CHAPTER 4

ANTIMICROBIAL ACTIVITY OF ROOT AND ROOT DERIVED CALLUS EXTRACTS
Introduction

Plants are the richest resource of drugs of traditional systems of medicine, modern medicines, nutraceuticals food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs (Hammer et al., 1999). The use of plants and plant products as medicines could be traced as far back as the beginning of human civilization. The earliest mention of medicinal use of plants in Hindu culture is found in “Rigveda”, which is said to have been written between 4500 - 1600 B.C. and is supposed to be the oldest repository of human knowledge. It is Ayurveda, the foundation of medicinal science of Hindu culture, in its eight division deals with specific properties of drugs and various aspects of science of life and the art of healing (Rastogi & Mehrotra, 2002).

WHO (2001) estimated that 80% of world population rely on medicinal plants for their primary health care needs. Out of the 3,50,000 plant species known so far, about 35,000 (some estimate up to 70,000) are used worldwide for medicinal purposes and less than about 0.5% of these have been investigated for their phytochemical and pharmacological potential (Hostettmann & Marston, 2002). This green inheritance thus represents an enormous reservoir of putative lead compounds to be discovered for various diseases. Plants are important sources of medicines and at least 25% of the prescription drugs issued in the USA and Canada contain bioactive compounds that are derived from or modeled after plant natural products (Farnsworth, 1984). Medicinal plants would be the best source to obtain a variety of drugs and therefore such plants should be investigated to understand better about their properties, safety and efficacy (Nascimento et al., 2000). Medicinal plants are major sources of obtaining antimicrobial drugs (Sofowora, 1986).

In addition to the alarming increase in the incidence of new and re-emerging infectious diseases, one major health concern is the resistance to existing antibiotics (Khanahmadi et al., 2010; Agbafor et al., 2011). Furthermore, novel antibiotics in the drug-development pipeline that offers significant benefits over existing drugs in lacking (Butler & Cooper, 2011). Examples of microorganisms that have developed antibiotic resistance were methicillin – resistant *Staphylococcus aureus* (MRSA) and vancomycin–resistant *Enterococcus faecium* (VREF).*S. aureus*, has acquired resistance to β- lactam antibiotics, such as penicillin and methicillin shortly after they
have been used for treatment (Kobayashi & DeLeo, 2011). Due to its virulence, its development of resistance to antibiotics and its generally expensive drugs and treatment, MRSA has become a serious public concern worldwide (Akhi et al., 2008; Monecke et al., 2011). In the US enterococci rank second to staphylococci as the most common cause of nosocomial infections. In addition, among the E. faecium recovered from US hospitals, more than 80% are vancomycin-resistant (Pannesso et al., 2010). At present the clinical development of potential drugs against gram-negative nosocomial infections that are significantly better than the existing drugs are still limited. The gram-negative bacteria are difficult to kill because they possess an additional outer membrane permeability barrier with multiple efflux pumps and antibiotic modifying enzymes (Butler & Cooper, 2011). As of this reporting, there is a sustained and urgent need to discover new antimicrobial compounds with novel action mechanisms.

It is widely known that plants possess healing properties (Cowan, 1999; Imaga, 2010; Agbafor et al., 2011). Such properties can be partly attributed to the diverse array of secondary metabolites (i.e., alkaloids, terpenes, phenolic compounds and cyanogenic glycosides) which are known to be essential for plants’ defense against microbial attack, or insect and animal predation (Cowan, 1999; Dixon, 2001; Kido et al., 2010; Oseni & Akindahunsi, 2011). Treatment of common infections with medicinal plants has been popular in developing countries due to its cheaper cost and claims for both its effectiveness and lesser side effects over synthetic drugs (Rojas et al., 2006; Dey et al., 2010; Butkhup & Samappito, 2011; Karim et al., 2011; Menghani et al., 2011).

A diverse range of compounds that offer potentials for the treatment of chronic and infectious diseases can be found most especially in traditional medicinal plants (Duraipandiyan et al., 2006; Karim et al., 2011; Sarwar et al., 2011). Well-known drugs that were derived from plants are taxol from Taxus brevifolia, vinblastine and vincristine from Catharanthus roseus, benzoin from Styrax tonkinensis and quinine from Cinchona pubescens (Mans et al., 2000). It is also well recognized that traditional medicine can be used along with synthetic pharmaceutical products for enhanced health management (Imaga, 2010).

Emergence of new and resistant fungal pathogens, increasing incidence in immunocompromised patients and environmental and health problems of chemical
fungicides dictate the search for novel antifungal agents for human diseases and plant protection (Sharma & Kumar, 2009). Consistency of biologic activity is essential requirements for the safe and effective use of therapeutic agents (Menghani et al., 2010).

Despite the existence of potent antibiotics, resistant or multiresistant strains are continuously appearing, imposing the need for a permanent search and development of new drugs. For Centuries plants have been used throughout the world as drugs and remedies for various diseases. These drugs serve as prototype to develop more effective and less toxic medicines (Sharma et al., 2009).

Medicinal plants are ingested as decoctions, teas and juice preparations to treat respiratory infections (Gonzalez, 1980). They are also made into a poultice and applied directly on the infected wounds and burns. One way to prevent antibiotic resistance of pathogenic species is by using new compounds that are not based on existing synthetic antimicrobial agents (Shah, 2005). It is necessary to evaluate, in a scientific base, the potential use of folk medicine for the treatment of infectious diseases produced by common pathogens. Medicinal plants might represent an alternative treatment in non-severe cases of infectious diseases. They can also be a possible source for new potent antibiotics to which pathogen strains are not resistant (Fabricant & Farnsworth, 2001).

