtered through muslin cloth. The filtrate thus obtained was further strained through Whatman No. 1 paper (Zore *et al*., 2004). The extraction was administered at temperature.

b. **Organic Extract**: Organic extract was prepared by Soxhlet extraction method following (Okeke *et al*., 2001). A thimble was prepared by employing a 0.5mm Whatman paper. About 50 gm of powder material was uniformly packed in a thimble and run in soxhlet extractor. It had been run upto 48 hour or 22 cycles until the solvent within the sippon table of an extractor become colourless. Then, extracts were filtered with the assistance of paper and solvent was evaporated from extract with the assistance of rotary evaporator to urge the syrupy consistency. The extract was then stored in refrigerator at 4ºC.

(C) PREPARATIONS OF EXTRACTS

(a) Extracts from bark and leaves of *Moringa oleifera*

Four different solvent were used for extracting phytomolecules.

1. Methanol
2. Chloroform
3. Ethyl acetate
4. Water

Methodology

50gm of dry powder of *Moringa oleifera* obtained from bark and leaves was put in a thimble. About 300 ml of different solvent were used in soxhlet apparatus for extracting of soluble phyto molecules from the plant. Soxhlet apparatus comprises of soxhlet, condenser and round bottom flask. It is heated on the heating mantle and evaporated solvent goes to soxhlet. Here, it is cooled by the water moving in the condenser

and then solvent moves back to round bottom flask with soluble bioactive components. Extract having volatile solvent were separated using rotary evaporator under reduce pressure. In order to start, the vacuum compressor was switched 'on' and extract were taken in a flask and was set to adopter and vaccum released nobe was closed. Flask was then dipped in water bath which rotate with attached vacuum handle. The evaporated volatile solvent was then passed through low temperature condensing region where volatile form of solvent condense to liquid and get collected with flask attached to bottom side. When the extract was concentrated, the rotor was switched off and vacuum was released to remove the flask. The concentrated extract was unloaded to sterilize collecting tube.

(b) Collection of Pathogenic microorganisms

The pure cultures of test bacterial and fungal strains used in the study were *Staphylococcus aureus* (MTCC No 740), *Citrobacter freundii* (MTCC No1658), *Bacillus megaterium (*MTCC No 428*), Pseudomonas fluorescens* (MTCC No. 103), *Rhizopus stolonifer* (MTCC No 2198) and *Microsporum gypseun* (MTCC No2819) fungal culture were used in the study. The test microorganisms were collected from Microbial Type Culture Collection (MTCC) situated at Institute of Microbial Technology, Chandigarh. The fungal pathogens were maintained in Potato Dextrose Agar (PDA) medium.

Preparation of 0.5 McFarland Standards

Add 0.5 ml of 0.048 M $BaCl_2$ (1.17% W/v Ba $Cl_2.2H_2O$) to 99.5 ml of 0.18 M H_2SO_4 (1% W/v) with constant stirring. Record the OD of the solution; it should be in the range of 0.08 - 0.1 at 0.25 nm. Standards are then distributed into the screw cap tubes of the same size and volume as those growing in the broth cultures. Seal the tubes tightly to prevent loss by evaporation store the standard in Amber tightly to present loss by evaporator store the standard in amber coloured bottle to prevent it from light at room temperature. Vigorously vortex the standard on a vortex mixer prior to use (NCCLS, 2004).

Test microorganism

The following test micro-organism included clinical isolates of bacterial and fungal pathogens are:

(i) *Staphylococcus aureus*

Scientific Classification

Domain	-	Bacteria
Kingdom	-	Eubacteria
Order	-	Bacillales
Family	-	*Staphylococcaceae*
Genus	-	*Staphylococcus*
Species	-	*aureus*

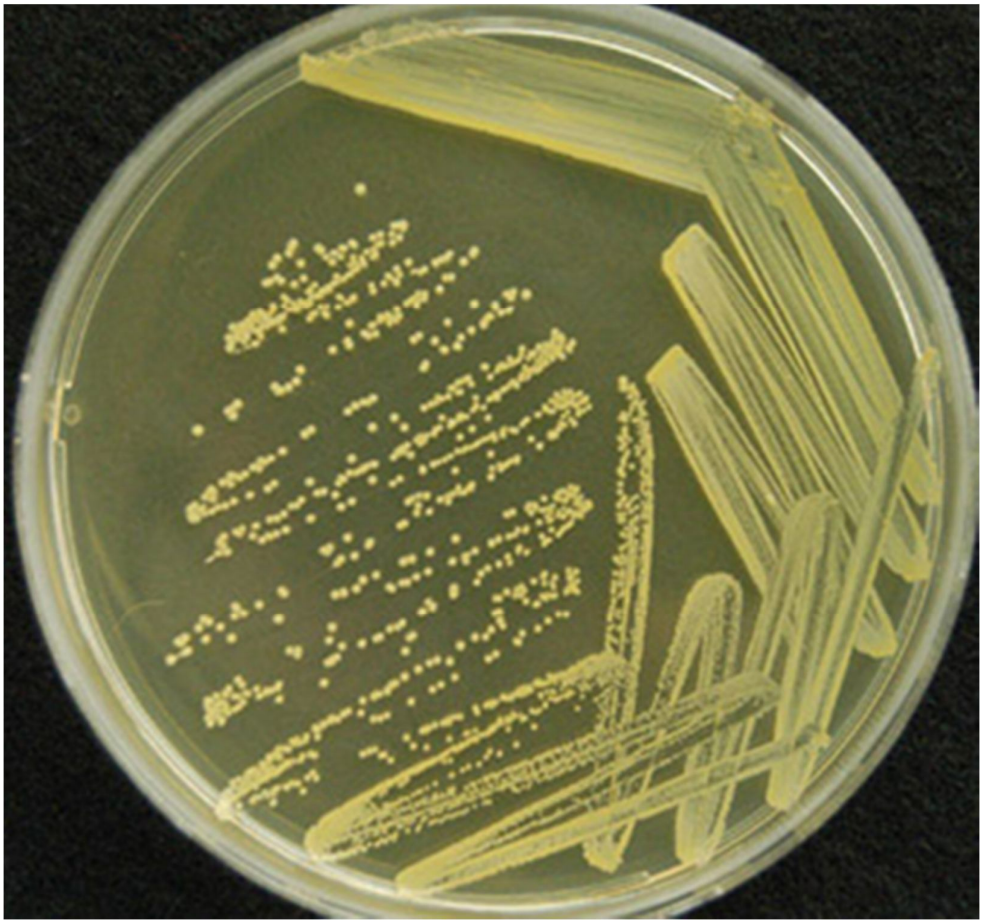

Fig. 5: *S. aureus*

Staph short form of a bacterium called staphylococcus. There are over thirty different species of staphylococcus bacteria which will cause infections starting from mild to life threatening. Most Staph infections are caused by a genus referred to as *Staphylococcus aureus*. Literally meaning "golden cluster seed," and is additionally referred to as Golden Staph. a number of the more common complications from Staph infections include toxic shock syndrome syndrome, skin infections, and pneumonia. A more serious infection occurs when Staph enters the bloodstream, this is often referred to as bacteremia. Identified as a lethal threat in 1999 the Staph infection, in additional serious cases, can cause death in up to twenty five percent of the patients that it attacks. People most susceptible to getting Staph infections include newborns and other people with skin disorders. More serious infections occur in people with surgical incisions, injecting drug users, people with a weakened immunity or a chronic disease like inflammatory bowel disease, cancer, and lung disease, diseases of the veins and arteries and diabetes.

Staph bacteria are usually diagnosed by its appearance alone since it most ordinarily causes skin infections. Some skin infections may become more serious and want a therapy that's more aggressive.

Staphylococcal gastrointestinal disorder is an illness of the bowels that causes nausea, vomiting, diarrhea, and dehydration. It’s caused by

eating foods contaminated with toxins produced by *Staphylococcus aureus*.

(ii) ***Citrobacter freundii***

Systematic description

Kingdom - Bacteria
Order - Enterobacteriales
Family - Enterobacteriaceae
Genus - *Citrobacter*
Species - *freundii*

C. freundii is the species belonging to family citrobacter that solely use citrate as a carbon source. The species was readily isolated from water, feces, and urine. It is belongs to normal flora of intestine but it may cause infections of the gall bladder, urinary tract,, middle ear. Different species of Citrobacter cause infections in immune compromised hosts and neonates. Citrobacter koseri (formerly Citrobacter diversus) is best referred to as the reason behind sepsis and meningitis resulting in central nervous system (CNS) abscesses in neonates and young infants. As an opportunistic pathogen, Citrobacter freundii is usually the reason for significant opportunistic infections, meaning that it doesn't generally cause disease in healthy human hosts. They only affect patients with a weak immunity, signifying that they have a chance to infect the person Whalen; Mully (2007). Therefore in patients with a suppressed immunity, Citrobacter species are known to cause a large kind of nosocomial infections of the respiratory system, urinary system, and therefore the

blood Whalen (2007). C. freundii also cause Hepatic, biliary and pancreatic diseases. The biliary tract is that the commonest site of infection by the C. freundii Sanchez *et al*., (1985).

(iii) *Bacillus megaterium*

Scientific classification

Kingdom	-	Bacteria
Order	-	Bacillales
Family	-	Bacillaceae
Genus	-	*Bacillus*
species	-	*megaterium*

Bacillus megaterium is a endospore forming, rod-shaped, Gram-positive bacteria used as a soil inoculant in agriculture and horticulture. Bacterium is arranged into the streptobacillus form. *Bacillus megaterium* is a rod shaped bacterium and one among the most important eubacteria found in soil. Groups of the bacteria are often found chained where the cells are joined together by polysaccharides on the cell walls. It survive in extreme environmental conditions because of the spores it forms. In favorable conditions the spores germinate. Sometimes these particular bacteria are often found on common surfaces that are frequently touched. *Bacillus megaterium* produces penicillin amidase used for creating penicillin. It produces enzymes for modifying corticosteroids, also as several amino acid dehydrogenases.

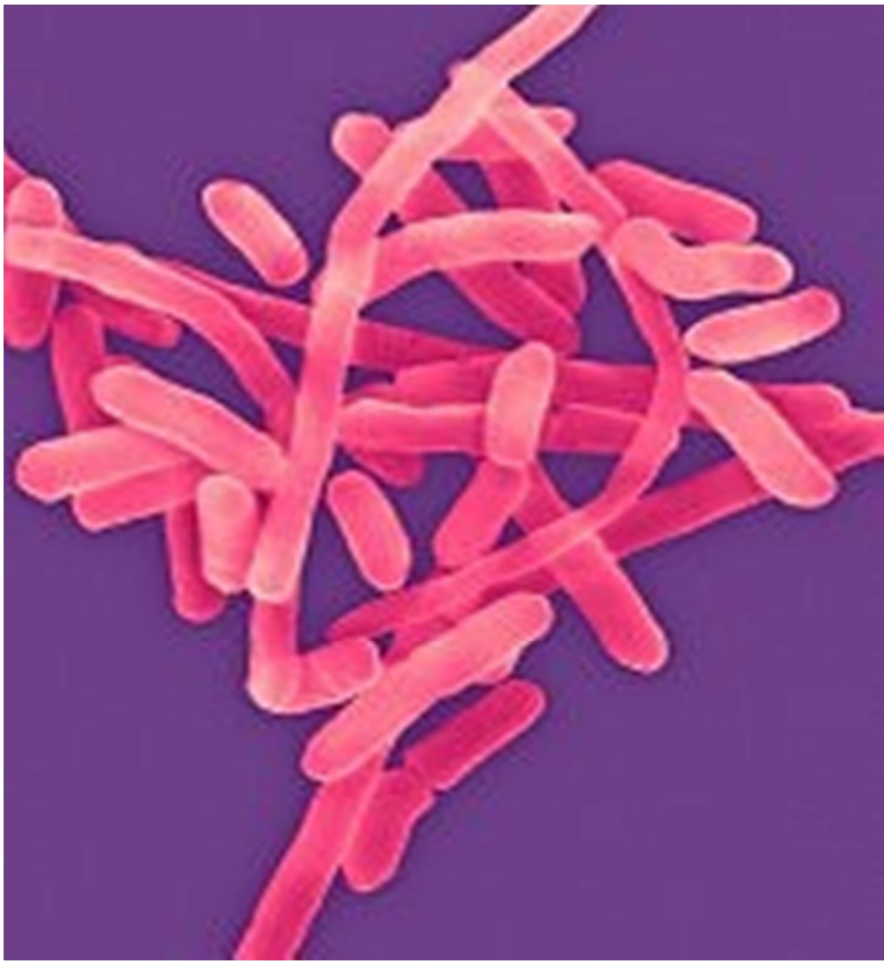

Fig. 6: *C. freundii*

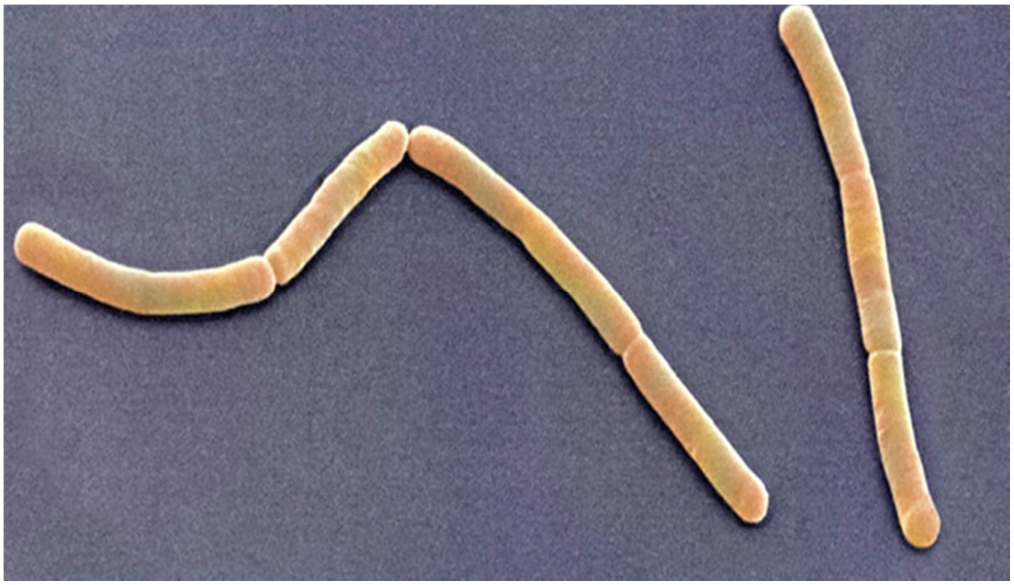

Fig. 7: *B. megaterium*

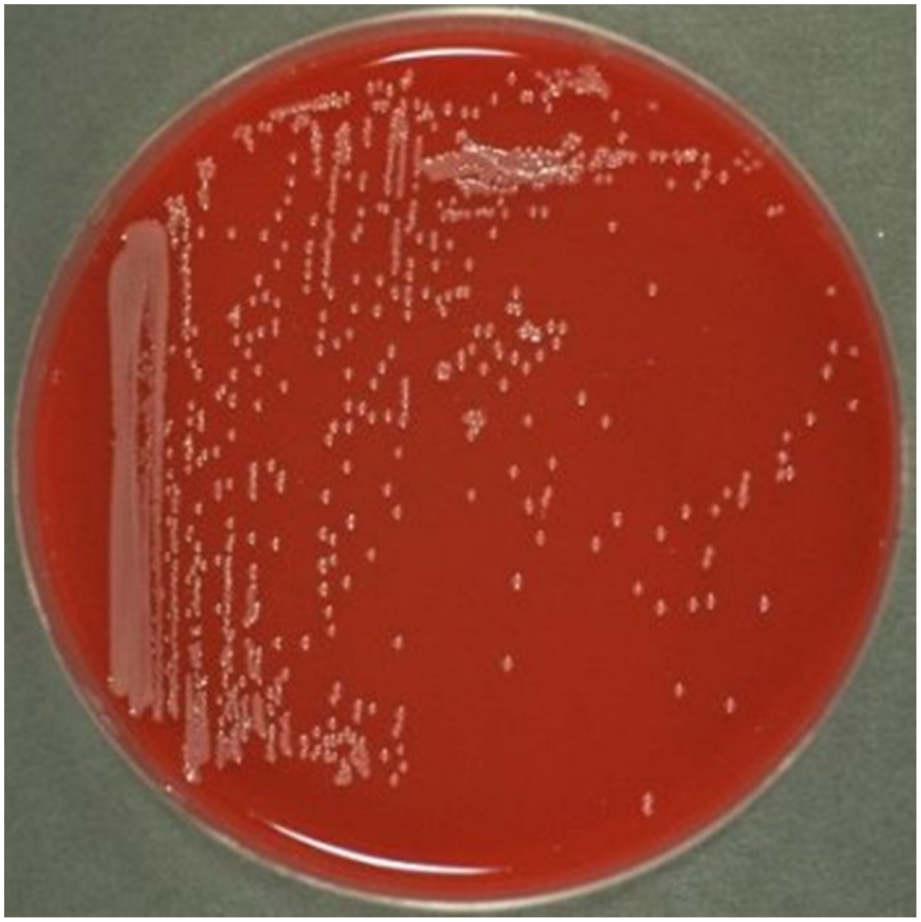

Fig. 8: *P. fluorescens*

Fig. 9: *R. stolonifer*

Fig. 10: *M. gypseum*

Fig. 11: Showing plant of *Moringa oleifera* growing at Khandari, Agra

(iv) *Pseudomonas fluorescens*

Scientific classification

Kingdom	-	Bacteria
Order	-	Pseudomonadales
Family	-	Pseudomonadaceae
Genus	-	*Pseudomonas*
Species	-	*fluorescens*

General characteristics

Pseudomonas fluorescens is a rod-shaped Gram-negative, bacterium. *P. fluorescens* is saccharolytic bacteria having multiple flagella, it tests positive for the oxidase test. *P. fluorescens* and other similar pseudomonads produce heat stable lipases and proteases. These enzymes cause spoilage of milk by causing bitterness, breaking casein and repines due to coagulation of proteins and production of slime. Jay d (2000) hemolytic activity is demonstrate *P. fluorescens* therefore it has been known cause infections during blood transfusions Gibb (1995).

