1.2 Introduction to *Pseudomonas aeruginosa*

Domain          Bacteria
Phylum          Proteobacteria
Class           Gammaproteobacteria
Order           Pseudomonadales
Family          Pseudomonadaceae
Genus           *Pseudomonas*
Species         *aeruginosa*

*Pseudomonas aeruginosa* is a ubiquitous gram-negative bacillus, 1.5-3μm x 0.5μm in size, aerobic, rod shaped bacterium with unipolar flegella and recognize exceedingly effective in colonizing a diversity of environments (Palleroni, 1992b; Rostamzadeh et al., 2016). Its production of pigments (yellow, blue, or rust-shaded) differentiates it from most other Gram-negative microorganism. Pyocyanin (blue pigment), flurescin (yellow pigment) both are not produced only by *P. aeruginosa* but also by other pathogenic species. A bright green color that diffuses throughout the medium due to the combined effect of pyocynin and flurescin (Ryan and Ray, 2004). *P. aeruginosa* grows at 37– 42 °C; but its growth at 42 °C differentiates it from other species. It is fail to ferment carbohydrates, but most of the strains oxidize glucose (Brooks et al., 2007).

1.2.1 Epidemiology

*P. aeruginosa* normally inhabit water, soil, and vegetation, isolated from the stool, skin and throat of healthy persons. They often colonize hospital food, sinks, taps, mops, and respiratory equipment. Occasionally, *P. aeruginosa* can colonize human body sites, with a preference for moist areas, such as the perineum, axilla, ear, nasal mucosa and throat; as well as stools. The prevalence of colonization by *P. aeruginosa* in healthy subjects is usually low,
but higher colonization rates can be encountered following hospitalization, especially amongst subjects treated with broad-spectrum antimicrobial agents. Spread is from patient to patient via contact with fomites or by ingestion of contaminated food and water (Baron, 1996).

1.2.2 Pseudomonas infections and symptoms

*Pseudomonas aeruginosa* causes contaminations in healthy individuals who are hospitalized or have a compromised immune system as a result of other infections. An assortment of human diseases is regularly associated with this bacterium:

- **Respiratory infection**: infection in respiratory track; symptoms include shortness of breath, fever, chills, increases heart rate, decrease appetite, malaise, systemic inflammatory response and pigmented cough or sputum.
- **Urinary infections**: infection in urinary track; symptom include pain or pressure in your back or lower abdomen, Cloudy, dark, bloody, or strange-smelling urine and fever or chills.
- **Pneumonia**: infection in the lungs; symptoms include chills, fever, cough that is productive, and difficulty breathing.
- **Bacteremia**: bacterial infection of the blood; symptoms include fever, chills, fatigue, muscle and joint pains.
- **Folliculitis**: skin infection; symptoms include itchy rash, bleeding ulcers, and headache.
- **Swimmer’s ear**: ear canal infection; symptoms include swelling, ear pain, itching, inside the ear, discharge from the ear, and difficulty hearing.
- **Eye**: symptoms include inflammation, pus, and pain.
1.2.3 Treatment

*P. aeruginosa* is a nosocomial pathogen and naturally resistant to many antibiotics and exhibited additional resistance after ineffective treatment, rarely, through alteration of a porin. It needs to usually be potential to coordinate treatment in line laboratory sensitivities, as opposed to choosing an antibiotic empirically. Antibiotics agents that have activity against *P. aeruginosa* may include:

- Aminoglycosides (gentamicin, amikacin, tobramycin, but not kanamycin)
- Cephalosporins (ceftazidime, cefepime, cefoperazone, cefpirome, ceftobiprole, but not cefuroxime, cefotaxime)
- Quinolones (ciprofloxacin, levofloxacin, but not moxifloxacin)
- Antipseudomonal Penicillins: carboxypenicillins (carbenicillin and ticarcillin) and ureidopenicillins (mezlocillin, azlocillin, and piperacillin). *P. aeruginosa* is intrinsically resistant to all otherpenicillins.
- Monobactams (aztreonam)
- Polymyxins (colistin and polymyxin B)
- Carbapenems (Imipenem, meropenem, doripenem, but not ertapenem)

These antibiotic agents should all be given by injection, with the exceptions of fluoroquinolones, aerosolized tobramycin and aerosolized aztreonam. Therefore, in most of the hospitals, use of fluoroquinolone is severely restricted to avoid the development of resistant strains of *P. aeruginosa*. In the uncommon occasions where contamination is superficial and restricted (for instance, ear diseases or nail contaminations), topical gentamicin or colistin might be used.
1.3 Introduction to *Candida* spp.

Kingdom  Fungi

Division  Class

Class  Saccharomycetes

Order  Saccharomycetales

Family  Saccharomycetaceae

Genus  *Candida*

Species  *albicans, tropicalis, glabrata, krusei* etc

The genus *Candida* belongs to the phylum *Ascomycota* and contains about 200 species. The most known species one of these is *Candida albicans*, but other clinically important species include *C. glabrata, C. tropicalis, C. parapsilosis* and *C. krusei*. Many *Candida* species occur as normal microbiota flora of the skin, gastro-intestinal tract and genitor-urinary tract. However, some *Candida* species have the potential to cause infection in humans and 75% of women have infected with vaginal yeast infection. The increase of immunocompromised host or treated with broad spectrum antifungals, *Candida* may overgrow and become infectious. *Candida* spp. behind these infections is most notably *C. albicans*. For this reason, pregnant women and diabetic patients are at the greatest risk for contracting a yeast infection. On the other hand stress, hormonal changes and changes in acidity can increase women’s chances of contracting a yeast infection ("Vaginal Candidiasis"). A yeast infection can be difficult upon a physical exam because the symptoms are similar to many other genital infections. Usually if a physician takes the time, diagnosis is done under a microscope ("The Basics of Vaginal Yeast Infections" 2015). Furthermore, identifying the etiological agent is complicated
because the various *Candida* spp. are indistinguishable from one another by microscopic observation.

### 1.3.1 Candida albicans

*C. albicans* is one of the very few fungal species causing disease in humans—millions of others do not. It is a member of the healthy microbiota, asymptotically colonizing the gastrointestinal (GI) tract, reproductive tract, oral cavity, and skin of most humans (Ganguly et al., 2011; Nobile and Johnson, 2015).

As previously reported, *C. albicans* is the most common *Candida* species isolated from the oral cavity of healthy subjects or from patients affected by different diseases. *Candida albicans* is basically characterized by the production of true hyphae which are detected by the germ tube test although this feature also found *C. dubliniensis*. Over the past decay, researchers have investigated the pathogenic attributes of *C. albicans* and these have recently been reviewed by a variety of authors (Calderone & Gow, 2002; Douglas, 2003; Naglik et al., 2003). In comparison with *C. glabrata* or *C. krusei*, *C. albicans* is more sensitive to commercial available antifungal agents, but some resistance has been reported to azole therapy in immunodepressed patients (Rex et al., 2000; Salehei et al., 2012) reported antifungal sensitivity against *Candida* spp. and found they were highly resistant to fluconazole and econazole antifungals.

### 1.3.2 Candida glabrata

Researchers are found that increased *C. glabrata* infection in hospitalised patients immunocompromised, indeed *C. glabrata* is the most commonly recovered non-*C. albicans* species from the oral cavity of HIV-infected patients (Junqueira et al., 2012). *C. glabrata* has a haploid genome and lacks the ability to switch to filamentous growth as like *C. albicans*. 
Several studies have noted that of *C. glabrata* are resistant to fluconazole and itraconazole (Moran et al., 2002; Panackal et al., 2006; Whaley et al., 2017). Sharifzadeh and Shokri (2016) reported, *C. glabrata* which were isolated from HIV patients are most sensitive to polyene drugs such as amphotericin B, nystatin and less sensitive to fluconazole.

### 1.3.3 Candida krusei

*Candida krusei* can replace *C. albicans* in the oral cavities of HIV patients, particularly after azole therapy (Ruhnke et al., 2000). Several studies have been found that *C. krusei* is inherently resistant to fluconazole, and that fluconazole prophylaxis may promote the proliferation of this organism (Rex et al., 2000).

### 1.3.4 Candida tropicalis

*C. tropicalis* infections have been reported in immunocompromised patients who have had chronic mucocutaneous candidiasis (Kothavade et al., 2010). In a large surveillance study conducted by Pfaller et al., (2009), *C. tropicalis* showed a moderate level of fluconazole resistance. There are many studies present on azole resistance, specifically in *C. albicans* and *C. tropicalis* (Brun et al., 2004; Yang et al., 2004; Vermitsky & Edlind, 2004).

### 1.3.5 Candida infections

Several *Candida* species are commensal and colonize the skin and mucosal surfaces of humans. Critically ill or otherwise immunocompromised patients are more prone to develop both superficial and life-threatening *Candida* infections (Hasan et al., 2009). *Candida* infections also constitute the most common fungal infections in AIDS patients. These patients predominantly develop oropharyngeal candidiasis, which can lead to malnutrition and interfere with the absorption of medication.
An infection caused by *Candida* is termed candidiasis or candidosis. Mycoses caused by these fungi show a wide spectrum of clinical presentations and can be classified as superficial, as with cutaneous and mucosal infections, to deep, widespread and of high severity, as is the case with invasive candidiasis. According to Colombo & Guimaraes (2003), the main transmission mechanism is through endogenous *Candidaemia*, in which *Candida* species that constitute the microbiota of various anatomical sites under conditions of host weakness behave as opportunistic pathogens. Another mechanism for transmission is exogenous, and this occurs mainly through the hands of health professionals who care for patients. Also indicated in the spread of infection are health-care materials, such as contaminated catheters and intravenous solutions (Ingham et al., 2012). *Candida* species are considered important pathogens due to their versatility and ability to survive in various anatomical sites. It was believed decades ago that yeasts passively participated in the process of pathogenesis in the establishment of fungal infection. Thus, organic weakness or an immunocompromised host was considered the only mechanism responsible for the establishment of opportunistic infection. Today, this concept has been modified. The current consensus is that these organisms actively participate in the pathophysiology of the disease process using mechanisms of aggression called virulence factors (Tamura et al., 2007).