Now a days enteric diseases in poultry industry cause low productivity, increased mortality and associated contamination of poultry products for human consumption. With increasing concerns about antibiotic resistance, the ban on sub-therapeutic antibiotic usage in Europe and the potential for a ban in US, there is an increasing interest in finding alternatives to antibiotics for poultry production. A public health concern associated with pathogenic bacteria is the increased incidence of strains that are resistant to antimicrobial agents. Those resistant microorganisms can be disseminated via animal feces to other animals. Resistance to antimicrobials is connected with genetic mechanisms. New trends in drug discovery from natural source emphasize on investigation of the marine ecosystem to explore numerous complex and novel chemical entities for the treatment of many disease such as cancer, inflammatory condition (Margret et al., 2009) arthritis, malaria and large variety of viral, bacterial, fungal disease (Ravikumar et al., 2005; Ravikumar et al., 2010a;
Despite tremendous progress in human medicines, infectious diseases caused by bacteria, fungi, viruses and parasites are still a major threat to public health. Their impact is particularly large in developing countries due to relative unavailability of medicines and the emergence of widespread drug resistance (Zampini et al., 2009). During the last two decades, the development of drug resistance as well as the appearance of undesirable side effects of certain antibiotics Okemo et al., (2003) has led to the search of new antimicrobial agents mainly among plant extracts with the goal to discover new chemical structures, which overcome the above disadvantages (Bouamama et al., 2006). Current research on natural molecule and products primarily focuses on plants since they can be sourced more easily and be selected based on their ethno-medicinal uses (Arora & Kaur, 2007).

A wide range of medicinal plants parts is used to extract as raw drugs and they possess varied medicinal properties. While some of these raw drugs are collected in smaller quantities by the local communities and folk healers for local used, many other raw drugs are collected in larger quantities and traded in the market as the raw materials for many herbal industries (Uniyal et al., 2006).

Clinical microbiologists have great interest in screening of medicinal plants for new therapeutics (Kumar et al., 2010). The active principles of many drugs found in plants are secondary metabolites. The antimicrobial activities of plant extracts may reside in a variety of different components, including aldehyde and phenolic compounds (Lai & Roy, 2004). The development of drug resistance in human pathogens against commonly used antibiotics has necessitated a search for new antimicrobial substances from other sources including plants (Erdogrul, 2002).

Antibiotics are one of our most important weapons in fighting bacterial infections and have greatly benefited the health related quality of human life since their introduction. However, over the past few decades these health benefits are under threat as many commonly used antibiotics have become less and less effective against certain illnesses not only because many of them produce toxic reactions but also due to emergence of drug resistant bacteria. It is essential to investigate newer drugs with lesser resistance. Systematic studies among various pharmacological compounds have
revealed that any drug may have the possibility of possessing diverse functions and thus may have useful activity in completely different spheres of medicine. Drugs derived from natural sources play a significant role in the prevention and treatment of human diseases. In many developing countries, traditional medicine is one of the primary health care systems (Farnsworth, 1993; Houghton, 1995).

Herbs are widely exploited in the traditional medicine and their curative potentials are well documented (Dubey et al., 2004). About 61% of new drugs developed between 1981 and 2002 were based on natural products and they have been very successful especially in the areas of infectious disease and cancer (Cragg & Newman, 2005a). Recent trends, however, show that the discovery rate of active novel chemical entities is declining (Lam, 2007).

Natural products of higher plants may give a new source of antimicrobial agents with possibly novel mechanisms of action (Shahidi, 2004; Runyoro et al., 2006). The effects of plant extracts on bacteria have been studied by a very large number of researchers in different parts of the world (Srinivasa Reddy et al., 2001). Much work has been done on ethnomedicinal plants in India (Maheshwari et al., 1986).

In the recent years, research on medicinal plants has attracted a lot of attentions globally. Large body of evidence has accumulated to demonstrate the promising potential of medicinal plants used in various traditional, complementary and alternate systems of treatment of human diseases. Plants are rich in a wide variety of secondary metabolites such as tannins, terpenoids, alkaloids, flavonoids, glycosides etc., which have been found in vitro to have antimicrobial properties (Cowan, 1999; Dahanukar et al., 2000).

Antimicrobial properties of medicinal plants are being increasingly reported from different parts of the world. The world health organization estimates that plant extracts or their active constituents are used as folk medicine in traditional therapies of 80% of the world’s population (Dilnawaz et al., 2011). Use of herbal medicines in Asia represents a long history of human interactions with the environment. Plants used for traditional medicine contain a wide range of substances that can be used to treat chronic as well as infectious diseases. The harmful microorganisms can be controlled with drugs and these results in the emergence of multiple drug resistant
bacteria and it has created alarming clinical situations in the treatment of infections. The pharmacological industries have produced a number of new antibiotics, resistance to these drugs by microorganisms has increased. In general, bacteria have the genetic ability to transmit and acquire resistance to synthetic drugs which are utilized as therapeutic agents (Towers et al., 2001). Therefore, present investigation is taken up to develop new antimicrobial agents which are effective against Gram positive and Gram negative bacteria as well as pathogenic fusarium spp.

**Review of Literature**

Infectious diseases are the world’s leading cause of premature deaths, killing almost 50,000 people every day. In recent years, drug resistance to human pathogenic bacteria has been commonly reported from all over the world (Piddock & Wise, 1989; Singh et al., 1992; Mulligen et al., 1993; Davies, 1994; Robin et al., 1998; Ahmad & Beg, 2001).