P. fluorescens is known cause of disease in humans, and generally impact patients with weak immune systems (e.g., patients on cancer treatment).several outbreak of *P. fluorescens* between year 2004 to 2006, observed in United States, involving almost 80 patients from six states. The contaminated heparinized saline flushes being used with cancer patients was the reason of infection. Beside this it also cause infection in plants like Pith necrosis diseases of tomato in Europe was caused by *P. viridiflava Pseudomonas corrugata, P. fluorescens* and *P. mediterranea*.

(v) *Rhizopus stolonifer*

Kingdom - Fungi
Order - Mucorales
Family - Mucoraceae
Genus - *Rizopus*
Species - *stolonifer*

Black bread mold is also known as *Rhizopus stolonifer*. It generaly contaminates food matter such as breads or soft fruits, grapes, strawberries for growth, nutrition and reproduction. *R. stolonifer* is a mass of mycelium having a fruiting structure and the vegetative filaments of the fungus. Most of the mycelium is composed of multinucleate hyphae. As the mold matures it begins to turn black they produce more mycelium through germination of the released mold's spores.

R. stolonifer also cause diseases in plant; through decomposition it breaks down organic matter. The parasite can quickly spread within a few days when kept in a moist environment, such as a piece of bread, its spores are commonly found in the air. The spores grow most rapidly and are able to germinate to their full potential at temperatures between 15°C and 30°C

(vi) *Microsporum gypseum*

Kingdom - Fungi
Order - Onygenales
Family - Arthrodermataceae
Genus - *Microsprum*
Species - *gypseum*

Microsporum

This species is the Causative organism behind diseases of the skin and hair such as *Tinea capitis* and *Tinea corporis*. *M. audouinii, M. canis (M. lanosum),* and *M. fulvum (M. gypseum)* are member of this species.

This genus *Microsporum canis, M.gypseum* mainly cause infection in animals while *M. canis* generally cause of Ringworm in cats and dogs and also can cause human infection beside above it less commonly cause infections in other animals including swine, cattle & goats. *M. gypseum* also causes ringworm infection in dogs, horses and rodents

(E) PROCEDURE FOR TESTING ANTIBIOGRAM: (DRUG SUSCEPTIBILITY TEST)

The pattern of resistance offered by microorganism against antimicrobial agents is termed as Antibiogram. The Kirby Bauer disk assay was used for testing antibacterial drugs against the test microorganisms for profiling their resistance pattern according to National Committee for Clinical Laboratory Standards (NCCLS) guidelines (NCCLS, 2009), the turbidity of activated culture of the test organism was visually adjusted using sterile saline solution (0.85% NaCl solution) to approximately that of 0.5 McFarland turbidity standard (1.5 x 108 CFU/ml). 90 mm sterile petri dishes were poured with 20 ml molten MH agar to mean depth of 4.00 ± 0.5 mm for carrying out the antibacterial resistance profiles. The inoculum is adjusted to 0.5

McFarland turbidity standards. Swabs of Sterile cotton were dipped into the suspension and for removing excess suspension is rotated against the wall of the test tube. For inoculating agar plates Cotton swabs were swabbed 4 times on each plate by rotating the plate at angle of 60ºC between swabs for ensuring even distribution of inoculum. Care was taken to prevent aerosols and the sides of the petriplate were avoided. The inoculum was allowed to be absorbed for a minimum of 3 minutes but not than quarter-hour before applying Kirby Bauer disks. Kirby Bauer disks were applied to the agar surface by employing a dispenser and applying gentle pressure with sterile forceps to make sure complete content of disc with agar.

Plates were incubated for twenty-four hours at 37ºC in BOD incubator susceptibility was measured as long as a lawn of microorganism was present to attain susceptibility plates were rested lid down on a black non-reflecting surface and therefore the diameter of the inhibition zone was measured to the closest whole millimeter by holding a caliper micrometer against the rear of the plates for isolating colonies within the zone of inhibition which have represented resistance visual examination of Plates were done.

In this technique standard antibiotic disc of HI-MEDIA was tested, listed in table 3.

Table 1: Culture media used for study

S.No.	Media	pH	Manufacturer's Details
1.	Mac-Conkey's agar	7.7	HI-MEDIA
2.	Muller – Hinton agar	7.4	HI-MEDIA
3.	Nutrient agar medium	7.2	Prepared in lab
4.	Nutrient broth	7.0	Prepared in lab
5.	Potato Dextrose agar medium	6.8	Prepared in lab
6.	Sabouraud's Dextrose agar medium	5.6	Prepared in lab

Table 2: Equipment used for study

S.No.	Equipments	Company
1.	Autoclave, Hot air ovens	Scientific equipment work
2.	Electronic analytical balance	Sartorius
3.	Laminar flow	Zenith
4.	Incubator	Toshiba
5.	Deep freezer and refrigerator	Sonyo
8.	Micropipettes	Tarson, Labmate,
9.	Soxhlet extractor, Rotary evaporate	Heidolph
10.	Glass wares	Borosil

Table 3: Details of antibiotic disc utilized for susceptibility test

S.No.	Antibiotics	Symbol
1	Cephalothin	Ch
2	Clindamycin	Cd
3	Co-trimoxazole	Co
4	Erthamicin	E
5	Gentamicin	G
6	Ofloxacia	Of
7	Penicillin-G	P
8	Vancomycin	Va

(F) PROCEDURE FOR TESTING ANTIMICROBIAL ACTIVITY:

Two methods were used to test antimicrobial activity of different plant extract, disc agar diffusion method (Mukherjee *et al.,* 1995) and MIC (minimum inhibitory concentrate) by Broth Dilution Methods (Eloff, 1998).

(i) Disc diffusion method

In this method, solution with different concentration 200 mg/ml, 100 mg/ml, 50 mg/ml, 25 mg/ml, 12.5 mg/ml, 6.25 mg/ml were prepared by serial dilution by dissolving Plant extract in suitable solvent. Empty sterile discs having a diameter of 6 mm were impregnated with 25µl of every serial dilution of extract solution. These impregnated disc, now contain different concentrations respectively of extract and so incubated for quarter-hour for correct diffusion of extract. On the opposite hand, aseptically close up some colonies from the pure culture was mixed (emulsify) in nutrient broth (7µl/ml broth). This broth was inoculated on entire surface of agar plate with this culture moistened cotton swab. Then await 5-6 minute after inoculation to permit the liquid culture to soaking to agar surface .With the assistance of sterile forceps, herbal extracts containing disc, placed on inoculated surface of agar plate. These plates were incubated for twenty-four hours at 37° C and zone of inhibition measured in millimeter.

(ii) Broth Dilution Methods (Eloff, 1998)

For determining Minimal inhibitory Concentration and determination of the death pattern (death kinetics) broth micro dilution technique was adopted.in this experiment 96 well micro titer plates and tetrazolium salt, 2,3,5-Triphenyltetrazolium Chloride (TTC) was used carried.

Well no A1 to H1 consisted of MH broth only and was the blank while well A3 to H3 was having the stock solution of the test extract (s) only and test extracts were serially diluted using MH broth was in the wells A4 to H4 till A9 to H9. Wells having 20 l of DMSO that is A12 to D12 were set control while E12 to H12 served as control over control. 100 l of MH broth were poured in all wells. 20 l of the herbal extract was transferred from stock test solution to the primary well i.e. well A4 to H4 which contains 100 l of MH broth. 20 l of the MH broth containing herbal extract was then transferred to following well to make serial dilutions. 100 l activated culture MH broth adjusted to 0.5 MacFarland was then added to all the wells except the blank. To all or any the dilutions, blank, control and control over control 5 l of 0.5 % TTC was further added. The ultimate volume of all the wells was 205 l. The sealed Microplate was incubated at 37°C.

PHYTOCHEMICAL ANALYSIS OF DIFFERENT CRUDE EXTRACT

For testing the presence of active principle such as flavonoids, tannins, triterpenoids, alkaloids, proteins and glycoside Standard protocols were used (Debela, 2002).

(i) Alkaloids

Mayer's test

Few drops of Mayer's reagent were added to 0.5 ml of sample was taken in a tube. It is shaked and allowed to stand for sometime. Presence of alkaloids was identified by cream colour precipitate in the sample.

(ii) Glycosides

Legal test:

Sample was treated with bit of pyridine during a tube. alkaline sodium nitropruside solution drops were added to sample. If blood red colour appears, then alkaloids were present within the sample.

(iii) Tannins and Phenolic compounds

Ferric Chloride test:

By adding some drops of ferric chloride to 0.5 ml of sample present in a test tube if blue green colour appear the presence of Tannins and Phenols in the sample confirmed.

(iv) Flavonoids

Alkaline reagent test

Some drops of sodium hydroxide solution (10 %) were added to the test solution (0.5 - 1 ml). confirmation of flavonoids was done by observing Formation of an intense yellow colour, which turns colourless on addition of some drops of dilute acid.

(v) Test of Steroids and Triterpenoids

Salkowski test

For detecting the presence triterpenoids and steroids some drops of concentration sulphuric acid was added about 0.5 to 1 ml of test solution in a test tube already treated with chloroform and shaken well. After waiting for some time appearance of red colour at the lower layer confirms triterpenoids while formation of yellow colour confirms steriods.

(vi) Carbohydrates

Benedict's test:

For testing carbohydrates about 0.5 to 1ml test sample was treated the with some drops of Benedict reagent (solution containing complex of alkaline cupric citrate) in a test tube and incubate in a water bath development of reddish and brown ppt. show that reducing sugars was present in the sample.

(H) STATISTICAL CALCULATIONS

In the present investigation, the following formula was used for different statistical calculation.

Mean $(\overline{X})$

$$\overline{X} = \frac{\sum x}{N}$$

where, $\sum x$ = *Sum of observations*

N = Total number of sample

Standard Deviation

$$SD = \sqrt{\frac{\sum (X - \overline{X})^2}{N - 1}}$$

where,

$(X - \overline{X})^2$ = Sum of the square of the deviation from the mean.

N = Total number of observations.

(I) THIN LAYER CHROMATOGHRAPHY (TLC)

Experiment was performed to separate different active molecules present sample on the basis of their solubility in different phases. Silica gel G 250 was coated on TLC plates (20 x 20cm) were used for separation of the extracts showing predominant antimicrobial activity in the prescreen and screen assays by using different combinations of mobile phases like Methanol, Methanol: Water, Acetone: Chloroform, Acetone: Ethyl Acetate: Chloroform at 28°C. The TLC chamber was conditioned for a period of 30 minutes prior to the run. 10 l of the herbal extract from the stock solution were loaded 1 cm above the TLC plate. The resolution of the extracts was recorded by their Rf values. The

mobile phase exhibiting the best resolution of the extracts was selected for use as a mobile phase for Mass separation by column chromatography (Salituro & Dufresne, 2000).

Result

Chapter- 5

RESULT

1. ANTIBIOGRAM (ANTIBIOTIC DRUG SENSITIVITY) OF*S.AUREUS C.FREUNDII, B. MEGATERIUM* AND *P.FLUORESCENS*

The antibacterial drug susceptibility of *S.aureus, C. freundii*, *B. megaterium*and *P. fluorescens*isolates were determined by using disc diffusion method and classified as resistant, intermediate and sensitive. In accordance to performance standards of disk susceptibility test (CLSI, Vol 29, No-3, Jan 2009) formally NCCLS. The results are given in table 4-5 (Figs. 12-15).

Observations reveled that from tested eight antibiotics *S.aureus* isolate was found to be resistant for cephalothin while it shows intermediate effect for Co-Trimoxazole, Penicillin-G and Vaneomycincefriaxone with inhibition zone diameter ranging between 8 - 12 mm *S.aureus* strain was highly sensitive to clindomycin, gentamicin, erythromycin, and ofloxacin producing the inhibition zone of 14 mm, 18 mm, 16 mm and 18 mm respectively (Fig. 12).

Similarly in case *C.freundi* from eight antibiotics tested, it was found to be resistant for cephalothin while it shows Intermediate for co-trimoxazole, clindomycin, erythromycin, and Vaneomycin with inhibition

zone diameter ranging between 7-12mm. *C. freundii* strain was sensitive gentamicin, ofloxacin and Penicillin-G with inhibition zone diameter ranging between 16 mm,18mm and 18 mm. (Fig. 13).

In case of, the *B. megaterium*isolate it was found that out of eight antibiotics, ofloxacin and Penicillin-G found to be resistant Intermediate for co-trimoxazole, clindomycin, erythromycin, and Vaneomycin with inhibition zone diameter ranging between 7-12mm *B. megaterium*strain was sensitive for gentamicin with inhibition zone diameter 13 mm (Fig. 14).

While, in case of *P. fluorescens* from eight antibiotics tested, the isolate did not show any resistant for any antibiotic but it has showed Intermediate activity for co-trimoxazole, clindomycin, erythromycin, and Vaneomycin with inhibition zone diameter ranging between 7-12mm. *P. fluorescens*strain was sensitive gentamicin, ofloxacin and Penicillin-G with inhibition zone diameter ranging between 16 mm,18mm and 17 mm. (Fig. 15). It appeared that isolates have become resistance to some antibiotics because of constant exposure with these antibiotics.

2. ANTIMICROBIAL PROPERTIES OF BARK AND LEAVES EXTRACT OF *M.OLEIFERA*

To study on antimicrobial properties of *M. oleifera* belonging to family moringaceae against selected micro-organisms was conducted. Extracts of *M. oleifera* bark and leave were prepared and diluted to

different concentrations such as (200 mg/ml, 100 mg/ml, 50 mg/ml 25mg/ml 12.5mg/ml 3.125mg/ml) and studied for the antimicrobial properties The pure cultures of test bacterial and fungal strains used was (*Staphylococcus aureus*, *Citrobacter freundi, Bacillus megaterium, Pseudomonas fluorescens Rhizopus stolonifer* and *Microsporum gypseun).* The individual diameter of inhibition were recorded which are shown in tables (6-13). It was observed that all extracts of *M. oleifera* leaves and bark prepared was effective against all selected bacterial and fungal micro-organisms. The antifungal and anti-bacterial activity of different extracts prepared from *M. oleifera* was described separately in paragraphs.

Antimicrobial activity of ethyl acetate bark extracts of *M. oleifera*

From the results given in table 6 it was observed that ethyl acetate bark extract was active against all the test microogranism the respective diameter of inhibition against *S. aureus* at different dilution used were (16.33, 14.33, 12.33, 11.67, 11.33, 7.66) mm, *B. megetarian* inhibition were (14.67*,* 13.00, 10.67, 9.00, 8.66, 7.67) mm, while *P. flouresence*, inhibition were (15.67, 12.00, 11.67, 10.66, 10.00, 6.67) mm. While ethyl acetate*,* were comparatively less effective against *C. freundii*their respective diameter of inhibition were (15.33, 13.67, 12.33, 11.33, 8.00) mm, and for fungal species ethyl acetate bark extracts were active against both *M. gypseum* and *R. stolonifer* the respective zone of inhibition at

different dilution for *R. stolonifer* were (9.67, 8.33, 7.66, 7.33, 7.00, 6.66) mm, and for *M. gypseum* were (10.66, 9.66, 8.66, 8.00, 7.67, 7.00) mm, respectively.

Antimicrobial activity of methanol bark extracts of *M. oleifera*

According to the result given in table 7 it was observed that the *M. oleifera* bark extract of methanol were found to be most active against*, P. flouresence* and *C. freundii* and the individual diameter zones of inhibition were recorded are (14.00, 12.00, 11.33, 10.66, 10.00, 9.66, 8.66) mm, and (13.67, 11.66, 10.66, 9.67, 9.33, 8.00, 7.33) mm, while methanolic extract were slightly less effective against *S. aureus, B. megetarian* their respective diameter of inhibition were (13.00, 12.67, 10.33, 8.66, 8.00, 7.00) mm and (9.66, 8.67, 8.00, 7.00, 6.67, 6.33) mm.

