Chapter 8
Antimicrobial Activity of Callus Extracts of
*Justicia adhatoda* L. in Comparison with Vasicine

8.1. ABSTRACT

The present work ascertains the antimicrobial activity of methanolic extracts of callus of *Justicia adhatoda* and vasicine against *Staphylococcus aureus*, *Streptococcus pyogenes*, *Serratia marcescens*, *Klebsiella pneumoniae*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Candida albicans*, *Cryptococcus neoformans* and *Aspergillus flavus*. It was determined by agar well diffusion method and paper disc diffusion method. The antimicrobial activity of the concentrated extracts was evaluated by the determination of the diameter of zone of inhibition against microorganisms. 25µg ml⁻¹ concentration was used to check the antimicrobial activity of callus extracts and vasicine. Minimum inhibitory concentrations and minimum microbicidal concentrations were determined against all the test pathogens. Sensitivity of the pathogens was also checked with four standard antibiotics, ciprofloxacin and ofloxacin for bacteria and nystatin and amphotericin B for fungi. Results of the phytochemical studies revealed the presence of alkaloids in the extracts were active against both bacteria and fungi. Minimum inhibitory concentration and minimum microbicidal concentration studies of the extracts on the test organisms showed that the lowest minimum inhibitory concentrations and minimum microbicidal concentrations were demonstrated against *S. marcescens*, *E. coli* and *P. aeruginosa* and the highest minimum inhibitory concentration was exhibited against *S. aureus*, *S. pyogenes*, *K. pneumoniae*. Among fungi *A. flavus* showed lowest minimum inhibitory concentration whereas *C. albicans* and *C. neoformans* showed highest minimum inhibitory concentration. The present study revealed that *J. adhatoda* has broad spectrum antimicrobial activity and a potential source of antimicrobial agents that could be useful for chemotherapy and control of infectious diseases.

**Key Words:** antimicrobial activity, agar well diffusion method, paper disc diffusion method, minimum inhibitory concentrations, minimum microbicidal concentrations.
8.2. INTRODUCTION

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 (Mahesh and Satish, 2008). It has been established that up to 25% of the drugs prescribed in conventional medicines are allied directly or indirectly to natural substances mostly of plant origin. In recent years, pharmaceutical companies have spent a lot of time and money in developing natural products extracted from plants, to produce more cost effective remedies that are affordable to the population (Doughari, 2006).

Traditionally used medicinal plants produce a variety of compounds of known therapeutic properties. The substances, that can either inhibit the growth of pathogens or kill them and have no or least toxicity to host cells, are considered candidates for developing new antimicrobial drugs. In recent years, antimicrobial properties of medicinal plants are being increasingly reported from different parts of the world. It is expected that plant extracts showing target sites other than those used by antibiotics will be active against drug-resistant microbial pathogens. However, very little information is available on such activity of medicinal plants (Ahmad and Beg, 2001).

A wide range of medicinal plant parts used for extract as raw drugs and they possess varied medicinal properties. The different parts used include root, stem, leaves, flower, fruit, twigs exudates and modified plant organs. While some of these raw drugs are collected in smaller quantities by the local communities and folk healers for local uses, many other raw drugs are collected in larger quantities and traded in the market as the raw material for many herbal industries. Although hundreds of plant species have been tested for antimicrobial properties, the vast majority of have not been
adequately evaluated (Mahesh and Satish, 2008). Considering the vast potentiality of plants as sources for antimicrobial drugs with reference to antibacterial and antifungal agents, a systematic investigation was undertaken to screen the antibacterial and antifungal activity from *J. adhatoda*.

8.3. REVIEW OF LITERATURE

Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from natural sources; many of these isolations were based on the uses of the agents in traditional medicine. This plant-based, traditional medicine system continues to play an essential role in health care, with about 80% of the world’s inhabitants relying mainly on traditional medicines for their primary health care (Owolabi *et al.*, 2007). According to World Health Organization, medicinal plants would be the best source to obtain a variety of drugs. Therefore, such plants should be investigated to better understand their properties, safety and efficacy (Nascimento *et al.*, 2000).