**1.3.6 Treatment**

Over the next 60 years, antifungal drug development has continued, and today we have several classes of antifungal drugs that are used in the clinic to treat fungal infections. There are 4 categories of antifungals according to their mode of action.
1.3.6.1 The polyenes

The class of polyenes includes several hundred different drugs, but the most commonly used ones are nystatin and amphotericin B (Odds et al., 2003). Polyenes target ergosterol, a major lipid in fungal plasma membranes. Through binding to ergosterol, AmB creates a pore that allows ions and other cellular constituents to diffuse across the membrane, ultimately leading to fungal cell death.

1.3.6.2 Flucytosine

Flucytosine, namely 5-flucytosine (5-FC), is active against Candida spp. (non filamentous fungi) but is ineffective against filamentous fungi such as A. fumigates. It is commonly used in combination with other drugs, particularly AmB, because when used as monotherapy, it is only fungistatic (causing arrest of fungal cell growth) and also fungal cells rapidly develop resistance against 5-FC. 5-FC targets the process of nucleic acid synthesis. 5-FC is first rapidly converted into 5-fluorouracil (5-FU) by the enzyme cytosine deaminase (Abaci and Haliki-Uztan, 2011).

1.3.6.3 The echinocandins

The echinocandins are the newest class of antifungals and consist of 3 FDA approved agents, caspofungin, micafungin and anidulafungin for treatment of invasive fungal infections like Candida. All echinocandins target the fungal cell wall by inhibiting the enzyme "-glucan synthetase, which is involved in the synthesis of the major cell wall polysaccharide, "-1, 3- glucan.

1.3.6.4 The azoles

The class of azole drugs is one of the major groups of antifungals used for the treatment of infectious diseases caused by fungi (Lass-Flörl, 2011). This group including ketoconazole,
triazole, fluconazole, voriconazole and posaconazole which are active against a wider range of fungal pathogens. Azoles target the fungal specific ergosterol biosynthesis pathway.

1.4 Occurrence of *P. aeruginosa* and *Candida* spp. in clinical samples

Motayo et al., (2012) processed a total of 91 ear swab samples were processed comprising 45 male and 46 female patients. 78(85.7%) isolates were recovered consisting 57(73%) gram negative bacteria, 20(25.6%) gram positive bacteria and 1 isolate of *Candida albicans*. It showed that *Pseudomonas aeruginosa* 38(48.7%) was the most predominant bacteria species isolated from ear infection and *Candida albicans* 1(1.3%) was the least prevalent.

Shanthi and Sekar (2009) analyzed the occurrence of *Pseudomonas* spp. in clinical samples. The common site of isolation was the respiratory tract (41.8%) followed by urinary tract (25.5%), wound (20%) and blood (12.7%).

Mangaiarkkarasi et al., (2013) processed a total of 5381 biological specimens for occurrence gram negative bacteria. Among 5381 specimens received, 1485 bacterial isolates were recovered from diverse biological specimens of both inpatients and out patients72% of the samples showed bacterial growth on culture and 15% out of them were *P. aeruginosa* isolates predominantly isolated from pus (64%) and urine (16.6%) samples. The other common gram negative organisms isolated were *E. coli* (57%), *Klebsiella* spp. (22%), *Proteus* spp. (3%) and others (3%).

Oliveira et al., (2008) isolated Seventy-six *P. aeruginosa* strains from the dental environment (5 strains) and water system (71 strains).

Magdy et al., (2013) reported occurrence of *P. aeruginosa* in clinical and environment swabs and they were isolated 31% strains from environment samples (200 samples) including wall, floor, medical appliances and the surroundings of out patients clinic. 43 % *P. aeruginosa*
strains which were isolated from clinical samples (400 samples) including urine (8%) and sputum (35%).

Premalatha et al., (2016) reported prevalence of *P. aeruginosa* in various clinical samples including sputum, pus, urine and others. They found out of 581 samples, 100 were positive to *P. aeruginosa*, the rate of bacteria was higher in pus samples (58) followed by blood (22), urine (12), sputum (4) and others (4).

Kirecci and Kareem (2014) isolated *P. aeruginosa* from burns, wounds, urine and ear specimens. The 25.53% of strains were isolated from burn materials, 9.09% from wound materials, 5.66% from urine, and 9.09 % from ear samples.

Senthamarai et al., (2014) studied 3760 total clinical samples for occurrence of the *Pseudomonas* isolates and 104 isolates of *P. aeruginosa* were isolated (2.76%). Among these samples, pus (47.11%) was the predominant sample of isolation, which was followed by sputum (36.53%), urine (12.5%) and blood (3.84%).

Salehei et al., (2012) isolated different *Candida* spp. from vaginal infected patients. They isolated 79.1% *C. albicans*, 5.9% *C. tropicalis*, 2.9% *C. krusei* and 11.9% *C. glabrata* respectively. Patel et al., (2012) reported 430 *Candida* isolates showing highest number from urine (30.5%), followed by sputum (28.9%) and blood (26%). 161(37.4%) and 269 (62.6%) were *C. albicans* and non *Candida albicans* spp.

Badiee and Alborzi (2011) isolated 595 *Candida* strains from various clinical samples with higher rate of *C. albicans* (48%), followed by *C. glabrata* (13.5%) and *C. Parapsilosis* (4.8%). The most sites of the isolate (70%) were mouth and lung (sputum and bronchoalveolar lavage), but *Candida* species were also isolated from the blood, cerebro
spinal fluid, sinus biopsy, eyes, pleural and abdominal tap. The most species isolated from the patients was *C. albicans* followed by *C. krusei, C. glabrata, C. kefyr, and C. parapsilosis*. Jayalakshmi et al., (2014) carried out a study to speciate *Candida* isolates from various clinical specimens at a tertiary care hospital. Among the hundred samples two species of *Candida* were isolated only from five samples. Twenty six confines were from HIV receptive patients and eighteen were from diabetic patients. Most regular isolate among all types of *Candida* was *C. albicans* (31.42%) trailed by *C. tropicalis* (26.66%).

Sajjan et al., (2014) reported occurrence of *Candida* spp. in various clinical specimens. The prevalence of *Candida* species was found to be 6.1%. The most common isolate found to be *Candida* albicans (65.0%) followed by non albicans *Candida* mainly *C. tropicalis* (24.3%), and *C. krusei* (10.7%). The highest number of isolates was from high vaginal swab isolated from vulvovaginitis constituting 42(40.8%). Among 5 ears swabs, 4(80%) were *C. albicans*, 1(18.18%) were *C. krusei* (20%). Among 4 samples of stool, 2(50 %) species were *C. albicans* and one (25%) each of *C. tropicalis* and *C. krusei*.

Jaggi et al., (2014) identified the various species of *Candida* from clinical specimens (urine, sputum, stool, pus, various body fluids, skin and corneal scrapings, medical implants and blood) suspected of Candidal infection. A total of 125 *Candida* isolates from various clinical specimens were obtained. *Candida* was mainly isolated from blood (42) and respiratory samples (25). The most common species of *Candida* isolated was *C. albicans* forming 44% of the total isolates. The non-albicans *Candida* species form the remaining 56% of the total isolates, thus stressing their emergence as major fungal pathogens.

Rathnapriya and Sulaiha (2016) studied various clinical samples to speciate the *Candida* isolates. They observed Non albicans *Candida* (86%) as compared to *Candida albicans*
(14%) among the total 50 Candida isolates. C. tropicalis had a higher prevalence rate of 30% followed by C. glabrata and C. krusei with a prevalence rate 22%.

Brandolt et al., (2017) assessed the prevalence of Candida spp. in the cervical-vaginal mucosa of patients. Among 263 patients 27% showed positive for Candida spp. relating to a pervasiveness of roughly 15% for both VVC and colonization. In 27% positive isolate from patients over 60% were identified as Candida albicans, while, non-albicans were isolated at a rate of 8.6% in symptomatic patients and 14.3% in asymptomatic patients.

Sukumaran et al., (2012) studied 50 Candida isolates from clinical samples. Out of 50 Candida isolates collected 62% were from males and 38% from females. The distribution of the clinical samples was urine 44%, exudate 32%, respiratory 14% and blood 10%. C. albicans (54%) was the most common species isolated from these samples. The distribution of non C. albicans was C. tropicalis (18%), C. krusei (12%), C. glabrata (10%), C. rugosa (6%). Among the non-albicans C. tropicalis was seen predominantly in urine and exudates.

1.5 Antibiotics and their over use

1.5.1 History and nature of antibiotics

The meaning of the word antibiotic in Greek is “against life”. Antibiotics are chemical substances produced by various species of microorganisms and other living systems that are capable of inhibiting the growth of or killing bacteria and other microorganism. The recent progress made in the chemistry of natural products has also contributed to the development of the method now in use for the isolation of antibiotics. The period 1885 - 1939 may be considered as one in which the foundation was laid for the development of our knowledge of Antibiotics. The production of chemical agents came to be recognized as responsible for the
inhibitory effect. These agents were at first designated as “lethal principles” and “Toxic Substances”. Their designation as “antibiotics” is only of recent origin.

The first systematic search for the antibiotics from micro organisms, made by Gratia and Dath in 1924, resulted the discovery of actinomycetin in strains of actinomycetes (fungus), and in the soil microorganisms. Actinomycetin was never used for the treatment of the diseases in patients but was used to lyses cultures of bacteria for the production of vaccines.

In 1929, Fleming published his observations on the effect of a fungal contaminant, identified at that time as penicillin rubrum but later as penicillin notatum, upon the growth of bacteria, Fleming designated the antibacterial product of the fungus as penicillin. The foregoing observations, in the studies of mixed cultures carried out before 1939, amply illustrated the act that numerous types of microorganisms, especially the bacteria, fungi and actinomycetes possess the capacity to inhibit the growth of other microorganisms. This inhibiting effect was shown to be due to the production of specific chemical substances later designated as antibiotics.