In case of fungal species, methanol extracts of bark were more active against *M. gypseum* the respective diameter of inhibition at different dilutions were (8.66, 8.33, 7.66, 7.33, 7.00, 6.67) mm in comparison to *R. stolonifer* inhibitions were recorded (9.00, 8.66, 8.33, 7.66, 7.00) mm, respectively.

Antimicrobial activity of aqueous bark extracts of *M. oleifera*

From the result given in table 8 it is observed that the antibacterial activity of the aqueous extracts of *M. oleifera* bark shows that higher activity against *P. fluorescence*. The inhibitory zone observed were (9.00, 8.67, 8.33, 8.00, 7.66, 7.00, 7.67) mm. while in case of*, C. freundii,*

*B. megetarian*and *S. aureus* diameter of inhibition was observed (11.33, 10.66, 9.66, 7.33, 8.33, 8.66) mm, (9.66, 8.67, 8.00, 7.66, 7.33, 6.67) mm, (9.00, 8.67, 7.67, 7.00, 6.33) mm, respectively.

For fungal species the bark aqueous extract of *M. oleifera* inhibited the growth of *M. gypseum*showing diameter of inhibition (9.00, 8.66, 8.33, 7.678, 7.33, 7.00, 6.67) mm and *R. stolonifer*was comparatively less effective the diameter of inhibition are observed (9.66, 8.33, 8.00, 7.66, 6.66, 6.33) mm, respectively.

Antimicrobial activity of chloroform bark extracts of *M. oleifera*

From the results given in table 9 it was observed that chloroform bark extract was active against all the test microogranism the respective diameter of inhibition against chloroform extracts *of M.oleirera*barkhad the maximum antibacterial activity against *P. fluorescence* the observed diameter of inhibition were (12.33, 10.67, 9.67, 9.33, 8.00, 7.00, 6.678) mm, and moderate antibacterial activity against *S.aureus* with diameter of inhibition were (15.00, 13.33, 11.00, 9.678, 9.00, 8.67) mm and the minimum antibacterial activity against *B. megetarian* observed diameter of inhibition were *(*14.67, 13.33 , 11.00 , 9.33 , 7.66) mm, against *C. freundii* diameter of inhibition were (12.67, 11.33, 10.67, 9.33, 8.66) mm respectively.

In case of fungus, the chloroform extracts had maximum antifungal activity against *R. stolonifer* the observed diameter of inhibition were

(9.33, 9.00, 8.33, 7.66, 7.00, 6.67, 6.33) mm, in comparison to the *M. gypseum* showed moderate activity and the diameter of inhibition were (9.33, 9.00, 8.33, 7.66, 7.00, 6.67, 6.33) mm, respectively.

Antimicrobial activity of different leaves extracts of *M. oleifera*

Different concentrations (200 mg/ml, 100 mg/ml, 50 mg/ml 25mg/ml 12.5mg/ml 3.125mg/ml) of *M. oleifera* leave extracts were prepared and studied for the antimicrobial properties The pure cultures of test bacterial and fungal strains used was (*Staphylococcusaureus*, *Citrobacterfreundi, Bacillus megaterium, Pseudomonas fluorescens Rhizopusstolonifer* and *Microsporumgypseun)* and diameter of inhibition were recorded which are shown in tables (10-13). It was observed that all extracts of *M. oleifera* leaves prepared was effective against all selected bacterial and fungal micro-organisms. The antifungal and anti-bacterial activity of different extracts prepared from *M. oleifera* leaves was described separately in paragraphs.

Antimicrobial activity of Ethyl acetate leaves extracts of *M. oleifera*

From the results given in table 10 it was observed that ethyl acetate leaves extract was active against all the test microogranism the respective diameter of inhibition against ethyl acetate extracts of *M. oleifera.* The leaves extract were found to be more active against *B. megetarian,S. aureus, P flouresence*and *C. freundii*. And the individual diameter zones of inhibition were recorded are (12.33, 11.33, 9.3, 8.00, 7.67, 7.33, 6.67)

mm, (10.33, 9.66, 9.33, 8.67, 8.00, 7.33) mm, (12.66, 11.00, 10.33, 10.00, 9.00, 7.67, 7.33) mm and (11.00, 10.00, 8.67, 8.00, 7.00, 6.67) mm while For fungal species ethyl acetate leaves extracts were more active against *M. gypseum*showing growth of inhibition (9.67, 8.66, 8.33, 8.00, 6.67, 7.33, 7.67) mm in comparison to the *R. stolonifer* showing of inhibition (8.67, 8.33, 7.67, 7.33, 7.00, 6.67) mm respectively.

Antimicrobial activity of Methanol leaves extracts of *M. oleifera*

From the results given in table 11 it was observed that methanol leaves extract was active against all the test microogranism the respective diameter of inhibition against methanol extracts.

Methanol extracts of *M. oleirera* leaves had the maximum antibacterial activity against *P. flouresence* then *B. megetarianS.aureus* diameter of inhibitions were (13.33, 10.33, 10.00, 9.67, 9.00, 8.66) mm, (12.00, 11.33, 10.00, 9.67, 8.33, 7.66, 7.33) mm, (11.33, 9.66, 9.00, 8.67, 8.00, 7.33, 7.00) mm, respectively and *C. freundii* showed slightlyless antibacterial activity as compare to above pathogen a with diameter of inhibition were (11.33, 10.33, 9.00, 8.00, 6.67, 6.33) mm.

In case of fungus the methanol extract had maximum antifungal activity against *R.stolonifer* and the diameter of inhibition were (9.66, 8.67, 8.33, 7.66, 7.33, 6.67, 6.33) mm, in comparison to the *M. gypseum* showing diameter of inhibition observed were (9.67, 8.67, 8.33, 7.67, 6.33, 6.66) mm repetitively.

Antimicrobial activity of aqueous leaves extracts of *M. oleifera*

From the results given in table 12 it was observed that aqueous leaves extract was active against all the test microogranism the respective diameter of inhibition.

The aqueous extracts of leaves had maximum antibacterial activity against all the four tested bacteria, *S. aureus, P. flouresence, C.freundii*and *B. megetarian*the respective diameter of inhibition were observed (10.00, 9.67, 9.00, 8.66, 8.33, 8.00, 7.67) mm, (9.00, 8.67, 8.33, 8.00, 7.67,7.33,8.33) mm, (8.66, 8.33, 8.00, 7.67, 7.00, 6.67, 6.33) mm and (10.66, 9.00, 8.67, 6.66, 8.00, 7.66, 7.00) mm respectively. Same result showed by aqueous leaves extract in case of fungal species both *R. stolonifer*, *M. gypseum* the diameter of inhibition observed were (8.66, 8.33, 7.67, 7.33, 7.00, 6.67, 6.30) mm, (8.67, 8.00, 7.66, 7.33, 7.00, 6.67, 6.33) mm respectively.

Antimicrobial activity of chloroform leaves extracts of *M. oleifera*

From the results given in table 13 it was observed that chloroform leaves extract was active against all the test microogranism the respective diameter of inhibition against the chloroform extract of *M.oleifera* leaves were found to be most active against the three bacteria out of four *C. freundii, P. flouresence* and *B. megetarian.* And the individual diameter of inhibition recorded were (16.33, 14.00, 12.00, 8.67, 7.67, 7.00) mm,

(14.00, 11.66, 10.66, 10.00, 9.00, 8.33, 7.33) mm, (12.67, 11.67, 10.00, 9.00, 8.67, 7.33, 7.00) mm respectively.

While itwere less effective against *Staphylococcus aureus*showing diameter of inhibition (8.66, 8.33, 7.33, 7.00) mm. In case of fungal species, chloroform extract of leaves were more active against *M.gypseum*dynamometer of inhibition were (9.67, 8.67, 8.33, 7.33, 6.33, 6.67, 7.00) mm, in comparison to *R. stolonifer*diameter of inhibition observed were (8.67, 8.33, 7.33, 7.67) mm respectively

3. PHYTOCHEMICAL ANALYSIS OF DIFFERENT EXTRACTS OF *MORINGA OLEIFERA*

Phytochemicals are, in the strictest sense of the word, chemicals produced by plants. Plants generally contain 10 phytoconstituents namely anthraglycosides, arbutin, bitter drugs, flavonoids, alkaloids, saponins, coumarins, phenol carboxylic acids, terpenes and valepotriates. These phytoconstituents confer specific characteristics and properties to plants. Therefore, the analysis of these constituents in plants would help in determining various biological activities of plants

Considering the importance of this plant, an attempt was made in the present study to carry out. Phytochemical screening of different bark and leaf extracts of *Moringaoleifera*. The preliminary phytochemical screening of the bark and leaf extracts using different organic solvent was reported Table 14 and 15.

The aqueous leaves extract of *M. oleifera* showed positive result for the presence of alkaloid, carbohydrate, tanins, flavonoid and steroids glycoside.

While two different leaves extracts of ethyl acetate and chloroform of *M. oleifera* showed positive result for the presence of alkaloid, carbohydrate and flavonoid,triterpnoid.Negative result showed for the absence of glycoside and tannin by chloroform extract. Absence of steroids was found in ethyl acetate extract. The methanolic leaves extract of *M. oleifera* showed positive result for the presence of alkaloid, carbohydrate, tanins, flavonoid and steroids. Glycoside and negative result for tanins.

The preliminary phytochemical screening of the bark extract using solvent were reported in Table 14.

The aqueous bark extract of *M. oleifera* showed positive result for the presence of alkaloid, tanins, and flavonoid triterpnoid negative result showed for the absence of glycoside, carbohydrate.

While two different leaf fractions of chloroform and ethyl acetate extract of *M. oleifera* showed positive result for the presence of alkaloid and tanin, triterpnoid and glycoside. Negative result showed for the absence of carbohydrate, flavonoid.

The methanolic leaves extract of *M. oleifera* showed positive result for the presence of alkaloid, carbohydrate, tanin, flavonoid and negative result for tanins steroids and glycoside.

The preliminary phytochemical screening of the leaves extract using solvent were reported in Table 15.

4. TLC (THIN LAYER CHROMATOGRAPHY)

Detection of different compounds in *Moringaoleifera* leaf and bark extract

The TLC plates were developed using a Camag twin-trough glass tank which was pre-saturated with the mobile phase chloroform-methanol (95:5) for alkaloids and the mobile phase ethyl alcholol - chloroform (1:3) for flavonoids are kept 1 hour and each plate was developed to a height of about 8 cm. The composition of mobile phase was optimized by using different mobile solvents of varying polarity. The TLC runs were made under laboratory conditions of 25 ± 50C and 50% relative humidity. After development, the plate was removed and dried and spots were visualized in UV light (UV cabinet, Camag, Switzerland) (Table - 15).

Table 4: Table showing the antibiogram of isolated *S. aureus* and *C. freundii*

S.No	Antibiotics	Symbol	*Staphylococcus aureus*		*Citrobacterfreundii*	
			Diameter of inhibition in (mm)	**Result**	**Diameter of inhibition in (mm)**	**Result**
1	Cephalothin	Ch	No Zone	Resistant	No Zone	Resistant
2	Clindomycin	Cd	14	Sensitive	12	Intermediate
3	Co-Trimoxazole	Co	8	Intermediate	7	Intermediate
4	Erythromycin	E	16	Sensitive	12	Intermediate
5	Gentamicin	G	18	Sensitive	16	Sensitive
6	Ofloxacin	Of	18	Sensitive	18	Sensitive
7	Penicillin-G	P	9	Intermediate	18	Sensitive
8	Vaneomycin	Va	10	Intermediate	8	Intermediate

Table 5: Table showing the antibiogram of isolated *Bacukkysnagaterium* and *P. fluorescence*

S.No	Antibiotics	Symbol	*Bacillus megaterium*		*Pseudomonas Fluorescence*	
			Diameter of inhibition in (mm)	**Result**	**Diameter of inhibition in (mm)**	**Result**
1.	Cephalothin	Ch	8	Intermediate	7	Intermediate
2.	Clindomycin	Cd	8	Intermediate	9	Intermediate
3.	Co-Trimoxazole	Co	7	Intermediate	7	Intermediate
4.	Erythromycin	E	10	Intermediate	10	Intermediate
5.	Gentamicin	G	12	Sensitive	16	Sensitive
6.	Ofloxacin	Of	No Zone	Resistant	18	Sensitive
7.	Penicillin-G	P	No Zone	Resistant	7	Sensitive
8.	Vaneomycin	Va	7	Intermediate	10	Intermediate

Table 6: Antimicrobial assay of *M. oleifera* ethyl acetate bark extract against various test microorganisms

Pathogens	Zone of inhibition in (mm)							
	200mg/ ml	**100mg/ ml**	**50mg/ ml**	**25mg /ml**	**12.5mg/ ml**	**6.25mg/ ml**	**3.125mg/ ml**	**Drug**
S.aureus	12.22.00 ± 1.52	12.61 ± 1.53	10.13 ± 0.57	8.36 ±1.53	8.20 ± 1.00	7.10 ± 0.58	-	20
C.freundii	13.30 ± 2.05	11.50 ± 2.60	10.03 ± 1.73	9.26 ±1.15	9.00 ± 1.00	7.66 ± 0.58	6.96 ± 0.57	19
B.megaterium	9.687± 1.52	8.67 ± 0.58	8.00 ± 1.00	7.74 ±2.00	6.67 ± 0.58	6.34 ± 0.57	-	19
P.fluorrescens	12.67 ± 1.15	11.66 ± 1.53	11.66 ± 1.52	9.67 ±1.15	9.23 ± 0.58	8.00 ± 1.00	7.43 ± 0.57	16
R.stolonifer	9.00 ± 2.00	8.66 ± 1.52	8.33 ± 1.15	7.63 ±0.05	7.20 ± 1.00	-	-	-
M.gypseum	8.66 ± 1.15	8.33 ± 1.00	7.66 ± 0.58	7.33 ±0.57	7.07 ± 1.00	6.69 ± 0.58	-	-

Table 7: Antimicrobial assay of *M. oleifera* methanol bark extract against various test microorganisms

Pathogens	Zone of inhibition in (mm)							
	200mg/ ml	**100mg/ ml**	**50mg/ ml**	**25mg/ ml**	**12.5mg/ ml**	**6.25mg/ ml**	**3.125mg/ ml**	**Drug**
S.aureus	12.99 ± 1.52	12.57 ± 1.53	10.43 ± 0.57	8.76 ± 1.53	7.90 ± 1.00	6.99 ± 0.58	-	21
C.freundii	13.99 ± 2.05	11.99 ± 2.60	11.23 ± 1.73	10.76 ± 1.15	9.99 ± 1.00	9.26 ± 0.58	8.45 ± 0.57	19
B.megaterium	9.66 ± 1.52	8.67 ± 0.58	8.00 ± 1.00	7.00 ± 2.00	6.67 ± 0.58	6.33 ± 0.57	-	19
P.fluorrescens	13.67 ± 1.15	11.66 ± 1.53	10.66 ± 1.52	9.67 ± 1.15	9.33 ± 0.58	8.00 ± 1.00	7.33 ± 0.57	17
R.stolonifer	9.00 ± 2.00	8.66 ± 1.52	8.33 ± 1.15	7.66 ± 0.05	7.00 ± 1.00	-	-	-
M.gypseum	8.66 ± 1.15	8.33 ± 1.00	7.66 ± 0.58	7.33 ± 0.57	7.00 ± 1.00	6.67 ± 0.58	-	-

Table 8: Antimicrobial assay of aqueous extract *M. oleifera* bark against various test microorganisms

Pathogens	Zone of inhibition in (mm)							
	200mg/ ml	**100mg/ ml**	**50mg/ ml**	**25mg/ ml**	**12.5mg/ ml**	**6.25mg/ ml**	**3.125mg/ ml**	**Drug**
S.aureus	10.00 ± 1.15	9.67 ± 0.58	8.67 ± 1.00	8.00 ± 0.57	7.33 ± 0.58	-	-	18
C.freundii	12.33 ± 0.58	11.66 ± 1.15	10.66 ± 0.58	9.66 ± 1.00	9.33 ± 1.15	8.33 ± 0.57	-	17
B.megaterium	9.66 ± 0.58	8.67 ± 1.15	8.00 ± 1.00	7.66 ± 1.15	7.33 ± 0.57	6.67 ± 0.58	-	18
P.fluorrescens	9.23 ± 1.00	8.67 ± 0.58	8.33 ± 0.57	8.00 ± 1.00	7.67 ± 0.58	7.00 ± 1.00	7.66 ± 0.58	14
R.stolonifer	9.66 ± 1.52	8.33 ± 1.15	8.00 ± 0.57	7.66 ± 0.58	6.66 ± 1.15	6.33 ± 0.57	-	-
M.gypseum	9.00 ± 1.73	8.66 ± 1.15	8.33 ± 1.00	7.67 ± 0.58	7.33 ± 0.57	7.00 ± 1.00	6.67 ± 0.57	-