Many studies indicate that in some plants there are many substances such as peptides, unsaturated long chain aldehydes, alkaloidal constituents, some essential oils, phenols and water, ethanol, chloroform, methanol and butanol soluble compounds. These plants then emerged as compounds with potentially significant therapeutic application against human pathogens, including bacteria, fungi or viruses (El astal *et al.*, 2005).

Plants have a great importance in our lives because they fulfil our basic needs for food, shelter, clothing, fuel, ornamentals, flavouring and medicine. Throughout the world, plants are used to treat various infectious diseases. Medicinal plants are important with respect to new drug and
pharmacological research development. The use of plants as medicines dates back to ancient times. Recently, the use of medicinal plants increased substantially (Khan et al., 2001). Medicinal plants play an important role for the management of different microbial infections (Shinwari et al., 2009). Kruti et al. (2011) stated that medicinal plants must be tested for microbiological contamination and foreign materials to assure quality. Plants that are being used in conventional herbal remedies should be investigated for their potential to produce new drugs with antimicrobial properties similar to those of modern medicines.

Experiments on the use of plant compounds against microbes were first documented in the late 19th century. Natural products perform various functions and many have interesting and useful biological activities. Researchers are turning their attention to natural products to develop better anticancer, antiviral and antibacterial drugs (Srinivasan et al., 2001). Antimicrobial properties of medicinal plants are being increasingly reported from different parts of the world. Many researchers have examined the uses of medicinal plants, but only a few studies have tested these Ethno-botanical findings in a laboratory setting to confirm the real antimicrobial properties of these plants (Bhattarai et al., 2008; Shakya et al., 2008). Medicinal plants can provide a wealth of antimicrobial agents, and hundreds have been investigated for biological activities. Local people collect raw materials in small quantities and use them to treat diseases. Raw materials are also collected in huge amounts and traded in the market place to supply herbal industries (Uniyal et al., 2006).

Infectious diseases are the leading causes of untimely death worldwide and they have become a global concern (Kumar et al., 2008; Mahady, 2005; Sakata et al., 2009). The clinical efficacy of many existing antibiotics
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is being threatened by rapid emergence of multidrug-resistant pathogens (Penner et al., 2005; Westh et al., 2004). Many infectious diseases have been known to be treated with herbal remedies throughout the history of mankind (Wadud et al., 2007). Natural products, either as pure compounds or as formulated with measured constituents of plant extracts, provide unlimited opportunities for emergence of new drug leads (Mukherjee and Wahile, 2006). There is a continuous and urgent need to discover new antimicrobial compounds with diverse chemical structures and novel mechanisms of action for new and re-emerging infectious diseases (Barbosa et al., 2009; Hazni et al., 2008; Kumar et al., 2008). Contrary to the synthetic drugs, antimicrobials of plant origin are not associated with many side effects (Mukherjee and Wahile, 2006) and have an enormous therapeutic potential to treat many infectious diseases.

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. 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 (Dabur et al., 2007). The rising incidence in multidrug resistance amongst pathogenic microbes has further necessitated the need to search for newer antibiotic sources (Doughari, 2006).

Nowadays, the development of resistance by a pathogen to many of the commonly used antibiotics provide an impetus for further attempts to
search for new antimicrobial agents to compact infections and overcome the problems of resistance and side effects of the currently available antimicrobial agents. The treatment of infectious diseases with antimicrobial agents continues to present problems in modern-day medicine with many studies showing a significant increase in the incidence of bacterial resistance to several antibiotics. Multiple drug resistance in human pathogenic microorganisms has developed due to indiscriminate use of commercial antimicrobial drugs commonly used in the treatment of infectious diseases. This situation forced scientist for searching new antimicrobial substances from various sources which are the good sources of novel antimicrobial chemotherapeutic agents (Dogan et al., 2010).