1.5.2 Antibiotic pollution

Antibiotic pollution in the environment may facilitate the development and spread of antibiotic resistance (Martinez, 2008). It has now become clear that man-made antibiotics agents can enter the environment via waste water treatment plant effluents, hospital and processing plant effluents, application of agricultural waste and bio solids to fields, and leakage from waste storage containers and landfills (Kümmerer, 2009). In surface waters receiving municipal waste-water, concentrations of antibiotics rarely exceed 1mg/l, but are more regularly in the low mg/l range. Antibiotic residues have also been found in marine environments. In several cases antibiotic residue concentrations exceeding 1 mg/l have been
detected in treated industrial effluents or recipient waters (Li et al., 2008; Sim et al., 2011). One of the difficulties of relating increased levels of resistance in the environment to antibiotic pollution, however, is the fact that antibiotic resistance genes can be co-released into the environment with antibiotic compounds. From an environmental health perspective, the selective pressure that antibiotic pollution may exert on clinically important bacteria is of particular concern. Several clinically relevant bacteria, such as *Escherichia coli* and the enterococci, occur and are able to grow in different environments (Moriarty et al., 2008). In the presence of environmental concentrations of antibiotics, they may face a selective pressure leading to a gradual increase in the prevalence of resistance.

As to in creature farming, administered drugs, their metabolites or debasement items achieve the amphibian condition by the use of compost or slurry to territories utilized agronomically, or by field – raised creature discharging straightforwardly on the land, took after by surface run – off, drifting or leaching in more profound layers of earth. In this way, soils can go about as a wellspring of anti-infection sullying of the oceanic condition. Mackie et al., (2006) distinguished both antibiotic tetracycline deposits and antibiotic tetracycline protection qualities in ground water affect by swine generation offices. Be that as it may, anti-infection agents contribution by farming use in the minor starting point of antimicrobial in the amphibian condition. Most of the investigated substances began from release of sewage into water ways just for a few example could an impact of creature cultivation on the occurrence of anti-toxins in surface water be expected. Anti-microbial agents from the fluoroquinolone group have additionally been found in hospitals effluent in different parts of the world. Amongst fluoroquinolones, ciprofloxacin was the most commonly found antibiotic in hospital effluent. High residue levels of fluoroquinolones in the aquatic environment can
cause genotoxic effects and can modify bacterial strains like *Salmonella typhimurium* at a residue level as low as 5μg/l for norfloxacin and 25μg/l for ciprofloxacin Hartmann et al., (1999).

Chang et al., (2010) studied Sewage samples from hospitals, nursery, slaughter house, waste water treatment plant. Results showed that the concentration of ofloxacin in hospital was the highest among all water environments ranged from 1.660 mg/l to 4.240 mg/l and norfloxacin (0.136–1.620mg/l), ciprofloxacin (0.011mg/l to 0.136mg/l), trimethoprim (0.061–0.174mg/l) were commonly detected. Expulsion scope of antibiotics in the sewage treatment plant was 18– 100% and the evacuation proportion of tylosin, oxytetracycline and antibiotic medication were 100%. Relatively higher removal efficiencies were observed for tylosin, oxytetracycline and Tetracycline (100%), while lower removal efficiencies were observed for trimethoprim (1%), Epi-iso-chlorotetraacycline (18%) and erythromycin-H 2O (24%).

Brown et al., (2006) studied Twenty-three samples of wastewater and 3 samples of Rio Grande water were analyzed for the presence of 11 antibiotics. Fifty-eight percent of samples had at least one antibiotic present while 25% had three or more. Hospital effluent had detections of sulfamethoxazole, trimethoprim, ciprofloxacin, ofloxacin, lincomycin, and penicillin G, with 4 of 5 hospital samples having at least one antibiotic detected and 3 having four or more. At the residential testing destinations, ofloxacin was found in spouting from helped living and retirement offices, while the understudy quarters had no identifies. Only lincomycin was detected in dairy effluent (in 2 of 8 samples, at 700 and 6600 ng/l). Civil wastewater had discoveries of sulfamethoxazole, trimethoprim, ciprofloxacin, and ofloxacin, with 4 of 6 tests having no less than one anti-toxin present and 3 having at least 3. The relatively high concentrations (upto 35,500 ng/l) of ofloxacin found in hospital and
residential effluent may be of concern due to potential genotoxic effects and development of antibiotic resistance.

Diwan et al., (2013) reported six of the eight antibiotics hospital wastewater from continuous and grab sampling methods. They observed the highest quantities of fluoroquinolones were released in winter followed by the rainy season and the summer. No temporal pattern in antibiotic release was detected.

Lagishetty and Nagarajan (2015) analysed hospital effluent samples for presence of antibiotic residues detected by high performance liquid chromatography (HPLC). They were reported Ciprofloxacin, Enrofloxacin, Oxytetracycline, Trimethoprim and Ampicillin were present in most of the samples and 90% of the samples contained residues of at least one of the investigated antimicrobials.

1.6 Antibiotic resistance
The discovery of antibiotics in the mid-twentieth century revolutionized the management and treatment of infectious disease caused by bacteria. Infections that would normally have been fatal were now curable. Since then, antimicrobial agents (antibiotics and related medicinal drugs acting on bacteria, viruses, fungi and parasites) have saved the lives and eased the suffering of millions of people. Today, antibiotics are crucial not only for the treatment of bacterial infections, but also for prophylactic coverage of high risk patients e.g. those in intensive care, organ transplants, cancer chemotherapy and prenatal care. However, these gains are now seriously jeopardized by the rapid emergence and spread of microbes that are resistant to antimicrobials. The mass production of penicillin in 1943 dramatically reduced illness and death from infectious diseases caused by bacteria. However, within four years, bacteria began appearing that could resist the action of penicillin. Pharmaceutical companies fought back by developing other types of antibiotics. After more than 50 years of widespread
use of these “miracle drugs”, antibiotics are no longer as effective as they once were. Usually all important microbial infections in throughout the world are becoming resistant. And even though pharmacological industries have produced a number of new antibiotics in the last three decades, resistance to these drugs by microorganisms has increased. Typically, all microorganisms have the hereditary capacity to transmit and secure protection from antimicrobial specialists like medications, which are used as remedial operators (Amenu, 2014). Several different epidemiological studies indicate that antibiotic resistance is increasing in clinical isolates. Being gram-negative bacteria, most *Pseudomonas* spp. are naturally resistant to penicillin and majority of related beta-lactum antibiotics, but a number are sensitive to piperacillin, imipenem, tobramycin or ciprofloxacin. Nowadays more and more resistance of *P. aeruginosa* are encountered in routine clinical practice, a serious problem, increase morbidity and mortality and also cost of treatment. *P. aeruginosa* show resistance towards antibiotics is due to a blend of factors, low absorption of metabolites on the microbial cell wall. It has the hereditary ability to express a wide collection of protection systems. It can wind up noticeably safe through transformations in the chromosomal qualities, which direct the protection qualities. Resistance can also supplemented by mutation in genes, from other organisms via plasmids, transposons and bacteriophages.

### 1.6.1 Causes for drug resistance

Major causes of antibiotics resistance are antibiotic over use, abuse, misuse, due to incorrect diagnosis. Antibiotic use in animal husbandry, causing some drug resistant microbes, which can be transmitted to humans. Increased globalization could also cause the spread of drug resistance. Finally, hospital settings often give rise to antibiotic resistant bacteria.
1.6.2 Types of drug resistance

1.6.2.1 Natural and acquired resistance

The resistance towards antibiotics can be of two types natural and acquired. Natural resistance implies that the microorganisms are 'inherently' resistant for example, Streptomyces has a few transformed genes in charge of resistance from its own particular antibiotic. Other cases incorporate organisms that do not have transport system and target for the antibiotics. In different cases, the resistance can be due to expanded efflux action. Acquired resistance is commonly caused by mutations in the genes, or by the acquisition of the plasmids or transposons, which carry the antibiotic resistance genes (Hoek et al., 2011).

1.6.2.2 Genetic and phenotypic resistance

Antibiotic resistance also divided into genetic drug resistance, most commonly discussed, and phenotypic resistance, which is a more slight type. Genetic resistance is caused by chromosomal mutations or presence of antibiotic resistance genes on moving genetic material like plasmids or transposons. Phenotypic resistance is due to changes in the microorganism physiological state, such as the stationary phase, antibiotic persisters, and the dormant state.

1.7 Antibiotic resistance in Pseudomonas aeruginosa and Candida spp.

Amutha et al., (2009) reported the highest resistance of Pseudomonas aeruginosa strains against ampicillin (85%) followed by amikacin (62.2%), gentamicin (48%), imipenem (5%), meropenem (17%) and ciprofloxacin (50.9%).

tam et al., (2010) found, 100% resistance to carbapenems and quinolones, 91% resistance against penicillins / cephalosporins and 21% against Aminoglycosides in Pseudomonas aeruginosa.
According to Li et al., (1994) report, *P. aeruginosa* is naturally resistant to -lactams, including broadplasmid spectrum cephalosporins, quinolones, chloramphenicol and tetracyclines, mainly because of the very low permeability of their cell wall.

Yetkin et al., (2006) found the percentage of resistance to cephalosporins against *P. aeruginosa*, was in the range of 27 to 88%. Javiya et al., (2008) reported resistance rate of 67.86% for ceftazidime, 50% for amikacin, and 69.64% for ciprofloxacin. They suggested that the use of amikacin should be limited to serious nosocomial infections in order to prevent further resistance.

Meharwal et al., (2002) reported a high resistance rate for ciprofloxacin (76.3%) followed by 65.8% for ceftazidime, and 50% for amikacin against *Pseudomonas* in UTI patients. Shah et al.,(2015) isolated *P. aeruginosa* from UTI patients with5.4% and found resistant drugs included ceclor(100%) and cefizox (100%) followed by amoxil/ampicillin (99.6%), ceflixime (99.6%), doxycycline (99.6%),cefuroxime (99.2%), cephradine (99.2%), cotrimoxazole (99.2%), nalidixic acid (98.8%), pipemidic acid (98.6%) and augmentin (97.6%).

Motayo et al., (2012) reported *Pseudomonas* isolates with 100% resistance, against Amoxicillin, and Erythromycin and 97.4% against chloramphenicol.

Rostamzadeh et al., (2016) observed rate of antibiotic resistance in *P. aeruginosa*. Isolates showed resistance maximum (99.5%) against trimetoprime sulfametoaxsole and ciprofloxacin (55.33%), amikacin (61%), imipenem (33%), isolated from clinical samples namely wound swab, urine samples, blood, trachea samples, eye swabs, ear swabs and pus.