Table 9: Antimicrobial assay of chloroform extract of *M. oleifera* bark against various test microorganisms

Pathogens	Zone of inhibition in (mm)							
	200mg/ ml	**100mg/ ml**	**50mg/ ml**	**25mg/ ml**	**12.5mg/ ml**	**6.25mg/ ml**	**3.125mg/ ml**	**Drug**
S.aureus	15.00 ± 1.00	13.33 ± 1.52	11.00 ± 1.73	9.67 ± 0.58	9.00 ± 1.00	8.67 ± 0.58	-	13
C.freundii	12.67 ± 1.53	11.33 ± 1.52	10.67 ± 0.57	9.33 ± 1.53	8.66 ± 0.57	-	-	14
B.megaterium	14.67 ± 2.08	13.33 ± 1.73	11.00 ± 1.00	9.33 ± 1.16	7.66 ± 0.58	-	-	16
P.fluorrescens	12.33 ± 1.53	10.67 ± 0.58	9.67 ± 0.58	9.33 ± 1.15	8.00 ± 1.00	7.00 ± 0.57	6.67 ± 0.58	17
R.stolonifer	9.33 ± 1.51	9.00 ± 1.00	8.33 ± 0.58	7.66 ± 1.15	7.00 ± 1.00	6.67 ± 0.58	6.33 ± 0.57	-
M.gypseum	9.00 ± 1.51	8.66 ± 1.52	8.33 ± 0.58	7.67 ± 1.15	7.33 ± 0.57	7.00 ± 1.00	-	-

Table 10: Antimicrobial activity of *M oleifera*. Ethylacetate leave extract against different test microorganisms

Pathogens	Zone of inhibition in (mm)							
	200mg/ ml	**100mg/ ml**	**50mg/ ml**	**25mg/ ml**	**12.5mg/ ml**	**6.25mg/ ml**	**3.125mg/ ml**	**Drug**
S.aureus	10.33 ± 0.58	9.66 ± 0.58	9.33 ± 0.57	8.67 ± 0.58	8.00 ± 1.00	7.33 ± 0.58	6.67 ± 0.57	19
C.freundii	11.00 ± 1.00	10.00 ± 1.00	8.67 ± 0.58	8.00 ± 1.15	7.00 ± 1.00	6.67 ± 0.58	6.33 ± 0.57	18
B.megaterium	12.33 ± 2.08	11.33 ± 1.52	10.33 ± 1.52	9.33 ± 1.16	8.00 ± 1.00	7.67 ± 0.58	7.33 ± 0.57	25
P.fluorrescens	12.66 ± 1.52	11.00 ± 1.00	10.00 ± 0.58	9.00 ± 1.00	7.67 ± 0.58	7.33 ± 0.57	6.67 ± 0.58	27
R.stolonifer	8.67 ± 1.51	8.33 ± 1.08	7.67 ± 1.52	7.33 ± 0.58	7.00 ± 1.00	6.67 ± 0.58	-	-
M.gypseum	9.67 ± 1.57	8.66 ± 0.58	8.33 ± 0.57	8.00 ± 1.00	7.67 ± 0.58	7.33 ± 0.57	6.67 ± 0.58	-

Table 11: Antimicrobial activity of *M oleifera*.methanol leave extract against different test microorganisms

Pathogens	Zone of inhibition in (mm)							
	200mg/ ml	**100mg/ ml**	**50mg/ ml**	**25mg/ ml**	**12.5mg/ ml**	**6.25mg/ ml**	**3.125mg/ ml**	**Drug**
S.aureus	11.33 ± 0.58	9.66 ± 0.57	9.00 ± 1.00	8.67 ± 0.75	8.00 ± 0.57	7.33 ± 0.58	7.00 ± 0.57	20
C.freundii	11.33 ± 1.15	10.33 ± 1.15	9.00 ± 1.00	8.00 ± 0.75	6.67 ± 0.58	6.33 ± 0.57	-	21
B.megaterium	12.00 ± 1.00	11.33 ± 1.00	10.00 ± 1.58	10.00 ± 1.00	9.67 ± 1.15	7.66 ± 0.58	7.33 ± 0.57	22
P.fluorrescens	13.33 ± 1.52	11.00 ± 1.00	10.33 ± 0.15	9.67 ± 1.15	9.00 ± 1.00	8.66 ± 0.58	8.33 ± 0.57	24
R.stolonifer	9.66 ± 2.30	8.67 ± 1.15	8.33 ± 0.58	7.66 ± 0.58	7.33 ± 0.57	6.67 ± 0.58	6.33 ± 0.57	-
M.gypseum	9.67 ± 2.25	8.67 ± 1.16	8.33 ± 1.00	7.67 ± 0.58	6.66 ± 0.58	6.33 ± 0.57	-	-

Table 12: Antimicrobial activity of *M oleifera*.aqueous leave extract against different test microorganisms

Pathogens	Zone of inhibition in (mm)							
	200mg/ ml	**100mg/ ml**	**50mg/ ml**	**25mg/ ml**	**12.5mg/ ml**	**6.25mg/ ml**	**3.125mg/ ml**	**Drug**
S.aureus	10.00 ± 1.98	9.67 ± 1.15	9.00 ± 1.00	8.66 ± 0.58	8.33 ± 0.57	8.00 ± 1.00	7.67 ± 0.58	18
C.freundii	8.66 ± 1.16	8.33 ± 0.58	8.00 ± 1.00	7.67 ± 0.58	7.00 ± 1.00	6.67 ± 0.58	6.33 ± 0.57	19
B.megaterium	10.66 ± 1.76	9.00 ± 2.00	8.67 ± 0.65	8.33 ± 0.57	8.00 ± 1.00	7.66 ± 0.58	7.00 ± 1.00	15
P.fluorrescens	9.00 ± 1.00	8.67 ± 1.15	8.33 ± 0.75	8.00 ± 0.11	7.67 ± 0.58	7.33 ± 0.41	6.66 ± 0.57	20
R.stolonifer	8.66 ± 1.51	8.33 ± 1.08	7.67 ± 0.58	7.33 ± 0.57	7.00 ± 1.00	6.67 ± 0.58	6.30 ± 0.57	-
M.gypseum	8.67 ± 1.15	8.00 ± 0.58	7.66 ± 0.57	7.33 ± 0.65	7.00 ± 1.00	6.67 ± 0.58	6.33 ± 0.57	-

Table 13: Antimicrobial activity of *M oleifera*. chloroform leave extract against different test microorganisms

Pathogens	Zone of inhibition in (mm)							
	200mg/ ml	**100mg/ ml**	**50mg/ ml**	**25mg/ ml**	**12.5mg/ ml**	**6.25mg/ ml**	**3.125mg/ ml**	**Drug**
S.aureus	8.66 ± 0.58	8.33 ± 0.57	7.33 ± 0.58	7.00 ± 1.00	-	-	-	13
C.freundii	16.33 ± 2.08	14.00 ± 2.00	12.00 ± 1.00	10.00 ± 1.00	8.67 ± 0.58	7.67 ± 0.57	7.00 ± 1.00	14
B.megaterium	12.67 ± 1.52	11.67 ± 1.52	10.66 ± 0.58	9.00 ± 1.00	8.67 ± 1.15	7.33 ± 0.75	7.33 ± 0.65	13
P.fluorrescens	14.00 ± 1.00	11.66 ± 1.52	10.00 ± 1.00	9.00 ± 1.00	8.33 ± 0.58	7.33 ± 1.52	7.00 ± 1.00	19
R.stolonifer	8.67 ± 1.52	8.33 ± 2.08	7.67 ± 0.58	7.33 ± 0.57	-	-	-	-
M.gypseum	9.67 ± 1.52	8.67 ± 1.15	8.33 ± 0.58	7.33 ± 0.57	7.00 ± 1.15	6.67 ± 0.58	6.33 ± 0.57	-

Table 14: Phytochemical analysis of different bark extract of *M. oleifera*

Plant	Plant part used for extraction	Extract	Alkaloid	Glycoside	Carbohydrates	Tannin	Flavonoid	Steroids	Triterpnoid
M. oleifera	Bark	Ethyl acetate	+	-	-	+	-	+	+
		Water	+	-	+	+	+	+	+
		Chloroform	+	+	-	+	-	+	+
		Methanol	+	-	+	+	+	-	+

Table 15: Phytochemical analysis of different leaf extract of *M. oleifera*

Plant	Plant part used for extraction	Extract	Alkaloid	Glycoside	Carbohydrates	Tannin	Flavonoid	Steroids	Triterpnoid
M. oleifera	Leaf	Ethyl acetate	+	-	+	+	+	-	+
		Water	-	+	+	+	+	+	-
		Chloroform	+	-	+	-	-	+	+
		Methanol	+	+	+	-	+	+	+

Table 16: Thin layer chromatographic analysis of crude extract of *Moringaoleifera* (bark)

Plant	**Solvent system**	**TLC pattern (bands)**	**Rf values**
Moringaoleifera (Bark)	(A) Alkaloid	2	0.61
			0.67
	(B) Flavonoid	1	6.51

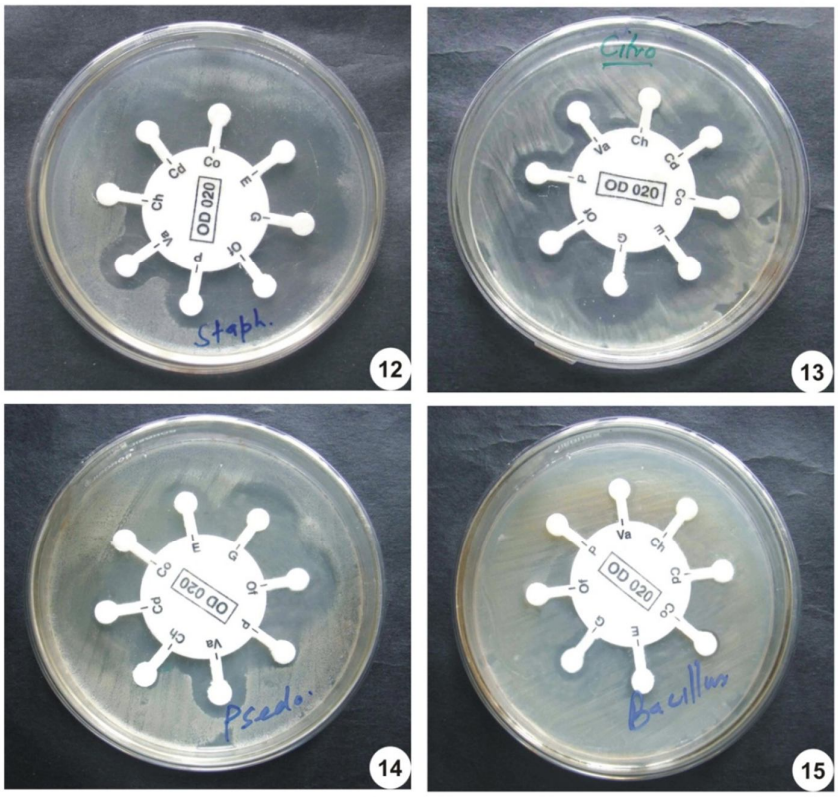

Plate 1: Fig. 12-15 showing antibigram of different test microorganisms.

(**Fig.12:** showing antibiogram of isolated *Staphylococcus aureus*; **Fig.13:** showing antibiogram of isolated Citrobacter freundii; **Fig.14:** showing antibiogram of isolated *Pseudomonas fluorescens*; **Fig.15:** showing antibiogram of isolated *Bacillus megaterium*)

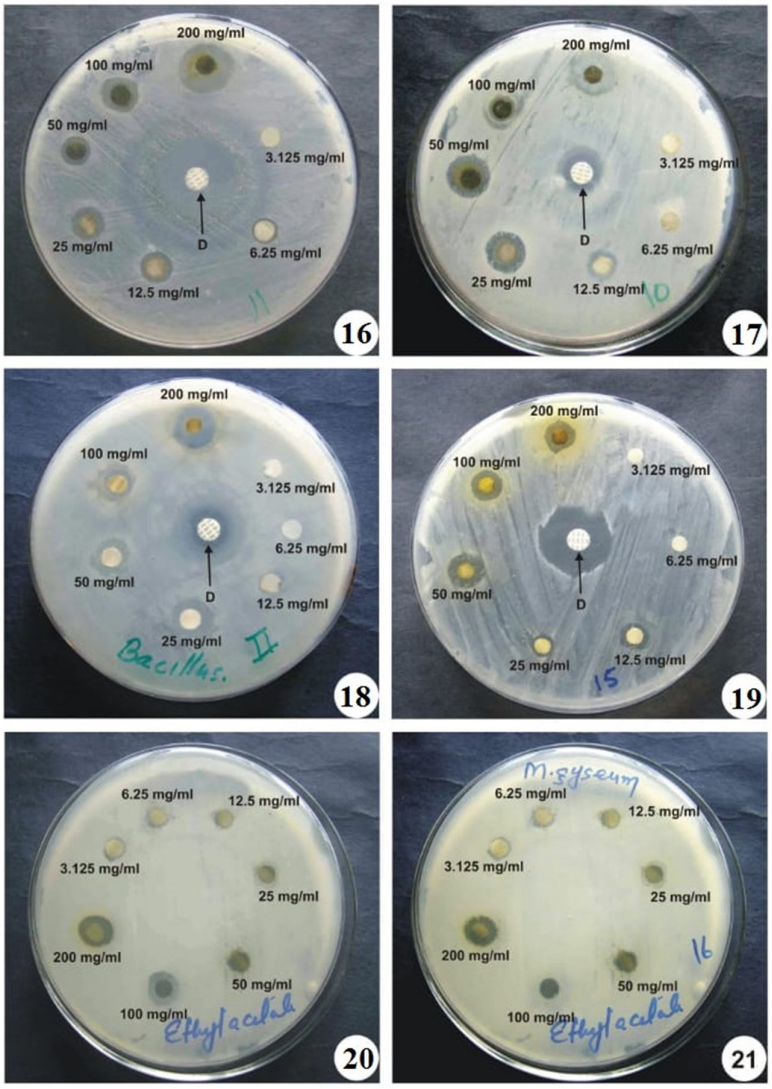

Plate 2: Fig. 16-21 showing antimicrobial activity of ethyl acetate bark extract of *Moringa olefera* against test microorganisms

(**Fig. 16:** zone of inhibition (in mm) of Ethyl acetate bark extract of *Moringa oliefera* against *Staphylococcus aureus*; **Fig. 17:** zone of inhibition (in mm) of Ethyl acetate bark extract of *Moringa oliefera* against *citrobactor freundii*; **Fig. 18:** zone of inhibition (in mm) of Ethyl acetate bark extract of *Moringa oliefera* against *Bacillus megaterium*; **Fig. 19:** zone of inhibition (in mm) of Ethyl acetate bark extract of *Moringa oliefera* against *Pseudomanas flurescens*; **Fig. 20:** zone of inhibition (in mm) of Ethyl acetate bark extract of *Moringa oliefera* against *Rhizopus stolonifer*; **Fig. 21:** zone of inhibition (in mm) of Ethyl acetate bark extract of *Moringa oliefera* against *Microsporum gypsem*)