Most clinical microbiology laboratories in this country now use the paper disc diffusion method for determining susceptibility of microorganisms to antibiotics and chemotherapeutic agents. Disc diffusion method for antibiotic susceptibility testing is the Kirby-Bauer method. A number of modifications of the test are employed (Bauer et al., 1966). This method is well documented and standard zones of inhibition have been determined for susceptible and resistant values. There is also a zone of intermediate resistance indicating that some inhibition occurs using this antimicrobial but it may not be sufficient inhibition to eradicate the organism from the body.

The antibacterial activity of crude methanolic extracts of medicinal plant parts was evaluated by using agar well diffusion method (Ahmad and Beg, 2001, Srinivasan et al., 2001).

Minimum inhibitory concentration (MIC) is defined as the lowest concentration of a drug that will inhibit the visible growth of an organism
after overnight incubation period; period is extended for organisms such as anaerobes, which require prolonged incubation for growth. The MIC is considered the ‘gold standard’ for determining the susceptibility of organisms to antimicrobials and are therefore used to judge the performance of all other methods of susceptibility testing. MIC is used in diagnostic laboratories to conform unusual resistance, to give a definitive answer when a borderline result is obtained by other methods of testing or when disc diffusion methods are not appropriate. The range of antibiotic concentrations for determining MIC is universally accepted to be in doubling dilution steps up and down from 1 mgL\(^{-1}\) as required (Andrews, 2001).

In the present study, the antimicrobial activity of methanolic extract of callus of \textit{J. adhatoda} was determined against Gram positive and Gram negative pathogenic bacteria and fungi along with pure vasicine and reference antibiotics.

**8.4. SPECIFIC OBJECTIVES**

This study was designed with the following objectives,

1. To determine the antibacterial activity of methanolic extract of callus of \textit{J. adhatoda} using disc diffusion method and agar well diffusion method.

2. To determine the antifungal activity of methanolic extract of callus of \textit{J. adhatoda} using disc diffusion method and agar well diffusion method.

3. To determine the minimum inhibitory concentration and minimum microbicidal concentration of methanolic extract of callus of \textit{J. adhatoda}
8.5. MATERIALS AND METHODS

8.5.1. PREPARATION OF CALLUS EXTRACTS

Hundred grams of calli were placed in hot air oven at a temperature of 50°C for 4 - 5 days till the weight became constant. Calli were regularly examined to check any fungal growth or rotting. The dried calli were powdered to obtain a very fine particle size using sterile clean mortar and pestle. Fifty grams of the powdered calli were soaked in 50 ml absolute methanol in 250 ml sterile conical flask, incubated at 37°C with shaking at 120 rpm for 30 minutes and kept for 24 hours. After 24 h, the extract was filtered rapidly through four layers of gauze. The content was then filtered with Whatman No.1 filter paper and the residue was again treated with 50 ml of absolute alcohol and incubated as mentioned earlier. It was repeated 3 times. The pooled up filtrates were evaporated to dryness using a desiccator. The dried extract was finally reconstituted in 5 ml of absolute ethanol and estimated the total concentration of alkaloid present in it as vasicine (Soni et al., 2008). Then packed in separate sterile glass vials as aliquots with 25 µg ml⁻¹ of vasicine and stored at 4°C until use. Aliquots of pure standard vasicine (SPIC India Ltd, Chennai) of same concentration was also prepared and stored.