Vijaya D. et al., (2011) reported sensitivity in *Candida* spp.100% sensitive to amphotericin B, clotrimazole, nystatin and ketaconozole. 87.5% of *C. krusei*, 36% *C. tropicalis*, 6.5% *C.
*C. albicans* were resistant to itraconazole. 25% of *C. krusei*, 28% *C. tropicalis* showed resistant to fluconazole. *C. dubliniensis* was resistant to itraconazole only.

Al-Abeid et al., (2004) showed that all tested *Candida* were susceptible to nystatin, miconazole, ketoconazole and fluconazole and *C. albicans* isolates were more susceptible to azoles than was *C. glabrata*.

Salehei et al., (2012) reported that 100% of non-*albicans* species were resistant to fluconazole, 64.3% to terbinafine and 57.1% to econazole.

Mangaiarkkarasi et al., (2013) reported drug resistance in *Pseudomonas* spp. Maximum susceptibility was observed to meropenam (100%), imepenam (82.6%), aztreonam (80%), followed by ciprofloxacin, tobramycin (77.7%), ceftazidime (57.5%) and ceftriaxone (57.1%). A high level of resistance was observed to ticarcillin (69.2%) and amikacin (50%).

Patel et al., (2012) reported azole group sensitivity in *Candida* species. 25.5% sensitivity was recorded in *C. albicans* and 18.7% in *C. tropicalis* while amphotericin B sensitivity varies from 75.6% to 100% to all *Candida* spp.

Brandolt et al., (2017) reported the prevalence of resistance against fluconazole and itraconazole by 42% and 48%, respectively; the minimal inhibitory concentration of miconazole ranged from 0.031 to 8 g/ml, and that of nystatin ranged from 2 to >16 g/ml. The high rate of resistance to triazoles observed in their study suggested the necessity of the association of laboratory exams to clinical diagnosis to minimize the practice of empirical treatments that can contribute to the development of resistance in the isolates.
1.8 Antibiotic resistance mechanism in bacteria

The most common antibiotic resistance mechanisms have been the alteration of target site, production of β-lactamases, intrinsic drug resistance (which includes low membrane permeability and an active efflux system) and acquired resistance.

1.8.1 Alteration of target site

Chemical modifications in the antibiotic target may result in reduced affinity of the antibiotic to its binding site (Sibanda and Okoh, 2007). Resistance to β-lactams is a result of modification in the active site of penicillin-binding proteins. The main mechanism in the development of resistance to fluoroquinolones is decrease in binding of the quinolones to enzymes because of changes in DNA gyrase enzyme and/or the topo isomerase enzyme. As the nucleotide and amino acid sequence of gyr A, gyr B, par C and par E genes needed for the synthesis of DNA topoisomerase are very similar to those of DNA gyrase enzyme, mutations occur in gyr B, par C genes and the resistance observed in P. aeruginosa is usually active against all quinolones (Algun et al., 2004).

1.8.2 Production of enzyme

β- Lactamases are enzymes that confer significant antibiotic resistance to their bacterial hosts by hydrolysis of the amide bond of the four-membered β-lactam ring (Sibanda and Okoh, 2007). The β-lactamases found in P. aeruginosa can belong to three different groups:

1. Narrow-spectrum active site-serine enzymes of molecular classes A and D (e.g., PSE-1, PSE-4 and some OXA-type enzymes) that efficiently degrade the antipseudomonal penicillins and cefoperazone, yet have no critical action against the other hostile to pseudomonal cephems, monobactams or carbapenems (Livermore, 1995; Nass et al., 1999)
2. Extended-spectrum active site-serine enzymes of molecular classes A and D (e.g., PER-1, VEB-1, GES-1, GES-2, various OXA-type enzymes and, although rarely, also TEM- and SHV-type extended-spectrum variants) that, in addition to penicillins, can also degrade the anti-pseudomonal cephems and monobactams but not carbapenems (Bradford et al., 2001 and Dubois et al., 2002).

3. Metallo-enzymes of molecular class B (e.g. the enzymes of the IMP, VIM, SPM and GIM type) that efficiently degrade virtually all the anti-pseudomonal β-lactams except monobactams (Docquier et al., 2003; Castanheira et al., 2004; Rossolini and Mantengoli, 2005).

1.8.3 Intrinsic drug resistance and efflux systems

*Pseudomonas aeruginosa* shows significant degrees of intrinsic resistance to a wide variety of antimicrobial agents including most β-lactams, chloramphenicol, tetracyclines and fluoroquinolones, due mainly to its low outer membrane permeability and to active efflux of antibiotics. A few reports have demonstrated that dynamic efflux can be a system of resistance for all antibiotics (Li et al., 2002). Multidrug-resistance efflux pumps are either chromosomally encoded or plasmid encoded and are ubiquitous proteins (Poole, 2007; Sun et al., 2014). They belong to five families of transporters namely; the major facilitator super-family (MFS), the adenosine triphosphate (ATP)-binding cassette (ABC) super-family, the small multidrug-resistance (SMR) family and the resistance nodulation-cell division (RND) super-family and the multidrug and toxic compound extrusion (MATE) family. Four *P. aeruginosa* multidrug-efflux systems have been reported, all of which are members of the resistance-nodulation-cell division (RND) family.
1.9 Drug resistance mechanism in fungi

*Candida* is the fourth leading cause of nosocomial, i.e. hospital derived, infections worldwide and can be acquired as early as 72 hours after hospitalization (Pfaller and Diekema, 2010). Generally, treating patients suffering from candidiasis is hampered by the fact that the immune system of these individuals is already compromised. Treating such patients has always been challenging, but was significantly improved in the 1950’s when the first antifungal agent, nystatin was isolated from a soil fungus. Over the next 60 years, antifungal drug development has continued, and today we have several classes of antifungal drugs that are used in the clinic to treat fungal infections. The next section describes these agents and groups them into 4 categories according to their mode of action.

1.10 Mechanisms of drug resistance against several classes of antifungal drugs

Clinical drug resistance has been reported for all 4 major classes of drugs namely azoles, polyenes, echinocandins and flucytosine.

1.10.1 Polyenes

This group including above hundred drugs but most common are Nystatin and Amphotericin –B which was discovered in 1954 and today marketed as Fungizone® by X-Gen pharmaceuticals (Pound et al., 2011). Polyenes are isolated from soil dwelling fungi of the genus *Streptomyces*. For instance, resistance to AmB is still rare despite extensive use over several decades. The exact rate of AmB resistance remains unknown, and although AmB resistance has been observed in clinical isolates as well as in laboratory strains, the mechanism of AmB resistance remains elusive. In some cases, AmB resistance has been linked to a significant reduction of ergosterol in the plasma membrane, thus leading to drug resistance because the target of AmB is reduced or even absent.
1.10.2 Flucytosine

Flucytosine, namely 5-flucytosine (5-FC), which is marketed as Ancobon® and distributed by Valeant pharmaceuticals Intl., was originally discovered in 1957 for its antitumor activity (Pound et al., 2011). 5-FC targets the process of nucleic acid synthesis, first rapidly converted into 5-fluorouracil (5-FU) by the enzyme cytosine deaminase (Waldorf and Polak, 1983). Resistance to 5-FC, on the other side, is very common. Known resistance mechanisms include decreased transport into the cell via the enzyme cytosine permease, alterations of metabolic enzymes (cytosine deaminase and uridine monophosphate pyrophosphorylase, uPRTase), as well as increased production of competitive pyrimidines. Mutations in the enzyme uPRTase seem to be the most commonly encountered event leading to 5-FC resistance in clinical isolates (Chandra et al., 2009).

1.10.3 Azoles

Probably the most-studied and best-understood mechanisms of drug resistance come from the use of azole drugs. Azole resistance is likely a consequence of extensive clinical and generally fungistatic (Vandeputte et al., 2011). At least 4 different types of azole resistance mechanisms have been described. The first one is facilitated by enhanced ATP-dependent efflux where the cell actively pumps the drug out of the cell. In such azole resistant isolates, genes encoding transporters containing the ATP binding cassette (ABC) have been found to be upregulated compared to susceptible isolates (Torelli et al., 2008). Genes that encode for such ABC transporters were first identified in C. albicans as CDR1 (standing for “Candida drug resistance 1”) and CDR2, but have now also been characterized in C. glabrata, C. tropicalis and in C. neoformans (Sanglard, 2002). There are at least two possibilities for how CDR1 and CDR2 can become upregulated in drug resistant C. albicans isolates. The first
results when a region within the coding sequence of \textit{CDR1} is mutated, leading to increased transcription initiation and mRNA stability. The second results from the hyper activation of \textit{TAC1}, a transcription factor that regulates \textit{CDR1} expression. Two mutations in particular, N972D and N977D, have been shown to cause hyperactivity of \textit{TAC1}, leading to drug resistance in clinical isolates of \textit{C. albicans} (Znaidi et al., 2007).

A second mechanism causing azole resistance is through the process of passive diffusion, where the cell uses the proton gradient across the plasma membrane to pump drugs out of the cell. This mechanism has been observed in several \textit{Candida} spp., including \textit{C. albicans}, \textit{C. tropicalis} and \textit{C. dubliniensis} and has been linked to one gene in particular, \textit{MDR1}. The mechanism behind \textit{MDR1}-coupled drug resistance has recently been elucidated by microarray studies. Comparing transcriptional profiles of a drug resistant clinical isolate and its susceptible parent strain, it was shown that \textit{MDR1} overexpression correlates with \textit{MRR1} overexpression. \textit{MRR1} is a transcription factor controlling \textit{MDR1} activity, and when \textit{MRR1} mutations, such as P683S or G997V were introduced in susceptible strains they became drug resistant.

A third mechanism of azole resistance includes the alteration of the drug target itself, \textit{ERG11}, and has been observed in all three major human fungal pathogens, \textit{C.albicans}, \textit{A. fumigatus} and \textit{C. neoformans}. Drug target alteration can occur in at least two different ways; either by overexpressing \textit{ERG11} or by mutating \textit{ERG11} so that drugs are less actively binding to it. Once a cell has acquired an \textit{ERG11} allele with such a resistance mutation, it is possible that the other allele also acquires this mutation through the process of gene conversion (Franz et al., 1998). Fortunately, some of the second-generation triazoles, like posaconazole, have been shown to be more insensitive to mutations within \textit{ERG11}, as was reported in a recent
publication where up to 5 of these mutations were required to decrease sensitivity to posaconazole in *C. albicans* isolates.