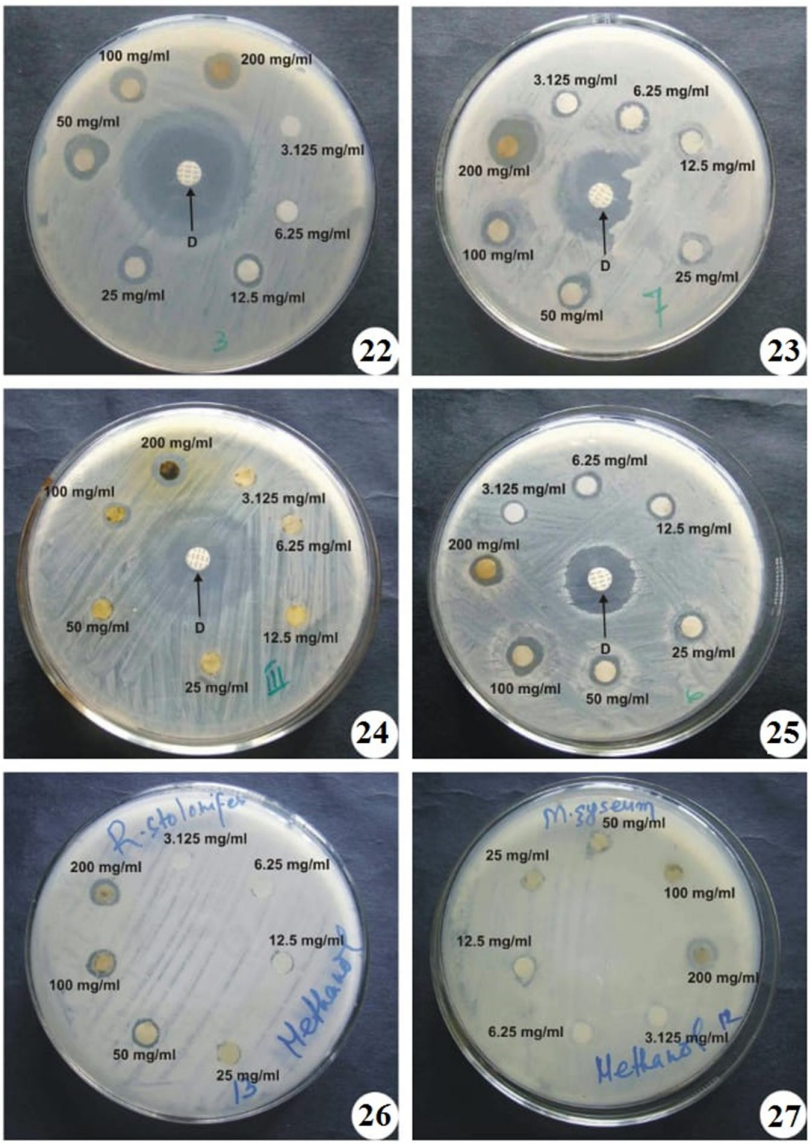

Plate 3: Fig. 22-27 showing antimicrobial activity of methanol bark extract of *Moringa oleifera* against test microorganisms

(**Fig. 22:** zone of inhibition (in mm) of methanol bark extract of *Moringa olifera* agaist *Staphylococcus aureus*; **Fig. 23:** zone of inhibition (in mm) of methanol bark extract of *Moringa olifera* agaist *Citrobacter freundii*; **Fig. 24:** zone of inhibition (in mm) of methanol bark extract of *Moringa olifera* agaist *Bacillus megaterium*; **Fig. 25:** zone of inhibition (in mm) of methanol bark extract of *Moringa olifera* agaist *Pseudomonas flurescens;* **Fig. 26:** zone of inhibition (in mm) of methanol bark extract of *Moringa olifera* agaist *Rhizopus stolonifer;* **Fig. 27:** zone of inhibition (in mm) of methanol bark extract of *Moringa olifera* agaist *Microsporum gypsem*)

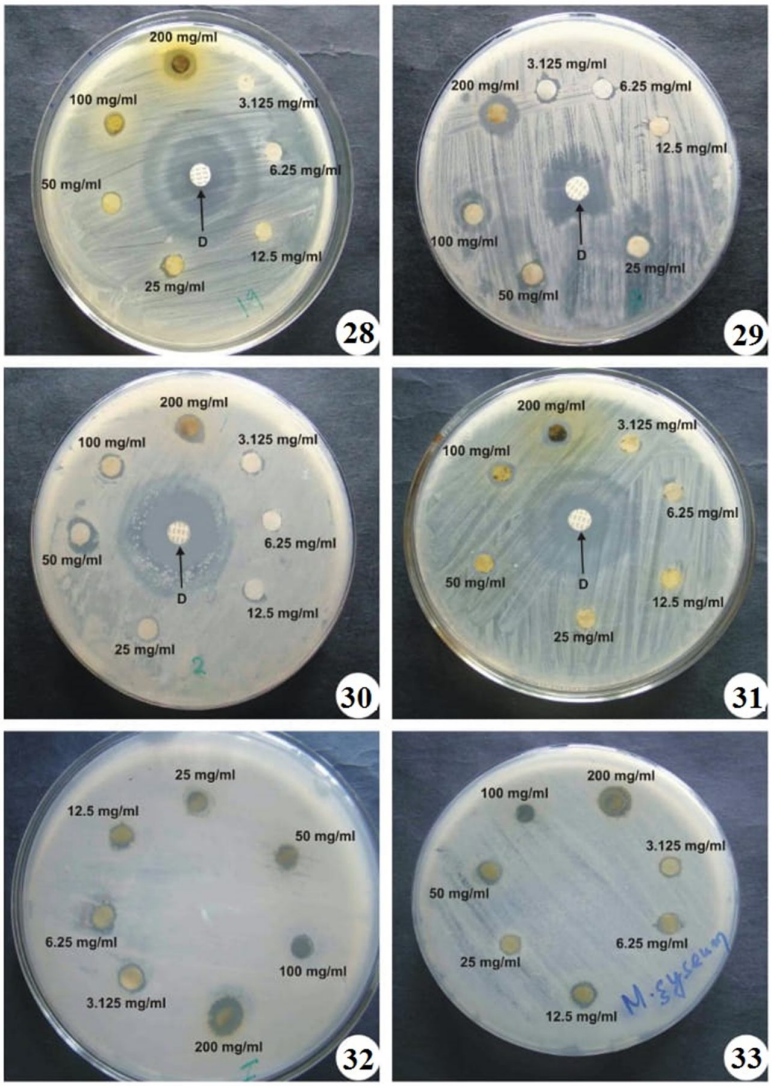

Plate 4: Fig. 28-33 showing antmicrobial activity of aqueous bark extract of *Moringa oleifera* against test microorganisms

(**Fig. 28:** zone of inhibition (in mm) of Aqueous bark extract of *Moringa oleifera* against *Staphylococcus aureus*; **Fig. 29:** zone of inhibition (in mm) of Aqueous bark extract of *Moringa oleifera* against Citrobacter freundii; **Fig. 30:** zone of inhibition (in mm) of Aqueous bark extract of *Moringa oleifera* against *Bacillus megaterium*: **Fig. 31:** zone of inhibition (in mm) of Aqueous bark extract of *Moringa oleifera* against *Pseudomonas fluorescens*; **Fig. 32:** zone of inhibition (in mm) of Aqueous bark extract of *Moringa oleifera* against *Rhizopus stolonifer*; **Fig. 33:** zone of inhibition (in mm) of Aqueous bark extract of *Moringa oleifera* against *Microsporum gypseum*)

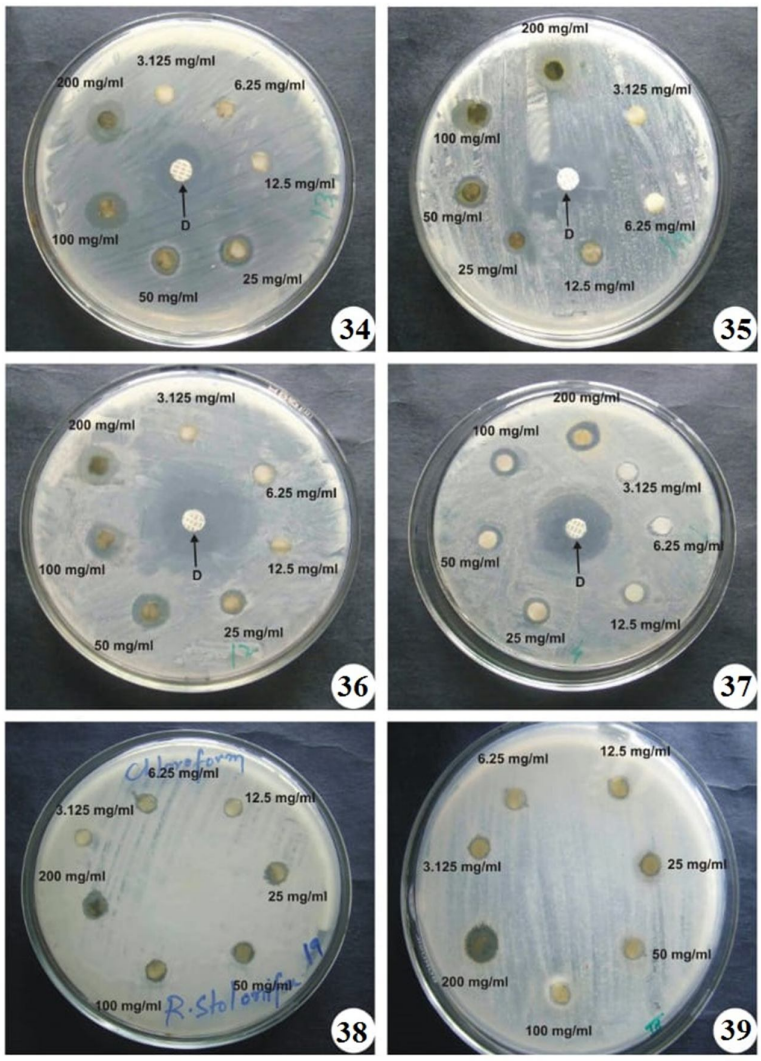

Plate 5: Fig. 34-39: Showing antimicrobial activity of chloroform leaves extracts of *Moringa oleifera* against test microorganisms.

(**Fig. 34:** zone of inhibition (in mm) of Chloroform bark extract of *Moringa oleifera* against *Staphylococcus aureus*; **Fig. 35:** zone of inhibition (in mm) of Chloroform bark extract of *Moringa oleifera* against *Citrobacter freundii*; **Fig. 36:** zone of inhibition (in mm) of Chloroform bark extract of *Moringa oleifera* against *Bacillus megaterium*; **Fig. 37:** zone of inhibition (in mm) of Chloroform bark extract of *Moringa oleifera* against *Pseudomonas fluorescens*; **Fig. 38:** zone of inhibition (in mm) of Chloroform bark extract of *Moringa oleifera* against *Rhizopus stolonifer*; **Fig. 39:** zone of inhibition (in mm) of Chloroform bark extract of Meringa oleifera against Microsporum gypsum)

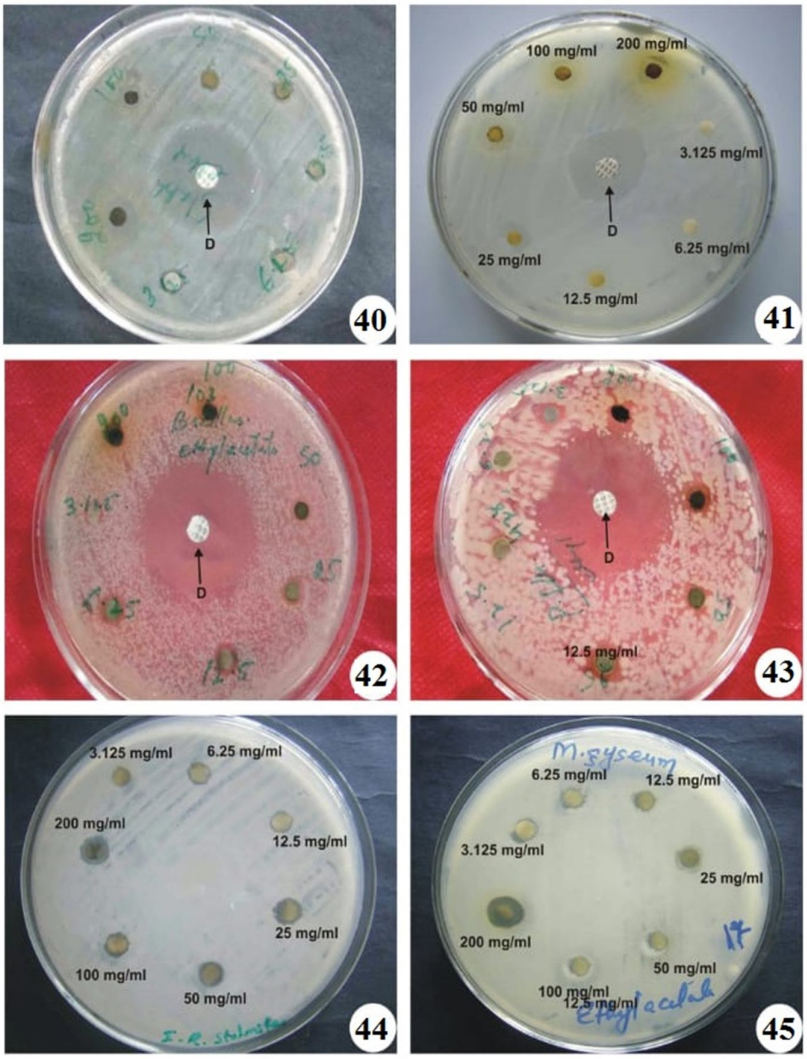

Plate 6: Fig 40-45 showing antimicrobial activity of ethyl acetate leaves extract of *Moringa oleifera* against test microorganisms.

(**Fig. 40:** zone of inhibition (in mm) of Ethyl acetate leaves extract of *Moringa oleifera* against *Staphylococcus aureus*; **Fig. 41:** zone of inhibition (in mm) of Ethyl acetate leaves extract of *Moringa oleifera* against *Citrobacter freundi*; **Fig. 42:** Showing zone of inhibition (in mm) of Ethyl acetate leaves extract of *Moringa oleifera* against Bacillus meguterium; **Fig. 43:** zone of inhibition (in mm) of Ethyl acetate leaves extract of Moringa cleifera against *Pseudomonas fluorescens*; **Fig. 44:** zone of inhibition (in mm) of Ethyl acetate leaves extract of Meringa oleifera against Rhizopus tclonifer; **Fig. 45:** zone of inhibition (in mm) of Ethyl acetate Seaves extract of Moringa oleifend against Microsporsan gypsum)

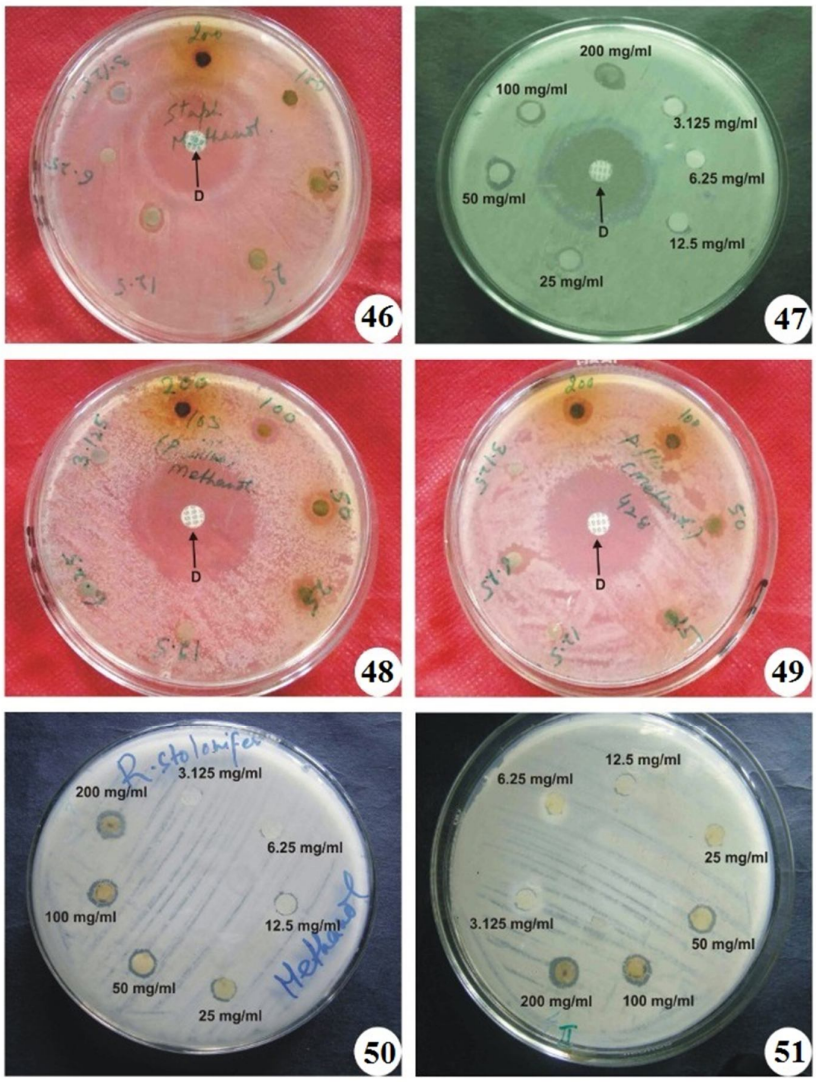

Plate 7: Fig. 46-51: Showing antimicrobial activity of methanol leaves extracts of *Moringa oleifera* against test microorganisms.