8.5.2. CULTURE AND MAINTENANCE OF TEST MICRO ORGANISMS FOR ANTIMICROBIAL STUDIES

Bacterial cultures of Escherichia coli, Serratia marcescens, Pseudomonas aeruginosa, Klebsiella pneumoniae, Staphylococcus aureus, and Streptococcus pyogenes and fungal cultures of Aspergillus flavus, Candida albicans and Cryptococcus neoformans were obtained from the Microbiology Laboratory of Presentation College of Applied Sciences, Puthenvelikkara, Ernakulam, Kerala. All the bacterial strains were
maintained on nutrient agar (NA, Hi-Media) at 37°C and fungi were maintained on Sabouraud’s Dextrose agar (SDA, Hi-Media) at room temperature. Bacteria were inoculated in nutrient broth and incubated at 37°C for 24 hours for doing the test. Mueller- Hinton Agar (MHA, Hi-Media) and SDA were used for testing the antibacterial and antifungal activity respectively. For S. pyogenes blood agar was used.

8.5.3. PREPARATION OF INOCULUM

Each 24 hour culture suspension of microorganisms was standardized to 25% transmittance at 560 nm using an ultraviolet (UV) -visible spectrophotometer for obtaining 10^6 colony forming units (CFU) ml^{-1}. McFarland standards were used as a reference to adjust the turbidity of microbial suspension so that the number of microorganisms will be within a given range. For the preparation of the 0.5 McFarland standard, 0.05 ml of barium chloride (BaCl_2) (1.17% w/v BaCl_2.2H_2O, E. Merck, India) was added to 9.95 ml of 0.18M H_2SO_4 (1.0% w/v, E. Merck, India) with constant stirring. The McFarland standard tube was tightly sealed to prevent loss by evaporation and stored for up to 6 months. To aid comparison the test and standard were compared against a white background with a contrasting black line (Andrews, 2001). Fungal isolates were standardized to 10^6 spores ml^{-1} by using spectrophotometer at 530 nm and were adjusted to 80% to 85% transmittance.

8.5.4. ANTIMICROBIAL ACTIVITY SCREENING

Agar-well diffusion methods (Ahmad and Beg, 2001), and paper disc diffusion methods (Kirbey-Bour method) (Bauer et al., 1966) were employed to determine the antimicrobial activities for methanolic extracts. Twenty microlitres of methanolic extracts of the calli at concentration of 25µg ml^{-1}
and pure vasicine with 25µg ml\(^{-1}\) concentration were used against the test microorganisms.

8.5.4.1. Antibacterial and Antifungal Screening by Agar Well Diffusion Method

Approximately 20 ml of sterile MHA and SDA was poured into sterile Petri plates and allowed to set. Plates were then seeded with 0.5 ml of a 24 h old bacterial culture and using a sterile glass (L) rod made a lawn culture. SDA plates were seeded with fungal cultures. The plates were allowed to dry. For doing agar well diffusion method, wells are made on the plate with the aid of a sterile hole puncture (8.0 mm diameter). Twenty microlitres of the callus extract and vasicine were poured into the respective wells. The plates thus prepared were left at room temperature for ten minutes, allowing the diffusion of the extracts into the agar. Then the plates with bacterial culture and fungal culture plates except *A. flavus* were placed in the incubator at 37\(^{0}\)C for 24 h. The plates with *A. flavus* were kept at room temperature for 48-72 h. After incubation the plates were observed for the antimicrobial activity of the callus extract and vasicine and were assessed by an inhibitory zone surrounding the well. The zone of inhibition was measured and expressed in millimetres.

8.5.4.2. Antibacterial and Antifungal Screening by Paper Disc Diffusion Method

Sterile MHA and SDA culture plates were prepared as agar well diffusion method. Sterile filter paper discs (diameter 6mm for bacteria and 13mm for fungi) impregnated with 20 µL of extract and vasicine at concentration of 25µg ml\(^{-1}\) were applied over each of the culture plates previously seeded with the 0.5 McFarland and 10\(^{6}\) CFU ml\(^{-1}\) cultures of
bacteria and $10^6$ spores ml$^{-1}$ of fungi respectively. Cultures were then incubated as in agar well diffusion method.