Finally, a fourth mechanism leading to azole resistance is alteration of the ergosterol biosynthetic pathway. There are two ways this can happen. The first is by deletion of *ERG3*, which leads to high levels of azole resistance in laboratory strains of *C. albicans* when tested *in vitro*. *ERG3* encodes for the 5,6-desaturase and, in the absence of azole drugs, converts episterol into non-toxic ergosta-5,7,24(28) trienol (Akins and Sobel, 2017). However, when *ERG11* is inhibited by azoles, 14-methyl intermediates accumulate and are converted by *ERG3* into toxic sterols like 14-methylergosta-8,24(28)-dien-3,6-diol (Kelly et al., 1995). Consequently, when *ERG3* is deleted there is less accumulation of such toxic sterols. The second mechanism of altered ergosterol biosynthesis is the upregulation of ergosterol genes, which leads to increased ergosterol content in the plasma membrane. This mechanism has been linked to the transcription factor *UPC2*. Two recent studies have demonstrated that hyperactivation of *UPC2* resulted in upregulation of not only ergosterol genes, including *ERG11*, but also other targets such as *CDR1* and *MDR1* in drug resistant clinical isolates in *C. albicans*. Introducing this *UPC2* mutation (G648D) into susceptible strains conferred drug resistance (Dunkel et al., 2008 and Znaidi et al., 2008).

### 1.10.4 Echinocandins

Resistance to the newest class of antifungals, the echinocandins, has also been described, despite the fact that all echinocandins have been in the clinic for less than a decade. However, the frequency of echinocandin resistance remains low. Clinical echinocandin resistance has been described for *C. albicans* and *C. parapsilosis*, while in *A. fumigatus* resistance could be engineered in a laboratory strain (Perlin, 2007). All mutations associated
with echinocandin resistance have so far been shown to lie in the gene encoding the enzyme 
"-glucan synthetase, *FKS1*, the target of the echinocandins.

### 1.11 Alternatives of antibiotics: medicinal plants

Antibiotics are the most important classes in therapeutic agents and have given an enormous impact on both of life expectancy and improve quality of life (Clark, 1996). After discovery of penicillin, other antibiotics isolated from microorganisms are introduced. However, the emergence of antibiotic resistant microorganism towards synthetic antimicrobial agents, the research in order to find an alternative antimicrobial agent became more extensive.

Plant products play an important role in the health care systems of the remaining 20% of the population mainly residing in developed countries. Currently, at least 119 chemical substances which are derived from 90 plant species can be considered as important drugs in many countries. Of these 119 drugs, 74% were discovered as a result of chemical studies directed at the isolation of the active substances from plants used in traditional medicine (Newman et al., 2000). A multitude of plant compounds is readily available over-the-counter from herbal suppliers and natural-food stores. There are about 350,000 species of plants growing on the earth and it is estimated that at least 5000 different of chemical compounds are present in a single species of plant. It is apparent that the secondary metabolites of plant origin constitute a tremendous resource for exploring useful drugs (Kuo and King, 2001).

Secondary metabolites, which plants employ to defend themselves against bacteria, fungi or viruses, can be used in almost the same way in medicine to treat microbial or viral infections. Secondary metabolites of plants are not compounds with random structures but active metabolites, which have been selected during evolution we can use them in medicine (Wink, 2008a).
Plants usually produce complex mixtures of secondary metabolites, whose compositions show substantial differences between developmental stages and organs. These mixtures are regularly composed of secondary metabolites from different classes. Most plants accumulate phenolic compounds (flavonoids and tannins), that are regularly accompanied by terpenoids (monoterpenes, sesquiterpenes, triterpenes or saponins. Much research has been done in crude extracts, fractions, essential oils and also isolated compounds to search antimicrobial compounds (Rios and Recio, 2005). The modern microbiological techniques demonstrate that higher plants frequently exhibit significant potency against human bacterial and fungal pathogens (Mitscher et al., 1987). The chemical structures of the antimicrobial agents found in higher plants belong to most commonly encountered classes of higher plant secondary metabolites (terpenoids, polyphenols, iridoids, saponins, polyenes, anthraquinones and some alkaloids) (Wink and Schimmer, 1999).

1.12 Medicinal plants and their oils as antimicrobials

1.12.1 *Allium sativum*

Kingdom Plantae

Division Magnoliophyta

Class Liliopsida

Order Liliales

Family Liliaceae

Genus *Allium*

Species *sativum*

*Allium sativum*, ordinarily known as garlic is a type of the onion family Alliaceae (Saravanan et al., 2010). *Allium sativum* is a natural plant being used as a food as well as folk medicine
for centuries in all over the world, in 1996, Reuter et al., described garlic a plant with various biological properties like antimicrobial, anti-cancer, antioxidant. As well as different properties such as antiviral, antifungal, expectorant, antiseptic and antihistamine (Hannan et al., 2011). And has a long folklore history as a treatment for cold, cough and asthma and is reported to strengthen the immune system. It has numerous therapeutic impacts, for example, bringing down of blood cholesterol level, antiplatelet accumulation, anti-inflammatory effect and restraint of cholesterol union (Shobana, 2009). Different garlic extracts demonstrated activity against Gram negative and Gram-positive bacteria including species of Escherichia, Salmonella, Staphylococcus, Streptococcus, Klebsiella, Proteus, Bacillus, clostridium, Helicobacter pylori and even acid-fast bacilli such as Mycobacterium tuberculosis. Allicin is thiosulfinate compound of garlic reported for its antibacterial activity. Allicin is proved to be anti-bacterial as it inhibits RNA synthesis (Hannan et al., 2011).

1.12.2 Azadirachta indica

Kingdom       Plantae
Order          Rutales
Suborder       Rutinae
Family         Meliaceae
Genus          Azadirachta
Species        indica

Azadirachta indica (A. indica) belonging to the Meliaceae family commonly usually as neem is the most versatile remedial plants having a wide range of biological activity (Adyanthaya et al., 2014). A. indica has been widely used in Ayurveda, Unani and Homoeopathic medicine and has turn into a part of modern medication. More than 140 compounds have
been isolated from different parts of it (Subapriya and Nagini 2005). Several biological active compounds are present in neem, like alkaloids, flavonoids, triterpenoids, phenolic mixes, carotenoids, steroids and ketones (Verkerk and Wright, 1993). Almost every part of the tree including *A. indica* tree, leaves, flowers, seeds, fruits, roots and bark have been used traditionally to treat various diseases and known to have several medicinal properties such as antimicrobial, antimalarial, antiulcer, antiparasitic and antiprotozoal. It is also been studied for antidiabetic, (Shukla et al., 1973) antifertility (Sinha et al., 1984) and antioxidant activity. The concentrated extract of different plant parts including bark, leaves, fruits and root have been utilized to control sickness, intestinal helminthiasis and respiratory disorders in youngster (Adyanthaya et al., 2014).

1.12.3 *Cordia dichotoma*

Kingdom Plantae
Division Magnoliophyta
Class Magnoliopsida
Order Lamiales
Family Boraginaceae
Genus *Cordia*
Species *dichotoma*

*Cordia dichotoma* Forst belonging to family Boraginaceae is medium sized tree with a short, usually crooked trunk (90-100 cm in girth) and bearing globose, grows in India, Srilanka and other warmer countries (Maisale et al.,2010). The fruits have been used in astringent, emollient, expectorant, anthelmintic, purgative and diuretic (Rastogi, 1993; Kuppast and Vasudev, 2006). A various medicinal properties like pain relieving, anti-inflammatory and
hepatoprotective have been reported. *C. dichotoma* is used as immunomodulator, anthelminitic, diuretic, antidiabetic, and hepatoprotective in folklore medicine. Seeds have disclosed the presence of α–Amyrin, betulin, octacosanol, lupeol–3–rhamnoside, β–sitosterol, β–sitosterol–3–glucoside, hentricontanol, hentricontanol, taxifolin–3,5–dirhamnoside, and hesperitin–7–rhamnoside (Srivastava and Srivastava, 1979; Mishra and Garg, 2011). The phytochemical examination of *C. dichotoma* bark and leaves demonstrated the presence of comparatively higher amounts of alkaloids, flavonoids, steroids, and terpenoids.

1.12.4 *Ocimum sanctum*

Kingdom Plantae

Order Lamiales

Family Lamiaceae

Genus *Ocimum*

Species *sanctum*

*Ocimum* is a genus of about 35 species of aromatic annual and perennial herbs and shrubs. *O. sanctum* grows up to 60 cm high with purple sub quadrangular branches and simple, serrate and hairy leaves. Flowers are purple in color. Fruits are smooth and not mucilaginous when wetted. It is propagated by means of seeds. Seeds are planted directly in the ground. Young plants are transplanted when they attain 8-10 cm height. Krishna Tulsi has purple leaves while Shri Tulsi has green leaves. Tulsi is used to reduce skin disorders, pain, swelling, headache and disease of the head and neck. Tulsi leaves are very useful for lung, intestinal and cardiovascular diseases. They are also effective in reducing stress, blood sugar and blood cholesterol. Prasad et al., (2012) have explained antibacterial, phytochemical and antioxidant potential of some *Ocimum* species. The phytoconstituents of *Ocimum sanctum* leaves extracts
by GC-MS analysis was reported by Devendran and Balasubramanian (2011). A comparative study of antimicrobial activity and phytochemical screening of aqueous and alcoholic leaf extract of Tulsi on *E. coli* is presented by Sadul Rama et al., (2012).