(**Fig. 46:** zone of inhibition (in mm) of methanol leaves extract of *Meringa oleifera* against *Staphylococcus aureus*; **Fig. 47:** zone of inhibition (in mm) of methanol leaves extract of *Moringa oleifera* against Citrobacter freundii; **Fig. 48:** zone of inhibition (in mm) of methanol leaves extract of *Moringa oleifera* against *Bacillus megaterium*; **Fig. 49:** zone of inhibition (in mm) of methanol leaves extract of *Moringa oleifera* against *Pseudomonas fluorescens*; **Fig. 50:** zone of inhibition (in mm) of methanol leaves extract of *Moringa oleifera* against *Rhizopus stolonifer*; **Fig. 51:** zone of inhibition (in mm) of methanol leaves extract of *Moringa oleifera* against *Microsporum gypseum*)

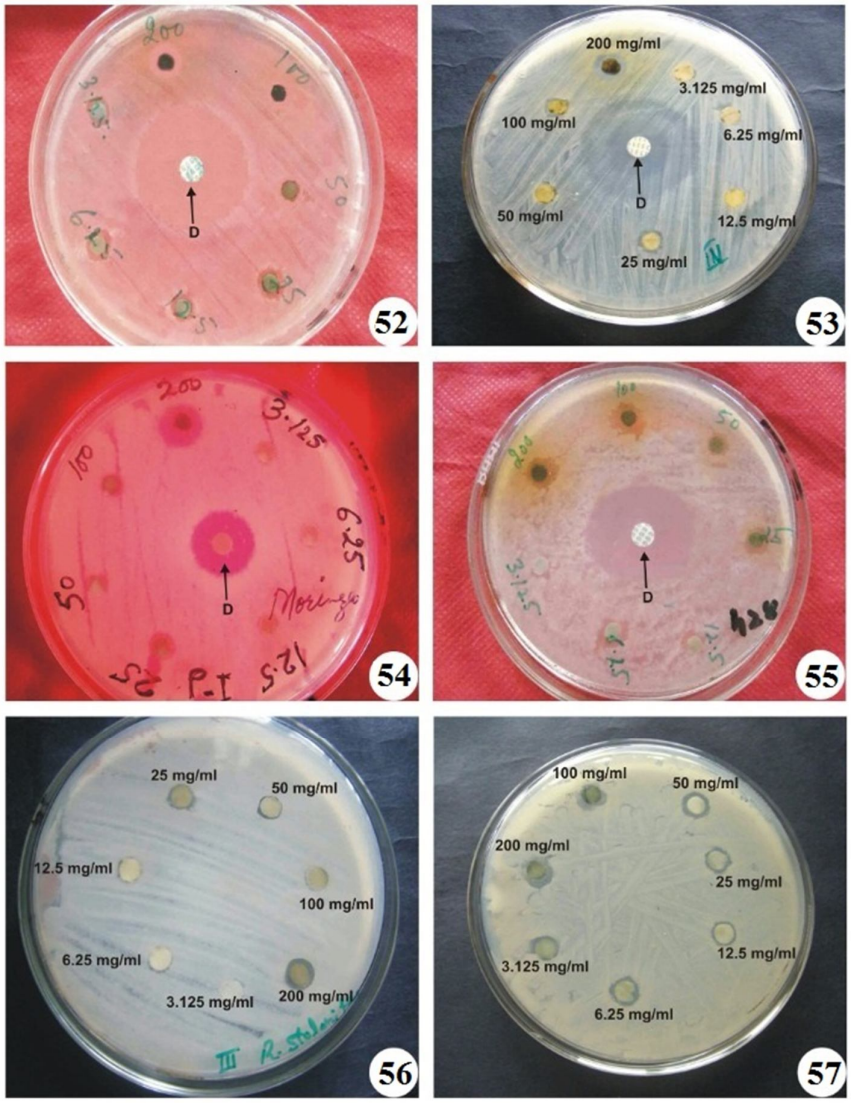

Plate 8: Fig. 52-57: Showing antimicrobial activity of aqueous leaves extracts of *Moringa oleifera* against test microorganisms.

(**Fig. 52:** zone of inhibition (in mm) of aqueous leaves extract of *Moringa oleifera* against *Staphylococcus aureus*; **Fig. 53:** zone of inhibition (in mm) of aqueous leaves extract of *Moringa oleifera* against Citrobacter freundii; **Fig. 54:** zone of inhibition (in mm) of aqueous leaves extract of *Moringa oleifera* against *Bacillus megaterium*; **Fig. 55:** zone of inhibition (in mm) of aqueous leaves extract of *Moringa oleifera* against *Pseudomonas fluorescens*; **Fig. 56:** zone of inhibition (in mm) of aqueous leaves extract of *Moringa oleifera* against *Rhizopus stolonifer*; **Fig. 57:** one of inhibition (in mm) of aqueous leaves extract of *Moringa oleifera* against *Microsporum gypseum*)

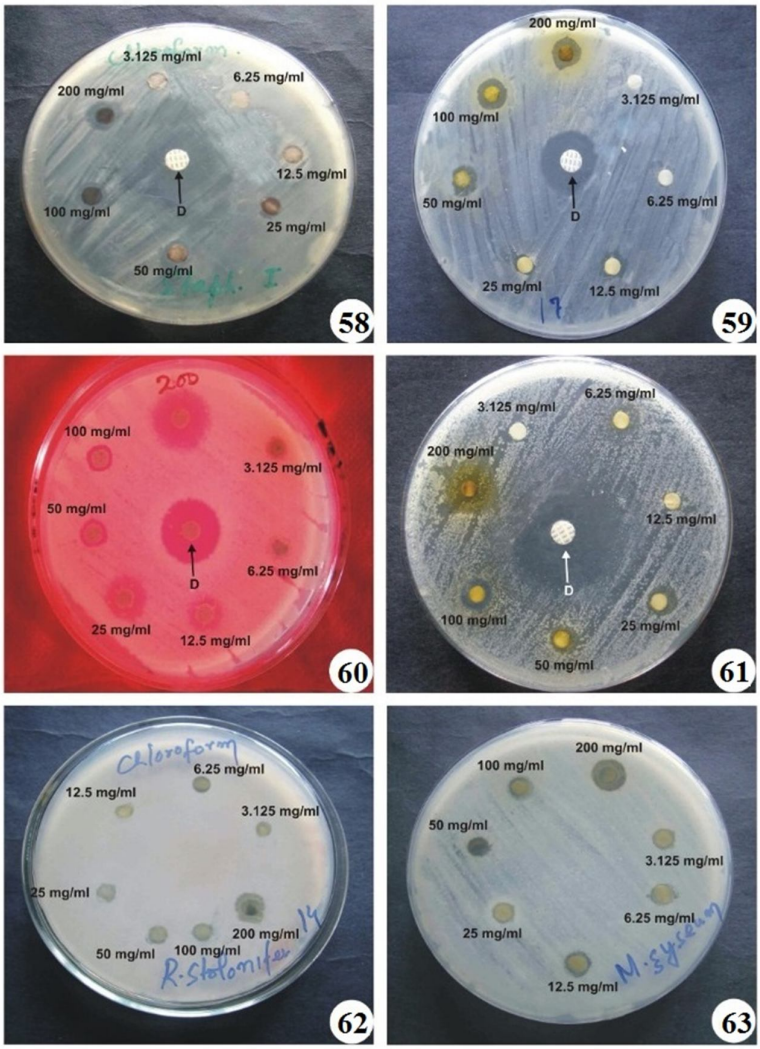

Plate 9: Fig. 58-63: Showing antimicrobial activity of chloroform leaves extracts of *Moringa oleifera* against test microorganisms.

(**Fig. 58:** zone of inhibition (in mm) of chloroform leaves extract of *Moringa oleifera* against *Staphylococcus aureus;* **Fig. 59:** one of inhibition (in mm) of chloroform leaves extract of *Moringa oleifera* against Citrobacter freundi; **Fig. 60:** rone of inhibition (in mm) of chloroform leaves extract of Moringa oleifers against Bacillus megateruim; **Fig. 61:** one of inhibition (in mm) of chloroform leaves extract of Moringa chloroform leaves extract of *Moringa oleifera* against *Pseudomonas fluorescens*; **Fig. 62:** zone of inhibition (in mm) of chloroform leaves extract of Maringa oleifera against *Rhizopus stolonifer*; **Fig. 63:** zone of inhibition (in mm) of chloroform leaves extract of Maringa oleifera against Microsporum gypsum)

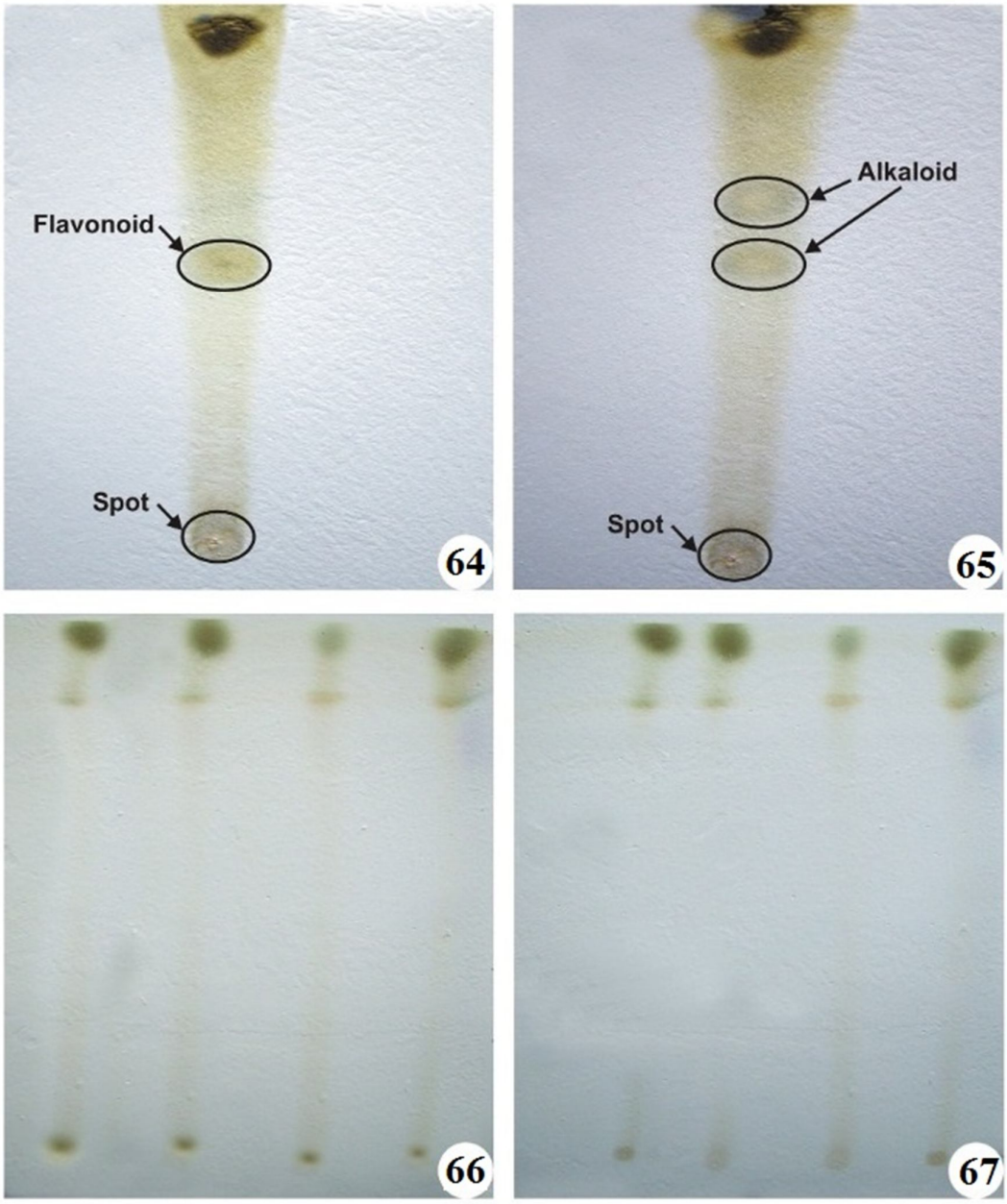

Plate 10: Fig. 64-67 showing thin layer chromatographic analysis of different extracts of leaves and bark of *M. olifera*

(**Fig. 64:** Aqueous Extract; **Fig. 65:** Methanolic Extract; **Fig. 66:** Ethyl Acetate Extract; **Fig. 67:** Chloroform Extract)

Discussion

Chapter- 6 DISCUSSION

Plants are important source of potentially useful structures for the development of new chemotherapeutic agents. The first step towards this goal is the *in vitro* antibacterial activity assay (Tona *et al.*1998) Previous studies have reported that various parts of *Moringa* roots, flowers, bark, and stem including seeds possess antimicrobial properties (Lockett *et al*., 2000; Anwar and Rashid, 2007).

The various extracts of *Moringa oleifera* namely ethyl acetate, methanol, aqueous and chloroform extracts of its leaves and bark were tested against *Staphylococcus aureus, citro freundii, Bacillus megetarian, pseudomonas flouresence,* for their antibacterial activity and against *R. stlonifer* and *M.gypseum* for their antifungal activity. The results are presented in table (6-13). The extracts of ethyl acetate, methanol, aqueous and chloroform of *Moringa oleifera* leaves and bark were found to be active against all the test organisms.

Results obtained from *in vitro* antimicrobial activity of *Moringa oleifera*. It was observed that the ethyl acetate extract of *Moringa oleifera* bark was more active against *Staphylococcus aureus, Bacillus megetarian, pseudomonas flouresence* ,and showing inhibition effect up to dilution of 6.25mg/ml while it was comparatively less active against

citrobacter freundii showing zone of inhibition up to dilution of 12.5mg/ml. Antimicrobial properties of bark of *Moringa oleifera* as shown in present study corroborate the earlier claims by Dewangan *et al.*, (2010) who reported that ethyl acetate has maximum antibacterial activity against *Staphylococcus aureus.* For fungal species ethyl acetate bark extracts were active against both *R.stolonifer* and *M.gypseum* showing zone of inhibition up to dilution of 6.25mg/ml. results are shown in table (6). Bhatnagar *et al.,* 1961 also reported that the bark extract of *M. oleifera* have antimicrobial activity.

Observations reveled that extract of methanol of *Moringa oleifera* bark shown higher inhibitory effect up to dilution of 3.125mg/ml against *Pseudomonas flouresence* and *Citro freundii* while when when the inhibitory is less in case of *Staphylococcus aureus, Bacillus megetarian* showing inhibitory zone up to dilution of 6.25mg/ml. similar findings are observed in the study of Rao *et al*, (2011) who evaluated antibacterial potential by using well diffusion technique of methanolic extract of *M.oleifera* and found that extract was most active against , while Devi *et al.*, (2011) also experimented to access the antibacterial potential of methanolic extracts of *Moringa oleifera* bark by agar well diffusion method against *Bacillus,* and *Staphylococcus aureus* that frequently cause enteric infections in humans, his observation reveled tha tbiomolecules

present in methanol extracts of *Moringa oleifera* bark, have shown greater inhibitory effect against the selected microorganisms.

In case of fungal species, methanol extracts of bark were more active against *M.gypseum* showing inhibitory effect at concentration of 6.25mg/ml in comparison to *R.stolonifer* inhibition at dilution of 12.5mg/ml as results are shown in table (7).

Results reveled that aqueous extracts of the bark demostrated strong Antibacterial potential against *pseudomonas flouresence* showing the zone of inhibitions at dilution of 3.125mg/ml while in case of, *Bacillus megetarian, Citrobacter freundii, Staphylococcus aureus* inhibitory zone was observed up at concentration of 6.25mg/ml. Antimicrobial properties of bark of *Moringa oleifera* as shows similarity with the earlier claims by Caceres *et al.* (1991) who reported antimicrobial activities of *Moringa oleifera*, bark *in vitro* against bacteria, yeast, dermatophytes and helminths by a disk-diffusion method. *Pseudomonas spp* and *Staphylococcus aureus* was found sensitive by aqueous extracts obtained from the bark. While similar findings was observed in study of Devi *et al.*, (2011) who found that aqueous extracts of *Moringa oleifera* bark have strong antibacterial potential against *Bacillus* and *Staphylococcus aureus* using agar well diffusion method beside this The aqueous extracts of *Moringa oleifera* bark, a have shown strong antibacterial activity against all selected microorganisms. However

Abdulmoneim *et al.*, (2011) also reported that aqueous extract have strong and superior antibacterial activity against the bacterial strains especially with regard to gram positive bacteria (*Staphylococcus aureus* and *Bacillus spp*). Antimicrobial properties of aqueous extract of *Moringa oleifera* bark as shown in present study should be due to the earlier claims by Ajaiyeoba, (2002) who indicate the polar solvent aqueous extract were more active then extract of other non polar solvent such as chloroform

For fungal species The aqueous extract inhibited the growth of *M.gypseum* showing zone of inhibition up to dilution of 3.125mg/ml and *R.stolonifer* is comparatively less active with zone of inhibition at dilution of 6.25mg/ml as results are shown in table (9). These scientifically divergent observations does not any way gives the impression that plants possess different biomolecules stored in different part of plant but it can be understand in the way these biomolecules exist in different concentration in different parts of the plant. Harbone (1982), has documented that active principles are stored in different plants and released in varying combination and strength. Miller (1973) has also documented that fats occur in all proportion of plant chloroform extracts of *M.oleifera* had the maximum antibacterial activity against *pseudomonas flouresence* showing zone of inhibition up to dilution of 3.125mg/ml and moderate antibacterial activity against *Staphylococcus*

aureus with zone of inhibition at 6.25mg/ml and the minimum antibacterial activity against *Bacillus megetarian' citro freundii* zone of inhibition up to 12.5mg/ml. However on evaluation of antifungal potential it is observed that chloroform extract most active against *R.stolonifer* showing inhibitory effect at of dilution of 3.125mg/ml in comparison to the *M. gypseum* showed moderate sensitivity to chloroform extracts up to the dilutions of 6.25mg/ml results are shown in table (10) Dewangan *et al.*, (2010) reported that the disc diffusion study revealed variable sensitivity to different extracts of *M. oleifera* against *Staphylococcus aureus, Pseudomonas spp* . In comparison of Ethyl acetate and aqueous extracts chloroform extracts showed intermediate sensitivity against above bacteria.