However, the situation is alarming in developing as well as developed countries due to indiscriminate use of antibiotics. The drug-resistant bacteria and fungal pathogens have further complicated the treatment of infectious diseases in immunocompromised, AIDS and cancer patients (Diamond, 1991; Rinaldi, 1991; Diamond, 1993; Mokoka et al., 2010).

Plants have a great potential for producing new drugs of great benefit to mankind. There are many approaches to search for biologically active principles in plants (Parekh & Chandren, 2006). Medicinal plants represent a rich source of antimicrobial agents. Wide range of different parts of medicinal plants was used for extract as raw drugs and they possess varied medicinal properties. Some of these raw drugs are collected in smaller quantities by local use while many other raw drugs are collected in larger quantities and traded in market as raw material for many herbal industries (Uniyal et al., 2006). The increasing failure of chemotherapeutics and antibiotic resistance exhibited by pathogenic microbial infectious agents have led to the screening of several medicinal plants for their potential antimicrobial activity (Elizabeth, 2005).

In addition, in developing countries, synthetic drugs are not only expensive and inadequate for the treatment of diseases but also have adulteration and side
effects. This kind of situation stresses the need to search a new drug for treating such infection (Sieradzki et al., 1999). Usually the discovery of new drug to cure pathogenic disorders is done by using pathogens obtained from commercial laboratories. The screening of drugs by using such microbial strains comparatively for many years may not be effective due to the evolution of resistant strains at high rate. So the antibacterial screening for any drug by using freshly collected clinical samples will be more potent. The revival of interest in plant derived drugs is mainly due to the current wide spread belief that “green medicine” is safe and more dependable than the costly synthetic drugs which may have adverse effect (Parekh & Chandren, 2006). Hence, researchers are increasingly turning their attention to natural products looking for new leads to develop better drugs against microbial infections (Saravanan et al., 2011). Nature has bestowed upon us a very rich botanical wealth and a large number of diverse types of plants growing wild in different parts of our country. In India several medicinal plants are used from ancient times to cure specific ailments (Alagesabooopathi, 2011).

The wide use of antibiotics in the treatment of bacterial infections has led to the emergence and spread of resistant strains. The emergence of multiple drug resistant bacteria (MDR) has become a major cause of failure of the treatment of infectious disease (Gibbons, 2005). As a result, society is facing one of the most serious public health dilemmas over the emergence of infectious bacteria displaying resistance too many and in some cases, effective antibiotic (Kepil, 2005) much like the situation in human medicine. The use of antibiotics in agriculture, livestock and poultry has accelerated the development of antibiotic resistant strains of microbial pathogens, potentially complicating treatment for plants, animals and human (White et al., 2002). The continues spread of multi drug resistant pathogens has become a serious threat to public health and a major concern for infection control practitioners worldwide (Simonsen et al., 2004). In addition to increasing the cost of drug regimes this scenario has paved way for the re-emergency of the high frequency of opportunity and chronic infection cases in developing countries (Ako-Nai et al., 2003). The slow pace of newer antibiotics development coupled with the availability of fewer antimicrobial actions centered on inhibition of ergosterol synthesis has provided the need to explore nature in search of phytotherapeutic agents work with novel targets and mode of actions. The practice of complementary and alternative
Antibiotics provide the main basis for the therapy of microbial infection. Since
the discovery of these antibiotics and their uses as chemotherapeutic agents, there was
a belief in the medical fraternity that this would lead to the eventual eradication of
infection diseases (Rosina et al., 2009). However, overuse of antibiotics has become
the major factor for the emergence and dissemination of multidrug resistant strains of
several groups of micro-organisms (Harbottle et al., 2006). In the light of the evidence
of rapid global spread of resistant clinical isolates, the need to find new antimicrobial
agent is of paramount importance. However, the past record of rapid, wide spread
emergence of resistance to newly introduced antimicrobial agents indicates that even
new families of antimicrobial agents will have a short life expectancy (Coates et al.,
2002).

Various workers had worked on the antibacterial activity of the plants used on
different medically important isolates but not on multi antibiotic resistant strains.
Recently, Ibrahim et al., (2009) assessed the antibacterial activity of Vernonia
amygdalina and Ocimum gratissimum leaves extract on selected food borne
pathogens. The high zones of inhibition at low concentration proved the plants to be
medically useful. Mbata and Saikia (2008) tested the antibacterial activity of the
extract of leaves of O. gratissimum on Listeria monocytogens. Their findings yielded
great significance in health delivery system, since it could be used as an alternative
treatment to orthodox antibiotics in the treatment of diseases caused by the bacterial
isolates especially as the frequent develop resistance to known antibiotics and reduce
the cost of obtaining health care as observed by Singleton and Lamuela Raventos
(1999). A study has been done on anti-hepatotoxic effects of root and root callus
extracts of Cichorium intybus L. and its results revealed that Cichorium intybus root
callus extract could afford a better protection against carbon tetrachloride induced
heptocellular damage as compared to the natural root extract (Zafar & Mujahid Ali,
1998).