### 1.12.5 Syzygium cumini

**Kingdom** Plantae  
**Order** Myrtales  
**Family** Myrtaceae  
**Genus** Syzygium  
**Species** *cumini*

*Syzygium cumini* Linn (family Myrtaceae), commonly known as “Jamun” have various phytoconstituents such as tannins, alkaloids, steroids, flavonoids, terpenoids, fatty acids, and vitamins due to promising a therapeutic value. The seeds of *S. cumini* have been reported to be rich in flavonoids, which account for the scavenging of free radicals and a protective effect on antioxidant enzymes (Ravi et al., 2004), and they have also been found to have high total phenolic content with significant antioxidant activity (Bajpai at al., 2005). Its leaves contain essential oils with a pleasant odour. The oil contains terpenes, dipentenes, sesquiterpenes, ellagic acid, isoquercitin, quercetin, kaempferol and myricetin in different concentrations (Rastogi and Mehrotra, 1990; Mohamed et al., 2013). The barks, leaves and seeds extracts of *S. cumini* have been reported to possess anti-inflammatory (Chandhuri et al., 1990), and antidiarrhoeal effects (Indira and Mohan, 1992). *Syzygium cumini* leaves extract has antimicrobial to some microorganisms and these extracts may be used in treatment of skin wounds (Valencia, 2004). Methanol extracts from *Syzygium cumini* leaves
were tested for antimicrobial activity and toxicity. *S. cumini* leaf extract inhibited the growth of bacteria.

### 1.12.6 *Trigonella foenum-graecum*

**Kingdom** Plante  
**Order** Fabales  
**Family** Fabaceae  
**Genus** *Trigonella*  
**Species** foenum-graecum

Fenugreek (*Trigonella foenum-graecum* L.) is one of the world’s oldest medicinal herbs belongs to the family Fabaceae. The seeds are rich in dietary fiber that it can bring down glucose levels in diabetes. Fenugreek seed is widely used as a galactagogue that is often used to increase milk supply in lactating women Sand cure breast cancer (Amin et al., 2005). Seed have been used for curing in tuberculosis, diabetes, atherosclerosis, constipation, high cholesterol, hypertriglyceridemia and externally it is used as a poultice for abscesses, boils, carbuncles, etc. Fenugreek seed is helpful for tuberculosis, diabetes, atherosclerosis, stoppage, high cholesterol, hypertriglyceridemia and remotely it is utilized as a poultice for abscesses, bubbles, carbuncles, and so forth.

Insulin is used to replace fat and reduce the calories of food. It is suitable for consumption by diabetes. The seeds of the fenugreek herb possess toxic oils, and other bioactive constituents of the fenugreek seed include volatile oils and alkaloids have been shown to be toxic to bacteria, parasites and fungi. Recent pharmocological investigation of the seed extract of this plant revealed anticancer and antifungal properties (Nandagopal et al.,2012; Palombo and Semple, 2001).
1.12.7 *Cymbopogon citratus*

Kingdom   Plantae  
Order      Poales  
Family     Poaceae  
Genus      *Cymbopogon*  
Species    *citratus*

*Cymbopogon citratus* commonly known as lemongrass are indigenous to India. It is a producer of essential oil with commercial value and also used as an important ingredient in Ayurveda an oldest system of medicine practiced in India for centuries to treat diseases and ailments (Singh et al., 2011). It has been used to cure various ailments such as cough, cold, spitting of blood, rheumatism, lumbago, digestive problems, bladder problems, leprosy, and as mouth wash for the toothache and swollen gums. It have antifungal activities (Khan and Ahmad, 2012), antibacterial activities (Cimanga et al., 2002), diuretic action (Gálvéz et al., 1998), gastroprotection mechanisms (Fernandez et al., 2012) and protective effect against different parasites (Santoro et al., 2007). Citral is the major constituent of lemongrass oil and widely used in flavors and fragrance, cosmetics, food and pharmaceuticals.

1.12.8 *Eucalyptus globulus*

Kingdom   Plantae  
Order      Myrtales  
Family     Myrtaceae  
Genus      *Eucalyptus*  
Species    *globulus*
*Eucalyptus* is an important ethnomedicinal plant belonging to the family Myrtaceae. There are more than 700 species that comprise this genus, most are native of Australia, though they are also widely cultivated throughout the tropics, especially in Asia and Central America as well as Africa (Akin et al., 2010) are used in China folk medicine for a variety of medical conditions. For examples, hot water extracts of dried leaves used as analgesic, anti-inflammatory and antipyretic remedies for the symptoms of respiratory infections, such as cold, flu, and sinus congestion and also known to contain bioactive products that display antibacterial, antifungal, analgesic and anti-inflammatory effects and antioxidative activities (Cheng et al., 2009).

It is used as a remedy for sore throat and other bacterial infection of the respiratory and urinary tracts. Essential oils of the leaves are used in the treatment of lung diseases while the volatile oils are used as expectorant. Eucalyptus oil obtained by steam distillation, leaves have Eucalyptol (1, 8-cineole) as its major constituent which is responsible for its various pharmacological activities (Ayepola and Adeniyi, 2008). Some studies have demonstrated that the oil and leaf extracts of Eucalyptus spp. have antifungal and repellent activity. Crude methanolic extract of *E. Camaldulensis* has been reported to inhibit the growth of *Candida albicans*. Also, it has been shown that ethanolic leaf extract of *Eucalyptus camaldulensis* had marked fungicidal effect against clinical dermatophytic fungal isolates; Microsporum gypseum and Trichophyton men-tagrophytes (Falahati et al., 2005).
1.12.9 *Melaleuca alternifolia*

Kingdom      Plantae  
Order       Myrtales  
Family  Myrtaceae  
Genus   Melaleuca  
Species  alternifolia

*Melaleuca alternifolia* oil (Tea tree) is obtained by steamdistillation from the Australian local plant *Melaleuca alternifolia* and commonly used as a topical antiseptic. It has a broad spectrum of antimicrobial activity against a wide range of pathogenic microorganisms like bacteria, viruses, and fungi, including yeasts and dermatophytes. It has more than 100 different phytocompounds, primarily terpenes mainly monoterpenes and sesquiterpenes (Turek and Stintzing, 2013).

1.12.10 *Rosmarinus officinalis*

Kingdom    Plantae  
Order      Lamiales  
Family  Lamiaceae  
Genus  Rosmarinus  
Species  officinalis

*Rosmarinus officinalis* is an aromatic plant and thus a flavouring agent, widely used in many foods to enhance the flavor. Its extracts have been introduced as preservatives in the food industry. Extract formulations are the only ones commercially available for use as antioxidants in the European Union and the United States, and they are available in market in different forms like oil-soluble form, as a dry powder, and in water-dispersible or water-
miscible formulations (Khansole, 2016). Rosemary separate details are the main ones financially accessible for use as cancer prevention agents in the European Union and the United States, and they are showcased in an oil-solvent frame, as a dry powder, and in water-dispersible or water-miscible plans. The secondary metabolites have been present in R. officinalis like phenolic diterpenes, carnosol, carnosic acid, methyl carnosate, rosmanol, and epirosmanol, and phenolic such as ferulic, rosmarinic, and chlorogenic and caffeic acids that have different biological activities, including antioxidant and antimicrobial activity (Klancnik et al., 2009).

1.12.11 Citrus reticulata

Kingdom Plantae
Order Sapindales
Family Rutaceae
Genus Citrus
Species reticulata

Citrus reticulata (Mandarin) is a small citrus tree with fruits resembling other oranges grown in China, India, and many other south Asian and Southeast Asian countries. Genus Citrus are belonging to Rutaceae family, known for their flavor, nutritional value and many medicinal properties. They are a diverse group of thin-skinned, easy-peeling fruit, consuming, largely due to the ease with which they can be eaten as compared to other types of citrus that are more difficult to peel. Oil is extracted by cold expression of the outer peel of the fruit; it has a light, fruity-citrus aroma, similar to orange essential oil. Mandarin oil is balancing, uplifting and calming; its properties include being anti-septic, digestive and anti-depressant. Mandarin is also used in soaps, cosmetics, perfumes and as a flavoring agent in liqueurs and
confectionery. The main compounds are limonene, gamma-terpinene and eucalyptol. Other compounds are geranial, neral, geranyl acetate, geraniol, beta-caryophyllene, nerol, alpha-terpinene, and neryl acetate have also been reported. Antibiotic, antifungal, antioxidant activity also reported against many microorganism (Boughendjioua and Boughendjioua, 2017).

1.12.12 Syzygium aromaticum

Kingdom Plantae

Order Myrtales

Family Myrtaceae

Genus Syzygium

Species aromaticum

Cloves (Syzygium aromaticum) are the aromatic flower buds of a tree belonging to family Myrtaceae. The tree is an evergreen, grows to a height from 10-20m, having oval leaves and flowers in numerous groups of terminal cluster. Cloves are used in medicine as a carminative, to increase hydrochloric acid in the stomach and to improve peristalsis. It is also used in dentistry where the essential oil of clove is used as anodyne for dental emergencies. In addition, the cloves are antimutagenic, anti-inflammatory, antioxidant, antiulcerogenic, antithrombotic and antiparasitic, antibacterial and anti-inflammatory (Kumar et al., 2014).
<table>
<thead>
<tr>
<th>Botanical name</th>
<th>Common name</th>
<th>Family</th>
<th>Plant extrac/oils used</th>
<th>Medicinal use</th>
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<td>Garlic</td>
<td>Liliaceae</td>
<td>Bulb extract</td>
<td>expectorant, antibacterial, antifungal</td>
</tr>
<tr>
<td><em>Azadiracta indica</em></td>
<td>Neem</td>
<td>Meliaceae</td>
<td>Leaf extract</td>
<td>Antiviral activity, antifungal activity</td>
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<td>Tulsi</td>
<td>Lamiaceae</td>
<td>Leaf extract</td>
<td>Antibacterial, antifungal</td>
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<td><em>Cordia dichotoma</em></td>
<td>Lasoda</td>
<td>Boraginaceae</td>
<td>Leaf extract</td>
<td>Wound healing Anti-diabetic, Analgesic, antibacterial, antifungal</td>
</tr>
<tr>
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<td>Jamun</td>
<td>Myrtaceae</td>
<td>Leaf extract</td>
<td>Diabetes and renal problems, anti-allergic, anti-inflammatory, antimicrobial</td>
</tr>
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<td><em>Trigonella foenum graecum</em></td>
<td>Methi</td>
<td>Fabaceae</td>
<td>Seed extract</td>
<td>Digestion, reduce blood sugar levels in diabetics, antimicrobial activity</td>
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<tr>
<td><em>Cymbopogon citratus</em></td>
<td>Lemon grass</td>
<td>Poaceae</td>
<td>Leaf oil</td>
<td>Relieving cough and nasal congestion and antifungal activity</td>
</tr>
<tr>
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<td>Neelgiri</td>
<td>Myrtaceae</td>
<td>Leaf oil</td>
<td>Sinus and allergies, antibiotic activity against pathogenic microbes</td>
</tr>
<tr>
<td><em>Rosmarinus officinalis</em></td>
<td>Rosemary</td>
<td>Lamiaceae</td>
<td>Leaf oil</td>
<td>Analgesic, antibacterial, anticancer, antifungal, anti-infection, anti-inflammatory, antioxidant</td>
</tr>
<tr>
<td><em>Syzygium aromaticum</em></td>
<td>Clove</td>
<td>Myrtaceae</td>
<td>Bud oil</td>
<td>Dental care, antiviral properties, antiseptic properties</td>
</tr>
<tr>
<td><em>Citrus reticulata</em></td>
<td>Medarian</td>
<td>Rutaceae</td>
<td>Peel oil</td>
<td>Antiseptic, antispasmodic, and diuretic properties, and is a laxative and digestive.</td>
</tr>
</tbody>
</table>
Akintobi et al., (2013) reported antimicrobial activity of garlic bulb (*Allium sativum*) extract on six pathogenic microorganisms tested. The water and ethanol extracts of the *Allium sativum* showed effective antimicrobial activity against bacteria namely *Proteus mirabilis*, *Salmonella typhi* and *Staphylococcus aureus*. The extracts were found to be ineffective against *Escherichia coli*, *Bacillus subtilis* and *Pseudomonas aeruginosa* with inhibition zone ranging from 7-19mm.