The disc diffusion study revealed that methanol and chloroform extracts showed maximum antibacterial activity against *Staphylococcus aureus***,** *pseudomonas flouresence* and *Citro freundii* and the aqueous extract demonstrated less inhibitory effect against the entire above selected microorganism while ethyl acetate extract showed moderate antibacterial activity against the test microorganism. In case of fungal species, aqueous and chloroform extracts of bark showed maximum antifungal activity, while moderate antifungal activity against the selected microorganism is observed on testing ethyl acetate and methanol extracts.

Results of *in vitro* antimicrobial activity of *Moringa oleifera* leaves showed that all the extracts ethyl acetate, methanol, aqueous and chloroform of leaves have been found active against selected bacterial strains and fungal strain. The antimicrobial potential of extracts might be justified by the presence of lipophilic compounds. These antimicrobial compounds might bind within or internal to the cytoplasmic membrane (Boyd and Beveridge, 1979-81) and affect the growth of filamentous fungi mainly by causing membrane permeabilization (Huang *et al*., 2000).

The *M. oleifera* leaves extract of ethyl acetate shown antibacterial potential against *B. megetarian, S. aureus, P. flouresence*, and *C. freundii* showing inhibitory zone till dilution of 3.125mg/ml while For fungal species ethyl acetate leaves extracts were more active against *M. gypseum* in comparison to the *R. stolonifer* showing inhibitory zone till dilution of 6.25mg/ml. results are shown in table (11).

Methanol extracts of *M. oleirera* leaves had the maximum antibacterial activity against *P. flouresence* then *B. megetarian, S. aureus* showing zone of inhibition up to dilution of 3.125mg/ml and *C. freundii* showed slightly less antibacterial activity as compare to above pathogen against with zone of inhibition at 6.25mg/ml In case of fungus the methanol extract had maximum antifungal activity against *R. stolonifer* showing zone of inhibition up to dilution of 3.125mg/ml in comparison to

the *M. gypseum* showing zone of inhibition up to dilution of 6.25mg/ml as results are shown in table (12) These results are in close agreement with other findings obtained by other workers Devi *et al.*, (2011) investigate the antibacterial activity of methanolic extracts of *Moringa oleifera* leaves by agar well diffusion method against *Bacillus,* and *Staphylococcus aureus* that frequently cause enteric infections in humans. The methanol extracts of *Moringa oleifera* leaves have shown strong antibacterial activity against the organisms tested.

The aqueous extracts of leaves found maximum effective against the selected bacteria, *S. aureus, P. flouresence* and *C. freundii, B. megetarian* showing inhibitory zone at dilution of 6.25mg/ml in comparison to the other extracts. Caceres *et al.* (1991) reported antimicrobial activities of *M. oleifera* leaves *in vitro* against bacteria, yeast, dermatophytes and helminths by a disk-diffusion method. *Pseudomonas* and *Staphylococcus aureus* is inhibited by the extract of fresh leaf. However Devi *et al.*, (2011) investigate the antibacterial activity of the aqueous extracts of *Moringa oleifera* leaves by agar well diffusion method against *Bacillus,* and *Staphylococcus aureus* that frequently cause enteric infections in humans. The aqueous extracts of *M. oleifera* leaves, have shown strong antibacterial activity .similar result was reported by Alam *et al.,* (2009). He investigated antibacterial potential of leaf juice and *M. oleifera* extracts by disc diffusion assay and

determined the minimum inhibitory concentration (MIC) against human pathogenic bacteria. The water extract of leaf found effective against the tested Gram negative bacteria *Pseudomonas spp*. and Gram-positive bacteria *S. aureus* and *Bacillus megaterium* while Thilzal *et al.,* (2010) reported that *M. oleifera* leaves water extract had no antibacterial activity against *S. aureus* and *Pseudomonas spp*. but our study revails that aqueous extract shows maximum antibacterial and antifungal activity. Our results are also supported by Caceres *et al.,* (1991). Who evaluated antimicrobial potential of *M. oleifera* roots, leaves, bark and seeds extracts by using disk-diffusion method. Observations reveled that different extracts are found effective against bacteria, yeast, dermatophytes and helminths by a. The aqueous extracts of fresh leaf inhibits the growth of *Pseudomonass* and *S. aureus*).

Presence of anthraquinones (9,10- dioxoanthracene) which is one of the naturally occurring phenolic compound is found in *M. oleifera* leaves and tend to have inhibitory effects. Presence of Terpenoids and steroids in *M. oleifera* leaves are described as being active against bacteria such as *S. aureus* (Cowan, 1999), *M. oleifera* leaves also contain alkaloids which are nitrogen-containing naturally occurring compound, commonly found to have antimicrobial properties due to their ability to intercalate with DNA of the microorganisms (Bennett *et al*., 2003) The

phytochemistry of *M. oleifera* shows that several organic compounds found in the pods and leaves differ despite their closeness (Duke, 1983).

However, Same result showed by aqueous leaves extract in case of fungal species both *M. gypseum* and *R. stolonifer* showing inhibitory zone at dilution of 3.125mg/ml as results are shown in table (12).

The *M. oleifera* leaves chloroform extract of were found to be most active against the three bacteria out of four *C. freundii, P. flouresence* and *B. megetarian* showing inhibitory zone at dilution of 3.125mg/ml while were less effective against *S. aureus* showing inhibitory zone at dilution of 25mg/ml. In case of fungal species, chloroform extract of bark were more effective against *M. gypseum* showing inhibitory zone at at dilution of 3.125mg/ml in comparison to *R. stolonifer* inhibition up to the dilution of 25mg/ml as results are shown in table (13).

On comparing antibacterial potential of extracts prepared with antibiotics selected as control we can concluded that the methanol and aqueous extracts of the bark and leaves of *M. oleifera* was less active in comparison of standard antibiotics. While the chloroform and ethyl acetate extracts of *M. oleifera* barkis found more active. Results justifies the use of *M. oleifera* bark and leaves extracts in traditional medicine, refined evaluation of active components is required using this plant to isolate and characterize the bioactive molecules and *in vivo* analysis

should be done to determine thier toxicity and to decide the optimum dose that can be used it as an antibiotic.

From results it can be conclude that extracts of *M. oleifera* possess antimicrobial potential and is active against bacterial species. The antimicrobial activity of the crude extract and supernatant might be due to the presence of lipophilic compounds that might bind within or internal to the cytoplasmic membrane (Body & Beveridge, 1979, 1981), and affect the growth of filamentous fungi mainly by causing membrane permeabilization (Huang *et al*., 2000). Observations reveled that effectiveness of extract decreases with lower dilution beside this different minimum inhibitory concentrations (MIC) values are obtained against different microorganism. The reason for susceptibility of the species towards concentration of the extracts, after which this extract damage that species which is not tolerable for it (Ordonez *et al*. 2006).

Phytochemical constituents such as alkaloids, flavonoids, tannins, phenols, saponins, and several other aromatic compounds are secondary metabolites of plants that serve a defense mechanism against prediction by many microorganisms, insects and other herbivores Bonjar *et al.,* (2004). On analysising results of experiment conducted revealed the presence of medicinally active constituents in the plant samples. The results of phytochemical analysis of the *M. oleifera* are summarized in Table 14 and 15. Observations demonstrated the presence of glycosides,

flavonoids, phenols, saponins, tannins and steroids. Presence of these phytochemicals could be responsible for the observed antimicrobial activity. These molecules are also known to possess antimicrobial potential as they act by different mechanism and exert antimicrobial action against microorganism.

Tannins exert antimicrobial effect by binding to proteins rich in proline amino acid and therefore inhibit the synthesis of protein Shimada (2006). Plants synthesis Flavonoids (hydroxylated phenolic substance) in response to microbial infection and so their presence in extracts is justified by their effectiveness as a antimicrobial substances against a wide array of microorganisms. Their activity is probably due to their ability to complex with extracellular and soluble proteins and to complex with bacterial cell walls Marjorie (2006).

Coumarins are also known act against gram positive bacteria and it is produced in carrots in response to fungal infection which could be attributed to its antimicrobial activity Hoult and Paya (1996). Antimicrobial property of saponin is due to its ability to cause leakage of proteins and certain enzymes from the cell Zablotowicz *et al.,* (1996). Antibacterial potential of Steroids was reported; the direct relation between membrane lipids and sensitivity towards steroidal compound gives an idea about the mechanism by which steroids binds with membrane lipid and causes leakages from liposomes Epand (2007).

Summary

Chapter- 7

SUMMARY

From the following observation and discussion some important facts have come to light, which are summarized below:

- *Moringa oleifera* Lam. Belongs to family Moringaceae. It is most monogeneric and generally distributed naturalized species also known as sahajan in Hindi, in English it is known as Horse radish. It's a fast growing, little, deciduous evergreen tree that sometimes reaches height up to 10 or 12 m. this plant is native to India, Pakistan, western and sub Himalayan tracts, Asia Minor , Arabia and Africa and is now widely found in Cambodia, Philippines, North and South America, Caribbean Islands and Central America.
- As almost every part of *M. oleifera* tree considered to have medicinal properties and are used in the treatment of rheumatism, ascites, snake bites and as a cardiac & circulatory stimulant therefore it is also known as a *'Miracle tree'*. Phytomolecules obtained from Leaves show anti-oxidant properties and are known to cures dry tumors, hallucinations, asthma and hiccups. For treatment of heart complaints, eye diseases, inflammation, dyspepsia, and enlargement of spleen the root and bark are useful. In the treatment of inflammations and

muscle diseases flowers are used. For the treatment of leprous ulcers Seed oil is found to be useful in.

- The antibacterial activity against the bacterial strains was studied by Disc diffusion method and Broth dilution methods. While antifungal activity was studied by Disc diffusion method only.
- The different bark and leaves extracts of test plants were made by Soxhlet extraction method. Seven different dilutions that are (200, 100, 50, 25, 12.5, 6.25 and 3.125) mg/ml were made from mother extractions. To test antimicrobial potential pure cultures of *S. aureus*, *C. freundii, B. Megaterium, P. fluorescens*, *R. stolonifer* and *M. gypseun*.
- For testing antibacterial potential Nutrient broth, Nutrient Agar medium and Muller-Hinton Agar medium were used while for testing antifungal potential PDA medium and Sabouraud's dextrose medium were used. The colony diameter was measured on 3rd day of incubation period as compared with control.
- The antibacterial activity of different leaves and bark extract of *M. oleifera* were assessed by measuring the diameter of growth inhibition zones with different dilutions (200,100, 50, 25, 12.5, 6.25 and 3.125 mg/ml) of different extract. The inhibition zone of ethyl acetate, methanol, aqueous and chloroform extracts of *M.oleifera* leaves and bark were found to be active against all the test organisms. Extracts of

M.oleifera shows in dose dependent inhibition; it means that extract is more active at higher dilutions while activity decreases with lower dilutions, the activity also get decreased. Beside this different value minimum inhibitory concentrations (MIC) were observed against different microbial species. the reason for different value minimum inhibitory concentrations (MIC) is due to different susceptibility of the different species towards the extracts, cause damage to that species which is cant have potential to tolerate it.

- The disc diffusion study revealed that methanol and chloroform extracts showed maximum antibacterial activity against *S. aureus*, *P. flouresence and C. freundii* and the aqueous extract showed antibacterial potential against all the above test microorganism. While ethyl acetate extract showed moderate antibacterial activity against the test microorganism. In case of fungal species, aqueous and chloroform extracts of bark showed maximum antifungal activity, while moderate antifungal activity against the test microorganism is shown ethyl acetate and methanol extracts.
- The phytochemical constituents of the *M.oleifera* was investigated and summarized in Table. 14 and 15. Observations revealed the presence of carbohydrates, flavonoids, glycosides, tannins and steroids. It can be concluded that these phytomolecules are responsible for the antimicrobial property observed. These phytomolecule exert

antimicrobial action by acting against microorganisms using different mechanism.

- Biomolecules such as steroids and Terpenoids found in *M. oleifera* leaves showed antibacterial potential against bacteria such as *S. aureus*. Beside this leaves of *M. oleifera* also possess alkaloids that are naturally occurring nitrogen-containing compound commonly show antimicrobial properties as they intercalate within microorganisms DNA. The phytochemical examination of pods and leaves of *M. oleifera* revealed the presence of several organic compounds.
- From the observation we get conclusion that various extracts of *Moringa oleifera* predominantly showed the antibacterial potential. The reason for this antimicrobial activity of the extracts may be due to the presence of lipophilic compounds that may bind to the cytoplasmic membrane and have an impact on the growth of filamentous fungi mainly by causing permeabilization of membrane.
- Till now many experiments have been conducted test antimicrobial potential of different parts of *M. oleifera*, but it important to isolate and identify new phytomolecules from different parts of the tree, that show antimicrobial potential.
- As *M. oleifera is* a valuable plant therefore there is need widely cultivate it in areas having favorable environmental conditions which

supports its optimum growth. By this we can maximize its yield and its different parts which can used for the welfare of mankind.

- Observations reveled that extract of *M. oleifera* had antimicrobial potential but it is not up to the mark as reveled by traditionally. However, *in vivo* experiments should be performed with the extract to confirm the *in vitro* observations that showed inhibitory potential in form of the zone of inhibition against the microorganisms.
- From the study we can conclude that phytomolecules obtained from *M. oleifera* can act as potent natural antimicrobial agents and can used for controlling the pathogenic bacteria by pharmaceutical industry.
- The present study reveals that the selected plant can act a less costly source of drug development and for treating human alignments. Many good effects inform of antifungal and antibacterial activities could be used in drug formulation. Experimental outcomes provide a support for its use in medicine and it can be suggested that further advance investigation required to completely exploring its potential.
- Further we can conclude that bark extracts of the plant showed high amount potential antimicrobial molecules which are found active against micro-organisms so these phytomolecule can be employed in the treatment of infectious diseases caused by resistant micro-organism. Investigating various naturally occurring organic

compounds and determination of their antimicrobial potential is present need as it may results in discovery of new drug and thus increases the spectrum of molecule used in treating bacterial and fungal infections in humans.

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Chapter- 8

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Apendix

Anoop Tiwari

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e-ISSN: 2455-667X

Annals of Natural Sciences
(Peer Reviewed and UGC Approved International Journal)
Vol. 4(1), March 2018: 114-117
Journal's URL: http://www.crsdindia.com/ans.html
Email: crsdindia@gmail.com

Annals of Natural Sciences

ORIGINAL ARTICLE

Evaluation of Antibacterial Potential of Different Extracts of *Moringa oleifera*

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ABSTRACT

The antibacterial potential of different extracts of Moringa oleifera was screened against four bacteria using disc diffusion assay. Lowest concentration of the extract, which inhibits any visual microbial growth after treatment with p-iodonitrotetrazolium violet, was considered to be minimum inhibitory concentration (MIC). Methanol extract of Moringa oleifera exhibited good activity against all the bacteria tested and the MIC was recorded in range of 9.375-37.5 µg/ml.The other extracts of Moringa oleifera showed antimicrobial activity in a range of 75-1200 µg/ml.

Key words: *Antibacterial Potential, Different Extracts, Moringa oleifera*

Received: 19th Feb. 2018, Revised: 23rd Feb. 2018, Accepted: 26th Feb. 2018

How to cite this article:
Tiwari A.K. and Srivastava M.P. (2018): Evaluation of Antibacterial Potential of Different Extracts of *Moringa oleifera*. Annals of Natural Sciences, Vol. 4[1]: March, 2018: 114-117.

INTRODUCTION

The number of multi-drug resistant microbial strains and the appearance of strains with reduced susceptibility to antibiotics are continuously increasing. This increase has been attributed to indiscriminate use of broad-spectrum antibiotics, immunosuppressive agent, intravenous catheters, organ transplantation and ongoing epidemics of HIV infection (Graybill, 1988; Ng, 1994; Dean and Burchard, 1996; Gonzalez *et al*, 1996). In addition, in developing countries, synthetic drugs are not only expensive and inadequate for the treatment of diseases but also often with adulterations and side effects. Therefore, there is need to search new infection-fighting strategies to control microbial infections (Sieradzki *et al*, 1999).