Saxena et al., (1994) documented antibacterial activity of these plants on
selected gram positive and negative bacterial isolates. Gislene et al., (2000) showed
that extracts of Zingiber officinale, Myristica fragrans, Ocimum gratissimum, thyme,
sage, rosemary, yarrow and guava showed antibacterial activity against antibiotic resistant bacteria such as *P. aeruginosa*, *K. pneumonia*, *Proteus sp*, *Shigella sp*. Suree and Pana (2005) found ethanolic extracts and essential oil of *Zingiber officinale* and *Myristicafragrans* to be effective against the *Enterobacteriaceae*. Also Seher et al., (2006) tested the methanolic extract of *Z. officinale* to be effective against *Proteus sp*, *Bacillus sp*, *Staphylococcus sp*, *Klebsiella sp*, *Listeria sp*, *Pseudomonas sp*, and *Streptococcus sp*. Koshy et al., (2009) found the ethanolic extract of *Z. officinale* and *M. fragrans* to be effective on *Bacillus sp*, *Pseudomonas sp* and *Staphylococcus sp*. For these reasons, researchers are increasingly turning their attentions to herbal products, looking for new leads to develop better drugs against MDR microbial strains (Braga et al., 2005). The antimicrobial properties of chloroform microbial extract of *Trianthema decandra* were studied against Gram positive, Gram negative and fungi by disc diffusion assay. Wound healing properties were determined using the excision wound model (Jaswanth et al., 2002a).

It is reported that crude extracts of *Trianthema decandra* L. (Aizoaceae) were studied for the antimicrobial and antioxidant potential. The antimicrobial property of the *Trianthema decandra* was studied against ten bacterial and two fungal strains using the disc diffusion method and minimum inhibitory concentration were determined for each strain, in which chloroform extract has shown bigger zone of inhibition (18.5 to 23.2 mm) at a concentration level of 1.5 mg disc⁻¹ and MIC at 39 µg/ml (Geethalakshmi et al., 2010a). In addition the antioxidant and antibacterial potential of *Trianthema decandra* was assessed and the results revealed significant activity in the ethyl acetate and methanolic extracts of roots and leaves of the plants (Sukantha et al., 2012b).

Sundaram et al., (2011) concluded that the plant *W. somnifera* is a potential candidate for antimicrobial agent to treat diseases. It is also reported that the *C. mangga*, *F. racemosa*, *V. negundo*, *O. basilicum*, and *E. elatior* are potentially good sources of antibacterial agents against the pathogens viz, *K. pneumonia*, *S. aureus*, *S. typhi*, *P. vulgaris* and *P. aeruginosa* (Renisheya et al., 2011).

Ravikumar et al., (2011) concluded that the bioactive compounds from the root extract of *C. serrulata* can be effectively used as an alternative poultry medicine to replace the conventional antibiotics of having adverse side effects. Ibrahim et al.,
(2011) determined that the ethanolic extracts of the *Ocimum gratissimum, Vernonia amygdalina, Zingiber officinale* and *Myristica fragrans* showed considerable antibacterial activity against the MDR bacterial strains used at low concentration of 50-200 mg/ml, thus can be used in the treatment of infectious disease caused by these MDR bacteria.

The results of a study conducted in Iran, showed that the essential oils of *Satureja bachtiarica, Echinophora platyloba, Thymus daenensis* and the ethanol extract of *Quercus branti* had antibacterial activities against *Streptococcus iniae*. In three methods of antibacterial test, the highest level of antibacterial activity was demonstrated by the essential oil of the aerial parts (leaves and stem) of *Satureja bachtiarica* (Pirbalouti Gasemi et al., 2011).

Screening of antibacterial potential of leaves and leaf derived callus extracts of *Solanum trilobatum L.* an important medicinal plant has been done by Natarajan and Kamalanathan (2012) and based on the results, it clearly indicates that most of the extracts were effective against *S. aureus, S. typhi, S. dysentriea, S. sonii, C. diptheriea* and *S. boydii*. The other solvent extracts (such as ethanol, acetone and ethyl acetate) showed moderate to least inhibitory effect against these organisms. The results from their findings indicated that *S. trilobatum* is one of the potential medicinal plant used for therapeutic purpose. Among the two extracts tested, callus extracts was found to be superior to field grown plants.

Natarajan et al., (2010) worked on antibacterial activity of leaf extracts of *Biophytum sensitivum* (L.) and they reported that all the extracts showed various levels of activity on different test organisms and their activity is quite comparable with the standard antibiotics. The acetone extracts showed remarkable antibacterial activity. This study fortifies that methanol and chloroform extracts found to be better antibacterial activity against all the test organisms than petroleum ether extract. The results from these investigations encourage that the plant extracts may be used as anti-infective agents.

Baby et al., (2010) screened methanolic root extract of *Passiflora foetida* contains various phytoconstituents such as carbohydrate, glycosides, phytosterol, flavonoids and phenolic compounds. The antibacterial activity in methanolic root
extract of *Passiflora foetida* by Kirby–Bauer disc diffusion method showed good antibacterial activity against gram negative organism.

The antibacterial and antifungal activities of several extracts of *centella asiatica* L. was investigated by Dash et al., (2011) for their activity against some human pathogenic microbes. The result demonstrated that the petroleum ether, ethanol and chloroform extracts of *Centella asiatica* have higher antimicrobial activities (average 12-19 mm zone of inhibition) than n-hexane and water extracts (average 8-14 mm zone of inhibition) whereas n-hexane extract showed no activity against *E. coli*. All the extracts showed better results against the tested fungal strains comparing with ketocanozole (10µg). The results obtained in the present study suggest that the different extracts of *Centella asiatica* revealed a significant scope to develop a novel broad spectrum of antibacterial and antifungal herbal formulations.