The antimicrobial activity of crude extract and methanolic extract of garlic were tested against *Escherichia coli*, *Staphylococcus aureus* and *Salmonella* ser. Typhi. The crude and methanolic extract both gave the same inhibition zone towards *E. coli*, the crude extract gave the maximum inhibition zone towards *S. aureus* and the methanolic extract gave the maximum inhibition zone towards *S. ser. Typhi* (Gaherwal et al., 2014).

Gull et al., (2012) observed the antibacterial effect of garlic (aqueous extract, methanol extract and ethanol extract) was evaluated by disc diffusion method against drug resistant *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Shigella sonnei*, *Staphylococcus epidermidis* and *Salmonella typhii*. The tested bacterial strains were most susceptible to the garlic aqueous extract followed by the methanolic extract and ethanolic extract.

Susmitha et al., (2013) reported antimicrobial assay of *Azadirachta indica* leaves extract (ethanolic, aqueous and acetone extract) against pathogenic bacteria. *Escherichia coli* and *Salmonella* spp. were observed sensitive against ethanolic extract of *A. indica* with inhibition zone 12mm and 8mm respectively but resistant against its aqueous extract.

Adyanthaya et al., (2014) showed the antimicrobial and antifungal activity of extract of *Azadirachta indica* (twigs) in different solvents namely petroleum ether, dichloromethane,
ethyl acetate, methanol and aqueous solvent against *S. mutans, S. mitis*, Lactobacilli and *P. intermedia* and fungus *Candida albicans*. They found that methanol extract of neem exhibited the highest antimicrobial activity with inhibition zone 10 – 20 mm against all microorganism tested at 500 mg/ml concentration, whereas the aqueous extract showed no activity at concentrations tested. The petroleum ether and methanol extracts of *A. indica* showed significant antimicrobial activity against all isolates tested. But the isolates were found sensitive with 8-14mm zone of inhibition to the ethyl acetate extract of *A. indica*.

Chauhan et al., (2015) compared antimicrobial effect of the aqueous, methanolic and ethanolic extracts of *Azadirachta indica* (Neem) leaves against Gram negative and Gram positive pathogenic bacteria causing urinary tract infections. Ethanolic extracts exhibited maximum antimicrobial activity ranged from 12 – 23 mm zone of inhibition against all tested Gram negative bacteria.

Lall et al., (2013) reported antibacterial and antifungal potential of methanolic and acetonic extracts of *Azadirachta indica, Saraca asoca* and *Curcuma longa* against *E.coli, B. subtilis, A. niger, A. fumigates*. The methanolic extracts of *Azadirachta indica* exhibited maximum antifungal activity with 20mm and 15mm zone of inhibition against *A niger* and *A. fumigates* respectively. Antibacterial activity of *A. indica* (methanolic extract) was observed maximum against *E. coli* and *B. subtilis* with inhibition zone 16 and 14mm respectively. Acetone extract of all plants showed lesser activity as compared to methanolic extracts against microorganisms tested.

Shenoy et al., (2014) reported antimicrobial activity of commercially available coconut oil, clove oil, castor oil, eucalyptus oil, neem oil and extracted oreganum oil against multidrug resistant nosocomial pathogens (*E. coli, Klebsiella pneumonia, Pseudomonas aeruginosa,*
Acinetobacter baumanii and Staphylococcus aureus) isolated from clinical samples. The oregano oil exhibited the maximum antibacterial activity with range 17.9±0.55mm zone of inhibition against the tested bacteria as compared to the control antibiotics. Eucalyptus oil (16.50±0.83mm – 18.17±0.76mm) and clove oil (12.08± 0.78 – 18.16± 0.85mm) also exhibited better antibacterial activity as compared to the neem oil, coconut oil and castor oil. The oils exhibited the highest antibacterial activity against E. coli and S. aureus as compared to other tested organisms.

Tarranum et al., (2014) studied antimicrobial activity of essential oils of (Cinnamomum zeylanicum, Cedrus deodara, Eucalyptus globulus, and Rosmarinus officinalis) against different pathogenic bacterial and fungal strains. Cinnamomum zeylanicum was found most effective (19-26mm) against all the bacterial and fungal strains. Cedrus deodara showed moderate activity against only three bacteria ranged from 10-17mm zone of inhibition but fungal strains showed resistance. Staphylococcus spp. showed sensitivity with 10mm and 9mm zone against Eucalyptus globulus and L. monocytogenes respectively. Rosmarinus officinalis showed activity against some of the bacterial strains with inhibition zone ranged from 10-17mm.

Mekonnen et al., (2016) screened in vitro antimicrobial activities of four plant essential oils (T. schimperi, E. globulus, R. officinalis, and M. Chamomilla) against bacteria and fungi. They reported that essential oils of T. schimperi, E. globulus, and R. officinalis were active against bacteria and fungi with inhibition zone ranging from 12 - 40mm diameter. The antimicrobial effect of M. chamomilla was found to be weaker and did not show any antimicrobial activity. The minimum inhibitory concentration values of T. schimperi were <15.75mg/ml for most of the bacteria and fungi studied. The minimum inhibitory
concentration values of the other essential oils were in the range of 15.75–36.33mg/ml against tested bacteria.

Gupta et al., (2016) reported antimicrobial activity essential oils Lemongrass (Cymbopogon flexuosus) against Pseudomonas aeruginosa, Staphylococcus aureus (MTCC96) and Bacillus subtilis (1429) and two fungal pathogens Aspergillus niger (MTCC2723) and Candida albicans except E coli. Mean inhibition zone diameter (mm) against bacteria was ranged 27-38 mm. B. Subtilis was the most sensitive bacterium to all essential oils. Essential oils also showed strong antifungal effects against both A. niger and C. albicans with mean inhibition zone diameter (mm) values 20-26 and 27-29 mm, respectively.

Deore and Namdeo (2013) reported the antibacterial activity of petroleum ether, chloroform, methanol and aqueous extracts of Cordia dichotoma against selected urinary tract pathogens such as Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae, Proteus mirabilis, Staphylococcus aureus and Proteus vulgaris. They found that the methanolic extract was more effective against Escherichia coli, Klebsiella pneumonia, Proteus mirabilis and Proteus vulgaris with a zone of inhibition of 22 mm, 21 mm, 22 mm and 21 mm diameter (at concentration 1000 μg) respectively and was least effective against Pseudomonas aeruginosa and Staphylococcus aureus with zone of inhibition of 12 mm and 11 mm (at 1000 μg) respectively.

Nariya et al., (2011) reported antimicrobial activity of bark of Cordia dichotoma extract and found that E. coli and P. aeruginosa were more sensitive to this extract as compared to S. aureus and S. pyogenes. The growth inhibition zone measured ranged from 10–20 mm for all the sensitive bacteria, and 12–21 mm for fungal strains. A. niger and A. clavatus found more
resistant than \textit{C. albicans}. The antibacterial and antifungal activity of the extract increased linearly with increase in concentration of extract (mg/ml) as compared with standard drugs.

The extracts and essential oils of \textit{S. cumini} leaves possessed antibacterial activity against Gram positive and Gram negative bacteria by Mohamed et al., (2013). The methanol extract exhibited the maximum activity with inhibition zones (18–24 mm) against \textit{Escherichia coli} ATCC35218, \textit{Staphylococcus aureus} ATCC 25923, \textit{Pseudomonas aeruginosa} ATCC 29212, \textit{Neisseria gonorrhoeae} ATCC 11778, \textit{Bacillus subtilis} ATCC 12228, \textit{Staphylococcus aureus} ATCC 9341, and \textit{Enterococcus faecalis} ATCC 9763 respectively. Both the methylene chloride extract and the essential oils showed moderate inhibition zones ranged from 13–16 and 12–14 mm.

Mulani et al., (2013) reported antimicrobial activity of \textit{Cordia dichotoma} (methanolic and acetone extract) against pathogenic microorganism such as \textit{Escherichia coli}, \textit{Staphylococcus aureus} and \textit{Candida albicans}. The methanolic leaf extract showed higher antimicrobial activity with inhibition zone 10.2mm, 8mm, 7.2mm against \textit{Staphylococcus aureus}, \textit{Candida albicans} and \textit{Escherichia coli} respectively as compared to acetone leaf extract.