Paper discs of 25µg ml$^{-1}$ of ciprofloxacin, ofloxacin, nystatin and amphotericin B (Hi-Media) were used as positive control for comparison in both methods. Sterilized paper discs without extracts or antibiotics were used as negative control for both. Antimicrobial activity was determined by measurement of zone of inhibition around each paper disc. Overall, cultured bacteria with halos equal to or greater than 7 mm and fungi with 10 mm halos were considered susceptible to the tested extract (Nascimento et al., 2000). For each organism three replicate trials were conducted.

8.5.5. DETERMINATION OF MINIMUM INHIBITORY CONCENTRATION (MIC)

The MIC of the extract was estimated for each of the test organisms in triplicates. To 0.5ml of varying concentrations of the extracts (25.0, 12.5, 6.25, 3.17, 1.58, 0.781 and 0.39 mg ml$^{-1}$) 2ml of nutrient broth was added and then a loopful of the test organism previously diluted, approximately to $10^6$ CFUml$^{-1}$ (for bacterial isolates) and $10^6$ spores ml$^{-1}$ (for fungal isolates), was introduced to the tubes. The procedure was repeated on the test organisms using the standard antibiotics. A tube containing nutrient broth seeded with the test organisms only, as described above, kept to serve as control. All tubes containing cultures were then incubated as mentioned above. After incubation the tubes were then examined for microbial growth by observing for turbidity. The MIC values were interpreted as the lowest concentration (highest dilution) of the sample, which showed no growth.
8.5.6. DETERMINATION OF MINIMUM MICROBICIDAL CONCENTRATION (MMC)

To determine the MMC, for each set of test tubes in the MIC determination, a loopful of broth was collected from those tubes which did not show any growth and inoculated on sterile NA (for bacteria) and SDA (for fungi) by streaking. NA and SDA plates streaked with the test organisms respectively were serve as control. Plates were then incubated as above. After incubation the concentration at which no visible growth obtained was noted as the minimum microbicidal concentration.

8.6. RESULT

8.6.1. ANTIMICROBIAL ACTIVITY

Phytochemical constituent present in the callus extract is alkaloid, mainly vasicine. Results of the antimicrobial activity of the callus extract and standard vasicine along with reference antibiotics are shown in Table 8.1 and 8.2 and Figure 8.1 and 8.2. The results showed that the callus extract was effective against organisms studied.

The antimicrobial activity of callus extract was variable according to various organisms. The inhibition zones ranged between 4.3 mm to 14.8 mm diameter (Table 8.1 and 8.2, Plate 8.1).

The results obtained in the present study relieved that _J. adhatoda_ callus extract possesses potential antibacterial activity against _S. aureus_, _S. pyogenes_, _S. marcescens_, _K. pneumoniae_, _E. coli_, and _P. aeruginosa_ and antifungal activity against _C. albicans_, _C. neoformans_ and _A. flavus_. When tested by disc diffusion method, the highest antibacterial activity of 12.3 mm recorded in _S. aureus_ and least activity recorded in _P. aeruginosa_ measured 4.3 mm (Table 8.1).
Antifungal activity of callus extract showed significant activity when compared with the standard vasicine and standard antibiotics. Among the three tested fungi highest antifungal activity of 14.8 mm was obtained against *C. albicans* and least activity of 9.06 mm against *A. flavus*. When compared to nystatin and amphotericin B better antifungal activity was obtained against *C. albicans* and *C. neoformans* (Table.8.2).

These results were compared with standard antibiotics used, nystatin and amphotericin B and ofloxacin and ciprofloxacin. But the extract showed higher activity than the given standard antibiotic against *S. aureus*, *K. pneumonia* and *E. coli* and antifungal activity against *C. albicans* and *C. neoformans*.