Bhalodia et al., (2011) reported that the microbial activity of hydroalcohol and chloroform extracts of flowers of *Cassia fistula* Linn. (An ethnomedicinal plant) were evaluated for potential antimicrobial activity against medically important bacterial and fungal strains. The antimicrobial activity was determined in both the extracts using agar disc diffusion method. Extracts were effective on tested microorganisms. The antibacterial and antifungal activities of solvent extracts (5, 25, 50, 100, 250µg/ml) of *Cassia fistula* were tested against 2 Gram positive, 2 Gram negative human pathogenic bacteria and 3 fungi respectively. The extracts showed broad spectrum of inhibition by showing antibacterial effect for both Gram positive and Gram negative human pathogen bacterial strains. Crude extracts of *Cassia fistula* exhibited moderate to strong activity against most of the bacteria tested. The tested bacterial strains were *S. aureus*, *S. pyogenes*, *E. coli*, *P. aeruginosa*, and fungal strains were *A. niger*, *A. clavatus*, *C. albicans*. The antibacterial potential of the extracts were found to be dose dependent. The phytochemical analysis of the plants was carried out. The antibacterial activities of the various parts of *Cassia fistula* were due to the presence of various secondary metabolites. Periyasamy and Rajkumar (2010) reported that among the different extracts, methanol extracts showed more antibacterial activity and moderate activity recorded with aqueous, ethyl acetate and chloroform extracts. *Achyranthes aspera* showed maximum antibacterial activity against all the tested bacteria than the other plants. All the bacteria were more susceptible to methanolic extracts than the other organic extracts.
Angalaparameswari et al., (2012) investigated the anti-microbial activity of aristolochic acid from the root of *Aristolochia bracteata*. From the methanolic & ethyl extracts of *Aristolochia bracteata*, aristolochic acid I was isolated and confirmed through IR, NMR & MS. The percentage purity of aristolochic acid I was determined by HPLC & UV method. Antibacterial activity of extracts of *Aristolochia bracteata* and the isolated compound was determined by disc diffusion method. The results revealed that the isolated aristolochic acid from methanolic extract was more pure than the compound from ethyl acetate extract. The various extracts (500 µg/disc) of *Aristolochia bracteata* showed moderate antibacterial activity with the average zone of inhibition of 7-18 mm by disc diffusion method. Among the extracts, ethyl acetate & methanol extracts were shown good anti-microbial activity and the growth of *E.coli* (18 mm) was strongly inhibited. Microbial assay of isolated compound (Aristolochic acid I) from ethyl acetate & methanol extracts were shown good antimicrobial activity and the zone of inhibition of both at higher concentration 50 µg/ml was similar with the standard aristolochic acid. It may be concluded that the isolated compound of aristolochic acid I has good anti-bacterial activity.

Antimicrobial activity of methanolic extract from rhizome and roots of *valeriana wallichii* has been studied by Mhaske et al., (2011) and the result revealed The methanolic extract from roots of *Valeriana wallichii* showed a good inhibition against all the bacterial Strains tested (MIC between 10&80 ug/ml). The Gram positive bacteria were sensitive with Gram negative bacteria and some common fungi.

A study on antimicrobial activity of *Moringa oleifera* (lam.) root extract and ethyl acetate extract showed high antibacterial activity against *Pseudomonas aeruginosa* (18.2 ± 0.2 mm). Chloroform extract were ineffective against *Escherichia coli* and *Proteus mirabilis*. Aqueous extract showed maximum number of inhibition against *Pencillium sp.*, (13.1 ± 0.2 mm) than other extracts and *Aspergillus niger* were ineffective in all the extracts except aqueous extract (Raj et al., 2011).

The results of a study on antimicrobial activity of root extracts of *Stellera chamaejasme* L. in China showed that crude ethanol extract, ethyl acetate fraction and methanol fraction antimicrobial activity against all tested strains. Among the five tested materials, the ethyl acetate fraction exhibited the highest inhibitory action against most of test microorganisms. The water-soluble crystalline was recorded.
moderate activity against three bacteria and twelve phytopathogens. However, the petroleum ether fraction only showed weak inhibitory effect against *Escherichia coli*, *Bacillus subtilis* and seven plant pathogen fungi (Ma et al., 2009).

Antimicrobial activity of secondary metabolites and lectins from plants has been worked by Paiva et al., (2010), they concluded that plant tissues contain secondary metabolites and lectins with antibacterial and antifungal activities and thus are sources of natural bioactive molecules to control pathogens that cause diseases in plants and humans.

The results of a study conducted in Amman-Jordan showed that most plant extracts studied had antibacterial and antifungal activities. The antibacterial activities with the best minimum inhibitory concentration (MIC) values were significantly produced by the aqueous extracts of *Eminium spiculatum* stems and *Lupinus varius*, seeds against *Pseudomonas aeruginosa* and by the ethanolic extracts of *Mandragora autumnalis*, fruits against *Escherichia coli*, and *Methicillin-resistant Staphylococcus aureus* (MRSA). Whereas, the highest significant antifungal activity with the best MIC value was produced by aqueous extracts of *L. varius* seeds against *Candida albicans*. However, leaf extracts of the tested plants were appeared to produce the least antimicrobial activity. It was concluded that the antimicrobial activity is associated with the used part of plant in addition to the type of solvent used for extraction. The antimicrobial effects of some plant extracts, in particular aqueous seed extracts of *L. varius* and ethanolic fruit extracts of *M. autumnalis*, may be used for the topical treatment of skin infections (Obeidat, 2011).

Evaluation of antimicrobial activity against the selected human pathogens (*Bacillus sp*, *Enterococcus faecalis*, *Escherichia coli*, *Klebsiella pneumoniae*, *Non-haemolytic Streptococci*, *Streptococcus epidermidis*, *Pseudomonas aeruginosa*, *Salmonella typhimurium*) by using natural leaves, roots and its respective calli has been done and result revealed that, increased inhibitory activities of callus extracts were found to be the best when compared to the natural plant material extracts. The study suggests the plant *Premna serratifolia* L. is an potent source for phytomedicine development in future (Ravinder Singh, 2011).