A number of higher plants have been used for centuries as remedies for human diseases. This has encouraged scientists to screen higher plants for various biological activities including antibacterial and antifungal effects (Eilert *et al.,* 1980; 1981; Omer and Elnima, 2003; Saadabi, 2006; Saadabi *et al.,* 2006; 2007; 2009). The acceptance of traditional medicine as an alternate form of health care and the development of microbial resistance to the available antibiotics has led researches to investigate the antimicrobial activity of medicinal plants (Hemmer *et al.,* 1999).

*M. oleifera*tree is also known as a 'Miracle tree' as almost every part of this tree possess products useful for humans. The leaves and pods are eaten. The plant is also reported to be medicinally important and almost all parts of the *M. oleifera*tree are considered to possess medicinal properties and are used in the treatment of ascites, rheumatism and venomous bites and as cardiac and circulatory stimulant (Shindano and Kasase, 2009). Leaves are also known to have anti-oxidant properties and are known to cures hallucinations, dry tumors, hiccups and asthma (Mehta and Agrawal, 2008). The root and bark are useful in treatment of heart complaints, eye diseases, inflammation, dyspepsia,

and enlargement of spleen. The flowers are known to cure inflammations and muscle diseases. Seed oil is known to be useful in treatment of leprous ulcers Fahey (2005).
During recent years, considerable work has been done to investigate the pharmacological actions of the leaves and seeds of *Moringaoleifera* on scientific lines. But only limited work has been reported so far on antibacterial activity of *Moringaoleifera*leaves and bark.. Therefore, it was considered worthy to investigate the antibacterial activity of *Moringaoleifera*leaves bark.

MATERIALS AND METHODS

PLANT MATERIALS

For conducting the present study, the plant material was collected from different locations of Agra and Plant parts (bark) devoid of contaminant parts were carefully collected and kept in polythene bags which were then subsequently sealed. The stored specimens were thoroughly washed with tap water. They were shade dried and ground with grinder to obtain course particle.

PREPARATION OF PLANT EXTRACTS

1. **Aqueous Extract:** For aqueous extract bark powder was separately homogenized with sterile distilled water at 1:8 w/v ratio in a pestle and mortar and filtered through muslin cloth. The filtrate thus obtained was further strained through Whatman No. 1 filter paper (Zore *et al.*, 2004). The extraction was carried out at room temperature.
2. **Organic Extract**: Organic extract was prepared by Soxhlet extraction method following (Okeke *et al.*, 2001). A thimble was prepared by using a 0.5mm Whatman filter paper. About 50 gm of powder material was uniformly packed in a thimble and run in soxhlet extractor. It was run upto 48 hour or 22 cycles until the solvent in the sippon table of an extractor become colourless. After that, extracts were filtered with the help of filter paper and solvent was evaporated from extract with the help of rotary evaporator to get the syrupy consistency. The extract was then stored in refrigerator at 4ºC.

ANTIBACTERIAL SCREENING

Antibacterial activities of the extracts were determined by the microbroth dilution assay as described by Buwa and Staden (2006). The water and ethanol plant extracts were dissolved in corresponding extracting solvents at a concentration of 2400 µg/ml. Acetone extracts were also dissolved in ethanol while the other extracts were dissolved in DMSO. Proper controls were kept for each experiment. The bacterial strains used as inocula were grown at 37^{o}C to get OD 0.6 at 600 nm and used for susceptibility testing. Lowest concentration, which inhibited any visual growth, was considered to be minimum inhibitory concentration (MIC).

RESULT AND DISCUSSION

Table 1: Antimicrobial activity of *M. oleifera* methanol bark extract against different test microorganisms

Pathogens	Zone of inhibition in (mm)							
	200mg/ml	100mg/ml	50mg/ml	25mg/ml	12.5mg/ml	6.25mg/ml	3.125mg/ml	Drug
S.aureus	13.00±1.52	12.67±1.53	10.33±0.57	8.66±1.53	8.00±1.00	7.00±0.58	-	20
C.freundii	14.00±2.05	12.00±2.60	11.33±1.73	10.66±1.15	10.00±1.00	9.66±0.58	8.66±0.57	18
B.megaterium	9.66±1.52	8.67±0.58	8.00±1.00	7.00±2.00	6.67±0.58	6.33±0.57	-	18
P.fluorrescens	13.67±1.15	11.66±1.53	10.66±1.52	9.67±1.15	9.33±0.58	8.00±1.00	7.33±0.57	16

Table 2: Antimicrobial activity of *M. oleifera* aqueous bark extract against different test microorganisms

Pathogens	Zone of inhibition in (mm)							
	200mg/ml	100mg/ml	50mg/ml	25mg/ml	12.5mg/ml	6.25mg/ml	3.125mg/ml	Drug
S.aureus	9.00±1.15	8.67±0.58	7.67±1.00	7.00±0.57	6.33±0.58	-	-	17
C.freundii	11.33±0.58	10.66±1.15	9.66±0.58	8.66±1.00	8.33±1.15	7.33±0.57	-	16
B.megaterium	9.66±0.58	8.67±1.15	8.00±1.00	7.66±1.15	7.33±0.57	6.67±0.58	-	18
P.fluorrescens	9.00±1.00	8.67±0.58	8.33±0.57	8.00±1.00	7.67±0.58	7.00±1.00	7.66±0.58	13

Plants are important source of potentially useful structures for the development of new chemotherapeutic agents. The first step towards this goal is the *in vitro* antibacterial activity assay (Tona *et al.*1998) Previous studies have reported that various parts of *Moringa* roots, flowers, bark, and stem including seeds possess antimicrobial properties (Lockett *et al*., 2000; Anwar and Rashid, 2007).

The various extracts of *Moringa oleifera* namely ethyl acetate, methanol, aqueous and chloroform extracts of its leaves and bark were tested against *Staphylococcus aureus, citro freundii, Bacillus megetarian, pseudomonas flouresence,* for their antibacterial activity and against *R. stlonifer* and *M.gypseum* for their antifungal activity. The extracts of methanol and water of *Moringa oleifera* leaves and bark were found to be active against all the test organisms.

Results obtained from *in vitro* antimicrobial activity of *Moringa oleifera*it was observed that the methanol extract of *Moringa oleifera* bark were found to be most active against *Citro freundii* and *pseudomonas flouresence* showing zone of inhibition up to dilution of 3.125mg/ml while it was observed that methanol extract of bark was slightly less active against *Staphylococcus aureus, Bacillus megetarian* showing zone of inhibition up to dilution of 6.25mg/ml. by Similar result was observed in the study of Rao *et al*, (2011) who investigated antibacterial activity of methanolic extract of *M.oleifera* by using well diffusion technique and reported that the most significant activity was seen against *S. aureus*, while Devi *et al*., (2011) investigate the antibacterial activity of methanolic extracts of *Moringa oleifera* bark by agar well diffusion method against *Bacillus,* and *Staphylococcus aureus* that frequently cause enteric infections in humans. The methanol extracts of *Moringa oleifera* bark, have shown strong antibacterial activity against the organisms tested.

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Paper Details:-
Title: Evaluation of Antibacterial Potential of Different Extracts of *Moringa oleifera*
Paper Received : 19.02.2018
Paper Revised : 23.02.2018
Paper Accepted : 26.02.2018

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Asian Journal of Agriculture & Life Sciences Vol. 3(2), April 2018: 79-81

Website: www.crsdindia.com/ajals.html e-ISSN: 2455-6149

ORIGINAL ARTICLE

Evaluation of Antifungal Potential of Different Extracts of *Moringa Oleifera*

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Received: 26th Feb. 2018, Revised: 7th March 2018, Accepted: 15th March 2018

ABSTRACT

The antifungal activity of different extracts of Moringa oleifera were tested against plant pathogenic fungi Rhizopus stolonifer and Microsporum gypseum by disc diffusion method. The plant leaves were extracted with various solvents like methanol, aqueous. Among the different extracts tested, the methanolic extracts showed maximum antifungal activity against.

Key words: *Antifungal, Medicinal plants, Moringa Oleifera*

INTRODUCTION

Concern has been expressed about the rising prevalence of pathogenic microorganisms which are resistant to the newer (or) modern antibiotics that have been produced in the last three decades (Cohen, 1992; Nascimento et al., 2000). Also, the problem posed by the high cost, adulteration and increasing toxic side effects of these synthetic drugs coupled with their inadequacy in diseases treatment found more especially in the developing countries cannot be over emphasized (Shariff, 2001). Coincidentally, the last decade has also witnessed increasing intensive studies on extracts and biologically active compounds isolated from plant species used for natural therapies or herbal medicine (Nascimento et al., 2000; Rios and Recio, 2005). Medicinal plants represent a rich source of antimicrobial agents. Plants are used medicinally in different countries and are a source of many potent and powerful drugs (Srivastava et al., 1996). A wide range of medicinal plant parts is used for extract as raw drugs and they possess varied medicinal properties. The different parts used include root, stem, fruit, twigs exudates and modified plant organs. While some of these raw drugs are collected in small 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 et al., 2006). Although hundreds of plant species have been tested for antimicrobial properties, the vast majority of them have not been adequately evaluated (Balandrin et al., 1985).

*M. oleifera*tree is also known as a 'Miracle tree' as almost every part of this tree possess products useful for humans. The leaves and pods are eaten. The plant is also reported to be medicinally important and almost all parts of the *M. oleifera*tree are considered to possess medicinal properties and are used in the treatment of ascites, rheumatism and venomous bites and as cardiac and circulatory stimulant (Shindano and Kasase, 2009). Leaves are also known to have anti-oxidant properties and are known to cures hallucinations, dry tumors, hiccups and asthma (Mehta and Agrawal, 2008). The root and bark are useful in treatment of heart complaints, eye diseases, inflammation, dyspepsia, and enlargement of spleen. The flowers are known to cure inflammations and muscle diseases. Seed oil is known to be useful in treatment of leprous ulcers Fahey (2005).

During recent years, considerable work has been done to investigate the pharmacological actions of the leaves and seeds of *Moringa oleifera* on scientific lines. But only limited work has been reported so far on antifungal activity of *Moringa oleifera* leaves and bark. Therefore, it was considered worthy to investigate the antifungal activity of *Moringa oleifera* leaves bark.

PLANT MATERIALS

For conducting the present study, the plant material was collected from different locations of Agra and Plant parts (bark) devoid of contaminant parts were carefully collected and kept in polythene bags which were then subsequently sealed. The stored specimens were thoroughly washed with tap water. They were shade dried and ground with grinder to obtain course particle.

PREPARATION OF PLANT EXTRACTS

1. **Aqueous Extract:** For aqueous extract bark powder was separately homogenized with sterile distilled water at 1:8 w/v ratio in a pestle and mortar and filtered through muslin cloth. The filtrate thus obtained was further strained through Whatman No. 1 filter paper (Zore *et al.*, 2004). The extraction was carried out at room temperature.
2. **Organic Extract**: Organic extract was prepared by Soxhlet extraction method following (Okeke *et al.*, 2001). A thimble was prepared by using a 0.5mm Whatman filter paper. About 50 gm of powder material was uniformly packed in a thimble and run in soxhlet extractor (Fig. 3). It was run upto 48 hour or 22 cycles until the solvent in the sippon table of an extractor become colourless. After that, extracts were filtered with the help of filter paper and solvent was evaporated from extract with the help of rotary evaporator to get the syrupy consistency. The extract was then stored in refrigerator at 4ºC.

ANTIFUNGAL ACTIVITY TEST

Antifungal activity was screened by disc diffusion method. The water and ethanol extracts of *Moringa oleifera* leaves bark were tested against *Rhizopus stolonifer and Microsporum gypseum*. The PDA medium was poured in to the sterile petriplates and allowed to solidify. The test fungal culture was evenly spread over the media by sterile cotton swabs. Then disc (6 mm) coated with 200µl of each extracts were transferred into the petri plate. The plates were incubated at 27°C for 48-72 hrs. After the incubation the plates were observed for formation of clear incubation zone around the disc indicated the presence of antifungal activity. The zone of inhibition was recorded.

RESULT AND DISCUSSION

Table 1: Antimicrobial activity of *M. oleifera* methanol bark extract against different test microorganisms

Pathogens	Zone of inhibition in (mm)							
	200mg/ml	100mg/ml	50mg/ml	25mg/ml	12.5mg/ml	6.25mg/ml	3.125mg/ml	Drug
R.stolonifer	9.00±2.00	8.66±1.52	8.33±1.15	7.66±0.05	7.00±1.00	-	-	-
M.gypseum	8.66±1.15	8.33 ± 1.00	7.66±0.58	7.33±0.57	7.00±1.00	6.67±0.58	-	-

Table 2: Antimicrobial activity of *M. oleifera* aqueous bark extract against different test microorganisms

Pathogens	Zone of inhibition in (mm)							
	200mg/ml	100mg/ml	50mg/ml	25mg/ml	12.5mg/ml	6.25mg/ml	3.125mg/ml	Drug
R.stolonifer	**9.66±1.52**	**8.33±1.15**	**8.00±0.57**	**7.66±0.58**	**6.66±1.15**	**6.33±0.57**	**-**	**-**
M.gypseum	**9.00±1.73**	**8.66±1.15**	**8.33±1.00**	**7.67±0.58**	**7.33±0.57**	**7.00±1.00**	**6.67±0.57**	**-**

Tona *et al.*1998 studied that plants are a valuable source of potentially supportive structures for developing novel chemotherapeutic drugs. In case of fungal species, methanol extracts of bark were more active against *M. gypseum* the respective diameter of inhibition at different dilutions were (8.66, 8.33, 7.66, 7.33, 7.00, 6.67) mm in comparison to *R. stolonifer* inhibitions were recorded (9.00, 8.66, 8.33, 7.66, 7.00) mm, respectively.

Fungal species the bark aqueous extract of *M. oleifera* inhibited the growth of *M. gypseum* showing diameter of inhibition (9.00, 8.66, 8.33, 7.678, 7.33, 7.00, 6.67) mm and *R. stolonifer*was comparatively less effective the diameter of inhibition are observed (9.66, 8.33, 8.00, 7.66, 6.66, 6.33) mm, respectively.

The ultimate conclusion of this study supports the traditional medicine use of different plant extracts in treating different infections caused by pathogenic fungi either by using a single or combined extracts. It also suggests that a great attention should be paid to medicinal plants which are found to have plenty of pharmacological properties that could be sufficiently better when considering a natural food and feed additives to improve human and animal health.

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Publication Certificate

To,

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Dear Sir/ Madam,

This is to certify that your paper has been published in **Vol. 3(2): April 2018** issue of A Peer-Reviewed and UGC Approved International Journal- **"Asian Journal of Agriculture & Life Sciences"** (ISSN: 2455-6149; UGC Serial No. 43795).

Paper: Evaluation of Antifungal Potential of Different Extracts of *Moringa Oleifera*
Paper Received: 26.02.2018
Paper Revised: 07.03.2018
Paper Accepted: 15.03.2018

With best Regards,

Arshad

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Exploring the Antimicrobial Properties of the Plants that are Commonly Being used as Traditional Medicines

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ABSTRACT

The emergence and spread of antibiotic resistance, as well as the evolution of new strains of disease causing agents, are of great concern to the global health community. Effective treatment of a disease entails the development of new pharmaceuticals or some potential source of novel drugs. Commonly used medicinal plants of our community could be an excellent source of drugs to fight off this problem. This study is focused on exploring the antimicrobial properties of the plants that are commonly being used as traditional medicines. The antimicrobial potential of four different plant extracts was screened against pathogenic microorganisms. Methanolic extracts of *Moringa oleifera* were subjected to a test of their antimicrobial properties by disc diffusion method. The result indicated that most of the extracts exhibited antimicrobial properties. The experiment confirmed the efficacy of some selected plant extracts as natural antimicrobials and suggested the possibility of employing them in drugs for the treatment of infectious diseases caused by the test organisms.

Key words: Antibacterial, disc diffusion, medicinal plants

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EVALUATION OF ANTIMICROBIAL POTENTIAL OF MEDICINALLY IMPORTANT PLANT VIZ., *MORINGA OLEIFERA* LEAF EXTRACTS AGAINST PATHOGENS

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ABSTRACT

An attempt was made to analyse the antimicrobial potential of medicinally important plant viz., *Moringa oleifera* leaf extracts against pathogens. Antimicrobial study was carried out by disc diffusion method against the pathogens by using the methanol and aqueous extracts. The result of the present study showed the presence of wide spectrum of antibacterial activities against all the bacterial pathogens studied. The present study demonstrates that the selected medicinal plants are potentially good sources of antibacterial against the pathogens. The phytochemical screening demonstrated the presence of different types of compounds like terpenoids, tannins, deoxy sugars, saponins, phenolic compouns, and flavonoids which may contribute for the antimicrobial action of the above medicinal plants.

Key words: Antibacterial, disc diffusion, human pathogens, medicinal plants