8.6.2. MINIMUM INHIBITORY CONCENTRATION (MIC) AND MINIMUM MICROBICIDAL CONCENTRATION (MMC)

Results of MIC and MMC are shown in Table.8.3. The result showed that *S. pyogenes* had the highest MIC (25 µg ml\(^{-1}\)) and MMC (25 µg ml\(^{-1}\)), while the lowest MIC of 3.125 µg ml\(^{-1}\) was shown by *S. marcescens* and *C. albicans* respectively.
Table 8.1. Antibacterial activity of methanolic extracts of callus of *J. adhatoda*, pure vasicine standard, and reference antibiotic discs

<table>
<thead>
<tr>
<th>Name of organisms tested</th>
<th>Zone of inhibition (mm)</th>
<th>Methanolic extract of callus of <em>J. adhatoda</em> (25µg ml&lt;sup&gt;-1&lt;/sup&gt;)</th>
<th>Pure vasicine standard (25 µg ml&lt;sup&gt;-1&lt;/sup&gt;)</th>
<th>Ofloxacin (25 µg ml&lt;sup&gt;-1&lt;/sup&gt;)</th>
<th>Ciprofloxacin (25 µg ml&lt;sup&gt;-1&lt;/sup&gt;)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Klebsiella pneumoniae</em></td>
<td>9.2±0.25</td>
<td>10.2±0.2</td>
<td>8.8±0.0</td>
<td>10.0±0.2</td>
<td></td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>12.2±0.2</td>
<td>12.5±0.2</td>
<td>9.1±0.1</td>
<td>11.0±0.0</td>
<td></td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td>4.3±0.5</td>
<td>6.0±0.2</td>
<td>2.0±0.0</td>
<td>8.0±0.0</td>
<td></td>
</tr>
<tr>
<td><em>Streptococcus pyogenes</em></td>
<td>8.9±0.3</td>
<td>9.8±0.2</td>
<td>7.0±0.1</td>
<td>8.2±0.1</td>
<td></td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>12.3±0.3</td>
<td>12.8±0.3</td>
<td>9.5±0.0</td>
<td>10.8±0.2</td>
<td></td>
</tr>
<tr>
<td><em>Serratia marcescens</em></td>
<td>8.5±0.25</td>
<td>8.2±0.2</td>
<td>7.8±0.1</td>
<td>9.0±0.1</td>
<td></td>
</tr>
</tbody>
</table>

Table 8.2. Antifungal activity of methanolic extract of callus of *J. adhatoda*, pure vasicine standard, and reference antibiotic discs

<table>
<thead>
<tr>
<th>Name of organisms tested</th>
<th>Zone of inhibition (mm)</th>
<th>Methanolic extract of callus of <em>J. adhatoda</em> (25 µg ml&lt;sup&gt;-1&lt;/sup&gt;)</th>
<th>Pure vasicine standard (25 µg ml&lt;sup&gt;-1&lt;/sup&gt;)</th>
<th>Amphotericine B (25 µg ml&lt;sup&gt;-1&lt;/sup&gt;)</th>
<th>Nystatin (25 µg ml&lt;sup&gt;-1&lt;/sup&gt;)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Aspergillus flavus</em></td>
<td>9.06±0.3</td>
<td>10.5±0.2</td>
<td>12.0±0.0</td>
<td>14.0±0.2</td>
<td></td>
</tr>
<tr>
<td><em>Candida albicans</em></td>
<td>14.8±0.3</td>
<td>14.2±0.2</td>
<td>11.0±0.2</td>
<td>12.0±0.2</td>
<td></td>
</tr>
<tr>
<td><em>Cryptococcus neoformans</em></td>
<td>11.1±0.2</td>
<td>11.5±0.2</td>
<td>10.0±0.1</td>
<td>11.0±0.2</td>
<td></td>
</tr>
</tbody>
</table>
Plate 8.1. Plates showing zone of inhibition in disc diffusion method
Plate 8.2. Plates showing zone of inhibition in agar well diffusion method