In a study which is carried out in the USA, concluded that defatted ethanol ether extract (DEE) of *Z. zanthoxyloides* should be analyzed further because of its
potential as a source of broad spectrum antimicrobial compounds. More importantly, this extract can be a source of compounds which can be used for treating infectious diseases caused by vancomycin-resistant *E. faecium* and methicillin-resistant *S. aureus*. DEE was shown to be inhibitory at the lowest concentration (5 µg µL⁻¹) tested in this study (Ynalvez *et al.*, 2012).

Several workers have reported that many plants possess antimicrobial properties in different plant parts i.e. flower, bark, stem, leaf, root, etc. Recently, a number of plants have been reported for antimicrobial properties across the world (Aswar *et al.*, 2007; Philip *et al.*, 2009; Irudayaraj *et al.*, 2010; Mandal *et al.*, 2010; Nitalikar *et al.*, 2010; Bragadeeswaran *et al.*, 2011; Johnson *et al.*, 2011; Kumar *et al.*, 2011; Madhumitha & Saral, 2011; Mariita *et al.*, 2011; Peixoto *et al.*, 2011; Raja *et al.*, 2011; Saad *et al.*, 2011).

The use of plant extract to treat infectious diseases has been extensively applied by people. Literature and our research work revealed great potential of plant for therapeutic purposes in spite of the fact that they have not been completely investigated. Therefore, more studies need to be conducted to search for new antimicrobial compounds once extracted and used in new therapeutic treatments, they should have their toxicity in vivo. The purpose of this study is to investigate the antimicrobial activity of root and root callus extract of *T. decandra* and its callus extract against human pathogenic bacteria and phytopathogenic fungi.

**Materials and Methods**

**Plant material**

Roots of *T. decandra* (Aizoaceae) were collected from Mysore, Karnataka, India. Specimen of the plant has been deposited in the herbarium of Department of Studies in Botany, University of Mysore, Mysore, Karnataka, India.

**Root callus**

Root callus were achieved on MS media supplemented with 1.0 mg/l BAP + 0.5mg/l NAA.
Preparation of solvent extracts

Thoroughly washed mature roots and root callus were shade dried and then powdered with the help of waring blender. Twenty-five grams of the powder was filled in the thimble and extracted successively with petroleum ether, chloroform, ethyl acetate and ethanol using a Soxhlet extractor for 48 hours. Likewise the same procedure was followed for root callus obtained from the roots of *T. decandra*. All the extracts were concentrated using rotary flash evaporator and preserved at 5°C in airtight bottles until further use.

Human pathogenic bacteria

Gram negative bacteria such as *Escherichia coli* (MTCC 7410), *Pseudomonas aeruginosa* (MTCC 7903), *Klebsiella pneumoniae* (MTCC 7407), *Alcaligenes faecalis* (MTCC 7416), *Enterobacter aerogenes* (MTCC 7325), *Proteus vulgaris* (MTCC 1771), *Proteus mirabilis* (MTCC 425), *Salmonella typhi* (MTCC 733), *Salmonella enterica* sub sp *enterica* (MTCC 3224), *Salmonella paratyphi* A (MTCC 735), *Salmonella typhimurium* (MTCC 1254), *Vibrio parahaemolyticus* (MTCC 451) and Gram positive bacteria such *Staphylococcus aureus* (MTCC 7443), *Staphylococcus epidermidis* (MTCC 435), *Bacillus subtilis* (MTCC 121) and *Bacillus cereus* (MTCC 1272) were obtained from MTCC Chandighar, India.

Plant pathogenic fungi

Important seed-borne pathogenic field and storage fungi associated with maize seeds such as *F. verticilliodes*, *F. anthophilum* *F. oxysporum* and *F. proliferatum* were isolated and cultured for the present study.

Antibacterial activity assay

Triphenyl Tetrazolium Chloride (TTC) method has been carried out by using Muller-Hinton Broth on a tissue culture test plate (96 wells) for bacterial cultures Sette et al., (2006). The stock solutions of the root and root callus extracts of *T. decandra* were diluted and transferred into the first well, and serial dilutions were performed in order to have concentrations in the range of 50 – 0.39 µg/ml. Chloramphenicol was used as the reference antibacterial agents. The inoculum was added to all wells and the plates were incubated at 36°C for 48 h. Antibacterial
activity was detected by adding 0.5% TTC (Triphenyl tetrazolium chloride, Merck) aqueous solution. MIC was defined as the lowest concentration of oil that inhibited visible growth, as indicated by the TTC staining (dead cells are not stained by TTC).

**Antifungal activity**

3-(4, 5-dimethyl-2-thiazolyl)-2, 5-diphenyl-2H-tetrazolium bromide (MTT) assay tests were carried out by Mosmann (1983) and Jahn et al., (1995) using tissue culture test plate (96 wells) for fungal cultures.

The fungal strains were grown at 28°C in potato dextrose broth (PDB) medium. The fungal cells were seeded in the wells of a 96-microtiter plate in Potato Dextrose Broth media at a density of 2 x10^3 cells (100 µl per well). Ten µl of the serially diluted extract was added to each well and the suspension was incubated for 24 h at 28°C. Ten µl of a 3-(4, 5-dimethyl-2-thiazolyl)-2, 5-diphenyl-2H-tetrazolium bromide (MTT) solution [5 mg/ml MTT in phosphate buffered saline (PBS), pH 7.4] was added to each well and the plates were incubated further at 37°C. Thirty µl of 20% (w/v) SDS solution containing 0.02 M HCl was then added and the plates were incubated at 37°C for 16 h to dissolve the formazan crystals that had formed. The turbidity of each well was measured at 570 nm using a microtitrator ELISA reader and observed for change in color. MIC was defined as the lowest concentration of extract that inhibited visible growth, as indicated by the MTT staining (dead cells are not stained by MTT). Bavistin was taken as a standard fungicide at a concentration of 0.5mg/ml.