\textit{Trigonella foenum-graecum} was recorded antimicrobial against human pathogen \textit{Staphylococcus aureus} (Mishra et al., 2016). The methanol and acetone extract of \textit{Trigonella} were effective against \textit{Staphylococcus aureus} at 100 mg/ml and 200mg/ml. activity of methanolic extract was recorded with inhibition zone 13mm at 200mg/ml but acetonic extract showed 10mm growth of inhibition. Phytochemical analysis also showed the presence of flavonoids, alkaloids, phenolic compound and carbohydrate.

highest antibacterial activity compared to other extracts. Petroleum ether extract of seeds showed antifungal activity against *Aspergillus niger* and *Candida albicans* with maximum zone of inhibition (20 mm) and (17mm) at the concentration 250 mg/ml.

Subramanian et al., (2014) reported antibacterial and antifungal activity of different leaf extracts of *O. tensanctum* in various solvents (Ethanol, Methanol, Ethyl acetate and chloroform) against *Escherichia coli*, and *Candida albicans*. Among four, methanol extracts showed highest activity with inhibition zone 22mm and 21mm against *Escherichia coli* and *C. albicans* respectively.

Antibacterial activity of leaf extracts of *Azadiracta indica* (Neem), *Cymbopogon citratus* (Lemon grass), *Mentha arvenis* (Pudina) and *Ocimum sanctum* (Tulsi) was evaluated against four human pathogens *Escherichia coli*, *Salmonella typhi*, *Shigella* sp. and *Staphylococcus aureus* by Shinde and Mulay (2015). Growth of all test pathogens was inhibited by methanol extracts of the plants ranged from 12-17mm, while chloroform extracts of the plants were found to be ineffective against all test pathogens. Phytochemical analysis of the alcoholic extracts showed the presence of alkaloids.

Naik et al., (2015) studied antimicrobial activity of *Ocimum sanctum* in Hexane, Acetone and methanol solvents against Gram negative and Gram positive bacteria. They found that acetone extracts had wide range of antibacterial activity (8mm inhibition zone) against bacteria, where as methanol extract showed slightly lower antimicrobial activity (7mm inhibition zone). Phytochemical screening of the plant leaves revealed the presence of saponins, alkaloids, flavonoids, cardiac glycosides, steroids, phenols and tannins.
1.13 Synergistic effect of antibiotics and plant extracts on microbes

Many plant extracts or products have been evaluated not only for direct antimicrobial activity, but also as resistance-modifying agents (Saiful et al., 2006). The enhancement of antibiotic activity or the reversal of antibiotic resistance by natural or synthetic nonconventional antibiotics affords the classification of these compounds as modifiers of antibiotic activity (Matias et al., 2011). Plant extracts and essential oils have been searched for their antibacterial, antifungal, antiviral, insecticidal, anticancer and antioxidant properties. There has been an increased concern in studying antimicrobial properties of plants essential oils after emergence of antibiotic resistance occurred. Although the active constituents may occur in lower concentrations, plant extracts may be a better source of antimicrobial compounds than synthetic drugs. The phenomenon of additive or synergistic effects is often crucial to bioactivity (Aqil et al., 2006) in plant extracts and in some cases; the activity is lost in purified fractions. Development of bacterial resistance to synergistic drug combinations, such as those found in plants, may be slower than for single drug therapies.

Chusri et al., (2014) studied synergistic effects of ethnomedicinal plants of Apocynaceae family and antibiotics against clinical isolates of *Acinetobacter baumannii*. They reported that 15 (5%) had synergistic effects, 23 (8%) had partial synergistic effects and 234 (86%) had no effects. Synergistic activity was observed mostly when the Apocynaceae extracts were combined with rifampicin or cefazolin. Interestingly, 10 out of 17 combinations between the extracts and rifampicin displayed synergistic or partial synergistic behaviors.

Ahmed et al., (2010) reported the susceptibility of Penicillin and Tetracycline separately and in combination (synergistic) against *Staphylococcus aureus*. It was found that the zone of inhibition was 23mm for tetracycline and 18 mm for penicillin. However, their synergistic
effect was much more effective and caused an inhibition that measured 27 mm in diameter. Apart from this these antibiotics were also applied in combination with various extracts (ethanolic) of *Salvadora persica*, a medicinal plant of repute. The highest inhibition was noticed (31.5 mm) when *S. aureus* was exposed to tetracycline + *Salvadora* stem extract. It was followed by tetracycline + leaf extract combination of *Salvadora persica* with a zone of inhibition of 30.0 mm.

The interactions between methanolic extract of *Acacia mearnsii* and eight antibiotics were investigated by Olajuyigbe and Afolayan (2012). Result showed that extract-kanamycin combination had zones of inhibition $\geq 20 \pm 1.0$ mm in all the bacteria tested (100%), followed by extract-chloramphenicol (90%) $>$ extract-ciprofloxacin= extract-tetracycline (70%) $>$ extract-amoxicillin (60%) $>$ extract-nalidixic acid (50%) $>$ extract-erythromycin (40%) $>$ extract-metronidazole (20%). The checkerboard showed synergistic interaction (61.25%), additivity/indifference (23.75%) and antagonistic (15%) effects. The synergistic interaction was most expressed by combining the extract with tetracycline, metronidazole, amoxicillin, ciprofloxacin, chloramphenicol and nalidixic acid against *E. coli* (ATCC 25922).

Atteia and Hussein (2014) revealed that the water extracts of *Syzygium aromaticum* and *Allium sativum* had a synergistic effect with different antibiotics (imipenem, levofloxacin, amikacin and erythromycin etc) and were able to suppress the *S. aureus* growth.

Lata and Gupta (2015) reported synergistic effect of antibiotics ampicillin and tetracycl in with methanolic leaf and stem extracts of *Azadirachita indica* and leaf extract of *Aloe vera* in combination against bacteria *E. coli*. 


1.14 Plasmids in bacteria

A plasmid is a circular, autonomously replicating DNA molecule. It may carry genes that can be beneficial to their host (e.g., genes which code for drug resistance, virulence factors etc.) (Carattoli, 2009). The resistance genes within the plasmids may be responsible for inactivation of antibiotics and the mechanisms involved are: 1. Drug modification, 2. drug degradation, 3. drug efflux and 4. drug target alteration. All these mechanisms can be regulated by the antibiotic resistance genes present on plasmids (Walsh, 2003). Based on their ability to move between different bacteria, plasmids are categorized into: 1. self-transmissible or conjugative, and 2. nonconjugative or mobilizable plasmids (Salyers and Amabile-Cuevas, 1997). Conjugative plasmids carry tra genes needed for conjugation and they do not need any external support for their gene transfer. By contrast, nonconjugative plasmids lack tra gene functions, so they depend on the tra genes of other conjugative plasmids for their gene transfer (Freifelder, 1987). For instance, when planktonic bacteria carrying plasmids come into contact with a biofilm population composed of recipient cells with no plasmids, planktonic bacteria will form biofilms and transfer infectious plasmids to the recipient. Plasmids can transfer antibiotic resistance and virulence factors, threofore plasmids involved in biofilm formation can produce infechions that are hard to treat (Ghigo, 2001). Since, R plasmids play a significant role in gene exchange between organisms, it is important to analyze the presence of R plasmids in environmental samples such as biosolids. *Pseudomonas aeruginosa* is a medically important species that is intrinsically resistant to many antibiotics. Many resistance genes that are carried by R plasmids in *P. aeruginosa* have evolved with the clinical use of different classes of antibiotics. In the late 1980s, before the
widespread use of third-generation cephalosporins, the common - lactamases TEM-1/2 and PSE-2 were found to be encoded by R plasmids (Xiong et al., 2013).

Ranjbar et al., (2011) analysed plasmid profile of 9 MDR *P. aeruginosa* which were isolated from burned patients from Tehran, Iran. All isolates had at least a single plasmid band. Plasmid DNAs showed 1 to 4 DNA bands ranging from 1.3 to larger than of 20 kbp and band with size 1.4 kbp were evident in 57.5% of the strains.

El-Sayed et al., (2015) observed that the resistance to antibiotics for all isolates due to the presence of plasmids. They studied plasmid profiles of multi drug resistant *Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae* and *Pseudomonas aeruginosa*. *S. aureus* showed presence of plasmid with molecular weight (23.130 kbp), *E. coli* showed presence of plasmid with molecular weight between (23.130 kbp and 9.416 kbp), *K. pneumoniae* showed presence of plasmid with molecular weight (23.130 kbp) and for *P. aeruginosa* showed presence of plasmid with molecular weight between (23.130 kbp and 9.416 kbp).

Shahcheraghi et al., (2003) reported the drug resistance in *P. aeruginosa* against tetracyclin, carbenicillin, amikacin, ceftazidime, sulfamethoxazol, ciprofloxacin, tobramycin, kanamycin, cefotaxime and gentamicin. They isolated plasmid of MDR isolates and found 95% harbored two megadalton (MDa) plasmids. The molecular weight of the larger plasmid was about 31.5 MDa and the other was 9.7 MDa.

Rustini et al., (2017) studied antibiotic resistance profile of 34 *P. aeruginosa* isolates. They found 3 isolates resistant to all antibiotics tested. Different multidrug resistant isolates showed plasmid various bands in different combinations. One isolate showed single plasmid band with 300bp, 12 isolates showed single plasmid band >1kbp and two isolates showed two plasmids band with size > 1kbp.
Kawita et al., (2015) studied plasmid profile of three bacteria *Pseudomonas, E. coli* and *Klebsiella*. Plasmids were isolated in 64% percent of isolates. All six *Klebsiella* isolates showed at least one plasmid band and another isolate showed 4 plasmid bands. Plasmids were detected in four *Pseudomonas* isolates and six *E. coli* isolates. The plasmids were obtained with sizes 2.7 kb, 5 kb, 8 kb, 8.7 kb, 9.4 kb and greater than 10 kb among the isolates studied. Two plasmids of size 2.7 kb and 5 kb were seen only in the *Klebsiella* isolates, while plasmids of size 8 kb, 8.7 kb and greater than 10 kb were seen among the *E. coli*, *Klebsiella* and *Pseudomonas* isolates.

Nikbin et al., (2007) reported 29.8% (31 out of 140 isolates) MDR *Pseudomonas aeruginosa* isolates harboring plasmids. 15 different patterns were recorded with 1, 2, 3 or 7 DNA bands and sizes of plasmids varied from 1.7 to 100 kb. The association between presence of certain plasmids and resistance to antibiotics was significant.