S. pyogenes  S. aureus  S. marcescens  K. pneumoniae

E. coli  P. aeruginosa  C. neoformans  C. albicans

A. flavus
Figure 8.1. Graph showing zone of inhibition against antibacterial agents

Figure 8.2. Graph showing zone of inhibition against antifungal agents
Table 8.3. Minimum inhibitory concentration (MIC) and minimum microbicidal concentration (MMC) of methanolic extracts of callus of *J. adhatoda*

<table>
<thead>
<tr>
<th>Sl.No</th>
<th>Name of the organisms tested</th>
<th>MIC (µg ml⁻¹)</th>
<th>MMC (µg ml⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><em>Klebsiella pneumoniae</em></td>
<td>6.25</td>
<td>6.25</td>
</tr>
<tr>
<td>2</td>
<td><em>Escherichia coli</em></td>
<td>3.125</td>
<td>6.25</td>
</tr>
<tr>
<td>3</td>
<td><em>Pseudomonas aeruginosa</em></td>
<td>Not Detected</td>
<td>Not Detected</td>
</tr>
<tr>
<td>4</td>
<td><em>Streptococcus pyogenes</em></td>
<td>25</td>
<td>25</td>
</tr>
<tr>
<td>5</td>
<td><em>Staphylococcus aureus</em></td>
<td>12.5</td>
<td>25</td>
</tr>
<tr>
<td>6</td>
<td><em>Serratia marcescens</em></td>
<td>3.125</td>
<td>6.25</td>
</tr>
<tr>
<td>7</td>
<td><em>Aspergillus flavus</em></td>
<td>3.125</td>
<td>6.25</td>
</tr>
<tr>
<td>8</td>
<td><em>Candida albicans</em></td>
<td>12.5</td>
<td>25</td>
</tr>
<tr>
<td>9</td>
<td><em>Cryptococcus neoformans</em></td>
<td>25</td>
<td>25</td>
</tr>
</tbody>
</table>

8.7. 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 vivo* antimicrobial activity assay. Many reports are available on the antiviral, antibacterial, antifungal, antihelminthic, antimolluscal and anti-inflammatory properties of plants (Mahesh and Satish, 2008). Some of these observations have helped in identifying the active principle responsible for
Emergence of multi drug resistance in human and animal pathogenic bacteria as well as undesirable side effects of certain antibiotics has triggered immense interest in the search for new antimicrobial drugs of plant origin. *P. aeruginosa* was the most resistant strain of all the bacteria used in this study. In fact, Gram negative bacteria, especially *P. aeruginosa* are frequently reported to have developed multi drug resistance to many of the antibiotics. Therefore, it is not surprising to learn that *P. aeruginosa* is the least responding bacterial strain to the tested plant callus extract (Dogan *et al.*, 2010).

Comparing the antimicrobial activity of the tested samples to that of reference antibiotics, the inhibitory potency of tested extracts could mostly be considered as important (Table 8.1 and 8.2). This is due to the fact that medicinal plants are natural origin, which means more safety for consumers, and are considered that they are being low risk for resistance development by pathogenic microorganisms.

The highest MIC and MMC values of *S. aureus* is an indication that either the callus extracts are less effective on some gram positive bacteria or that the organism has the potential of developing antibiotic resistance, while the low MIC and MMC values for other bacteria is an indication of the high efficacy of the callus extracts (Table 8.3).

Present study revealed that the callus extracts inhibited bacterial growth but their effectiveness varied. Plant based antimicrobials have enormous therapeutic potential as they can serve the purpose with lesser side effects that are often associated with synthetic antimicrobials. Moreover
further exploration of plant derived antimicrobials is needed today. Thus, the study ascertains the value of plants used in Ayurveda, which could be of considerable interest to the development of new drugs.

The demonstration of activity against both Gram-negative and Gram-positive bacteria and fungi is an indication that the callus of *J. adhatoda* can be a source of bioactive substances that could be of broad spectrum of activity. Thus the broad spectrum of antimicrobial activity by *J. adhatoda* may help to discover new chemical classes of antibiotic substances that could be useful for chemotherapy and control of infectious diseases.