**Results**

*T. decandra* L. root and root callus extracts of different solvents viz., petroleum ether, chloroform, ethyl acetate and ethanol tested for antibacterial activity, chloroform and ethanol extracts of root callus showed significant activity against Gram positive bacteria particular on *B. subtilis*, *B. cereus* and *Staphylococcus aureus* when compared to crude root extracts (Table 4.1). The result showed significant activity in root callus extract of *T. decandra* compared to the root extract. Petroleum ether and ethanol extract of *T. decandra* showed an MIC of 12.5µg/ml against all the Gram positive bacteria tested whereas 50µg/ml crude chloroform extract and ethyl acetate was required to inhibit *B. subtilis* and *Staphylococcus epidermidis* respectively.
*Trianthema decandra* root callus extract of chloroform, ethyl acetate and ethanol showed significant inhibition at 3.12 to 6.25µg/ml against the tested Gram positive bacteria. However petroleum ether root callus extract showed MIC around 12.50 µg/ml against pathogens tested but did not show any activity against *Staphylococcus aureus* and *Staphylococcus epidermidis*.

Root callus extract showed highly significant antibacterial activity against Gram negative bacteria than the crude root extract (Table 4.2). Root extract of petroleum ether showed a MIC of 25 to 50 µg/ml against all the Gram negative bacteria. Root extract of ethyl acetate and ethanol extract showed lowest MIC of 3.12 to 12.50 µg/ml when compared to petroleum ether and chloroform. No inhibitory activity was observed in petroleum ether root callus extract against *Alcaligenes faecalis*, *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus vulgaris*, *Salmonella enterica sub sp. enterica*, *Salmonella paratyphi A*, *Salmonella typhi* and *Salmonella typhimurium* but inhibited all the other tested Gram negative bacteria at MIC of 50 µg/ml. Significant activity was observed in root callus extract of chloroform, ethyl acetate and ethanol with MIC of around 3.12 to 12.50 µg/ml.

*T. decandra* root and root callus extracts of different solvents viz., petroleum ether, cholorform, ethyl acetate and ethanol were tested for antifungal activity against *Fusarium verticilliodes*, *F. anthophilum*, *F. oxysporum* and *F. proliferatum*. *Trianthema decandra* root callus extracts of chloroform, ethyl acetate and ethanol showed highly significant activity against all the tested *Fusarium spp.* with a lowest MIC of 3.12 µg/ml when compared to root extract of *T. decandra* than with a MIC of 25.50 to 6.25 µg/ml (Table 4.3). Antifungal activity was not observed in petroleum ether root callus extract against the tested *Fusarium spp.* Chloroform and ethyl acetate root extract of *T. decandra* did not show any activity against *F. proliferatum*. Bavistin showed complete inhibition even at a lowest concentration of 0.001mg/ml.

**Discussion**

The history of medicine includes many ludicrous therapies. Nevertheless, ancient wisdom has been the basis of modern medicine and will remain as one important source of future medicine and therapeutics. The future of natural products drug discovery will be more holistic, personalized and involve wise use of ancient and modern therapeutic skills in a complementary manner so that maximum benefits can
be accrued in the management of plant and human disease management. Lag phase for botanical medicine is now rapidly changing for a number of reasons. Problems with drug-resistant microorganisms, side effects of modern drugs, and emerging diseases where no medicines are available, have stimulated renewed interest in plants as a significant source of new medicines.

Fungal deterioration of stored seeds and grains is a chronic problem in the Indian storage system because of the tropical hot and humid climate. The presence and growth of fungi may cause spoilage of food and its quality and quantity. In-spite of use of all available means of plant protection, about 1/3 of the yearly harvest of the world is destroyed by pests and loss due to this is expected to be nearly $300 billion per year (Chandler, 2005). Today, there are strict regulations on chemical pesticide use, and there is political pressure to remove the most hazardous chemicals from the market (Pal & Gardener, 2006). Although restrictions are being imposed to protect food quality and the environment, chemicals are still our only recourse at present to prevent diseases of food crops. In recent years, the need to develop disease control measures as alternative to chemicals has become a priority of scientists’ worldwide (Reddy et al., 2009).

Traditional knowledge will serve as a powerful search engine and most importantly, will greatly facilitate intentional, focused and safe natural products research to rediscover the drug discovery process. The appeal of using natural products for medicinal purposes is increasing, and at present, researchers aim to produce substances with anti-tumor, anti-viral, hypoglycemic, anti-inflammatory, anti-parasite, antimicrobial, tranquilizer and immune modulating activities through tissue culture technology. Advances in the area of cell cultures for the production of medicinal compounds has made possible the production of a wide variety of pharmaceuticals like alkaloids, terpenoids, steroids, saponins, phenolics, flavonoids, and amino acids (Singh, 2011).

The finding of the present investigation is an important step towards crop protection strategies for fungal disease management particularly caused by *Fusarium spp.*, and also on human pathogens. Geethalakshmi et al., (2010a) have given detailed account on *Trianthema decandra* for its phytochemistry and various biological properties of the extract and the constituents might provide incentive for proper
evaluation of the use of the plant in medicine. In the present work callus extract of chloroform, ethyl acetate and ethanol extract showed highly significant activity when compared with crude extract. This tends to express that the active ingredients is an effective antibiotic and active principle or compound may be more in callus than the roots of *T. decandra*. The results of the present investigation is successful in identifying the nature of the bioactive principle and its solubility, which will help in further isolation and characterization of the active principle responsible for the activity for development of new antimicrobial agents for preventive treatment of serious microbial disease infections in both human beings and animals along with plant fungal diseases.

The results of the present study showed that, root and root derived callus extracts of *T. decandra* especially ethyl acetate extract possess bioactive compounds with antimicrobial activity against many pathogens. It is suggested that the ethyl acetate extract of root and its callus revealed a significant scope to develop a novel broad spectrum of antimicrobial drug formulation and can be used to carry out further pharmacological evaluation to be used as antimicrobial agents/drugs.
Table 4.1: Minimal inhibitory concentration (µg/ml) of different solvent extracts of root and root callus extracts of *T. decandra* and chloramphenicol against gram positive bacteria

<table>
<thead>
<tr>
<th>Extracts</th>
<th>Solvents</th>
<th>Test Pathogens (Gram positive Bacteria)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td><em>B. cereus</em> (MTCC 1272)</td>
</tr>
<tr>
<td>Root extract</td>
<td>Petroleum ether</td>
<td>12.50</td>
</tr>
<tr>
<td></td>
<td>Chloroform</td>
<td>25.00</td>
</tr>
<tr>
<td></td>
<td>Ethyl acetate</td>
<td>06.25</td>
</tr>
<tr>
<td></td>
<td>Ethanol</td>
<td>12.50</td>
</tr>
<tr>
<td>Root callus extract</td>
<td>Petroleum ether</td>
<td>12.50</td>
</tr>
<tr>
<td></td>
<td>Chloroform</td>
<td>03.12</td>
</tr>
<tr>
<td></td>
<td>Ethyl acetate</td>
<td>06.25</td>
</tr>
<tr>
<td></td>
<td>Ethanol</td>
<td>03.12</td>
</tr>
<tr>
<td></td>
<td>Chloramphenicol 1mg/ml</td>
<td>03.12</td>
</tr>
</tbody>
</table>

Note: Values are mean of triplicates
NA - no activity
Table 4.2: Minimal inhibitory concentration (µg/ml) of different solvent extracts of root and root callus extracts of *T.decandra* and chloramphenicol against gram negative bacteria

<table>
<thead>
<tr>
<th>Extracts</th>
<th>Solvent</th>
<th>Test Pathogens (Gram negative Bacteria)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Root extract</td>
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<td></td>
</tr>
<tr>
<td>Petroleum ether</td>
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<td>25.00</td>
</tr>
<tr>
<td>Chloroform</td>
<td>25.00</td>
<td>12.50</td>
</tr>
<tr>
<td>Ethyl acetate</td>
<td>12.50</td>
<td>06.25</td>
</tr>
<tr>
<td>Ethanol</td>
<td>06.25</td>
<td>03.12</td>
</tr>
<tr>
<td>Root callus extract</td>
<td>Petroleum ether</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td>Chloroform</td>
<td>03.12</td>
</tr>
<tr>
<td></td>
<td>Ethyl acetate</td>
<td>06.25</td>
</tr>
<tr>
<td></td>
<td>Ethanol</td>
<td>12.50</td>
</tr>
<tr>
<td>Chloramphenicol (1mg/ml)</td>
<td>03.15</td>
<td>03.15</td>
</tr>
</tbody>
</table>

Note: Values are mean of triplicate, NA -no activity

1. Alcaligenes faecalis (MTCC 7416)
2. Enterobacter aerogens (MTCC 7325)
3. Escherichia coli (MTCC 7410)
4. Klebsiella pneumoniae (MTCC 7407)
5. Proteus mirabilis (MTCC 425)
6. Proteus vulgaris (MTCC 1771)
7. Pseudomonas aeruginosa (MTCC 7903)
8. Salmonella enterica sub sp. enterica (MTCC 3224)
9. Salmonella paratyphi A (MTCC 735)
10. Salmonella typhimurium (MTCC 1254)
11. Salmonella typhi (MTCC 733) and
12. Vibrio parahaemolyticus (MTCC 451)
Table 4.3: Minimal inhibitory concentration (µg/ml) of different solvent extracts of root and callus root extract of *T. decandra* and Bavistin against *Fusarium* spp.

<table>
<thead>
<tr>
<th>Extracts</th>
<th>Solvents</th>
<th>Test phytopathogenic <em>Fusarium</em> spp</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td><em>F. verticilliodes</em></td>
</tr>
<tr>
<td>Root extract</td>
<td>Petroleum ether</td>
<td>12.50</td>
</tr>
<tr>
<td></td>
<td>Chloroform</td>
<td>06.25</td>
</tr>
<tr>
<td></td>
<td>Ethyl acetate</td>
<td>06.25</td>
</tr>
<tr>
<td></td>
<td>Ethanol</td>
<td>12.50</td>
</tr>
<tr>
<td>Root callus extract</td>
<td>Petroleum ether</td>
<td>NA</td>
</tr>
<tr>
<td></td>
<td>Chloroform</td>
<td>03.12</td>
</tr>
<tr>
<td></td>
<td>Ethyl acetate</td>
<td>03.12</td>
</tr>
<tr>
<td></td>
<td>Ethanol</td>
<td>03.12</td>
</tr>
<tr>
<td>Bavistin</td>
<td></td>
<td>0.001</td>
</tr>
</tbody>
</table>

Note: Values are mean of triplicate, NA- no activity