Chapter - 2

Review of Literature
Pathogenic bacteria infecting cow and buffalo milk that disease is known as Mastitis. Mastitis is one of the most recurrent and important problem in dairy production. Mastitis is characterized by mammary gland inflammation of cow and buffalo udder and is caused mainly by pathogenic bacteria that attack on the udder, multiply and produce toxins that are harmful to the mammary tissue.

**Pathogenic bacteria**

The most common causative organisms of udder disease of cow and buffalo include *Staphylococci* (*Staphylococcus aureus* and *Straptococcus epidermidis*), *Streptococci* (*Streptococcus agalactiae, Staphylococcus dysgalactiae, Staphylococcus uberis* and *Staphylococcus bovis*) and Coliforms (mainly *Escherichia coli* and *Klebsiella pneumoniae*). Coagulase Negative *Staphylococcus* (CNS) is also prevalent bacterial pathogen in udder infections (Ahmed, 1966; Ghumman, 1967; McDonald et al., 1979; Kapur et al., 1992 and Allore, 1993).

The main etiological agents responsible for mastitis infections can be divided into different groups of organisms depending on the source of the organism involved. These include contagious pathogens, environmental bacteria, opportunistic bacteria and other organisms that cause mastitis less frequently. Contagious mastitis microorganisms are usually found on the udder or teat surface of infected cows and are the primary source of infection between uninfected and infected udder quarters, usually during milking. *Staphylococcus aureus* is the species most frequently isolated from bovine mastitis, a disease responsible for noteworthy economic losses all over the world (Oliveira et al., 1998 and Philpot, 1999).

The microorganisms caused more infection are *Staphylococcus aureus* (Coagulase positive *Staphylococci*), *Streptococcus agalactiae* and the less common sources of infection caused by *Corynebacterium bovis* and *Mycoplasma bovis*. Opportunistic pathogens result in mild forms of mastitis and include coagulase negative *Staphylococci* (CNS). The coagulase test correlates well with
pathogenicity and strains that are coagulase-negative are generally regarded as non-pathogenic (Quinn et al., 1999; Ahmad, 2001; Akram, 2002; Khan, 2002 and Dego et al., 2002).

*Staphylococci* occur commensally and may be isolated from milk but usually illicit a minor immune response in cattle and infections caused are slight. *Staphylococcus aureus* is major pathogen followed by *Micrococcus* spp., *Bacillus cereus*, *Staphylococcus epidermidis*, *Klebsiella* spp., *Escherichia coli* and *Corynebacterium* spp. Coagulase negative *Staphylococcus* has traditionally been considered to be minor mastitis pathogens, especially in comparison with major pathogens such as *Staphylococcus aureus*, *Streptococci* and Coliforms. The main reason for this is that mastitis caused by coagulase positive *Staphylococcus* is very mild and usually remains subclinical (Contreras et al., 2003, Vliegher et al., 2003; Pitkala et al., 2004; Nascimento et al., 2005; Sudhan et al., 2005; Taponen et al., 2006, Tenhagen et al., 2006; Sharma et al., 2007 and Jemeljanovs et al., 2008).

Many infective agents have been implicated as cause of mastitis in cattle. Contagious mastitis is caused by *Streptococcus agalactiae* and *Staphylococcus aureus* who living on skin of teat and inside the udder, but environmental mastitis caused by environmental pathogens *Escherichia coli*, *Streptococcus uberis*, *Klebsiella* spp., who found in soil and feed. Subclinical mastitis with the load of major mastitis pathogens, result found majority of *S. aureus*, Coagulase negative *Staphylococcus*, *E. coli*, *S. agalactiae* followed by *Streptococcus uberis*, *Streptococcus dysgalactiae*, *Staphylococcus epidermidis*, *Staphylococcus saprophyticus* and *Staphylococcus chromogenes*. Coagulase negative *Staphylococcus*, *Streptococci* and *E. coli* are the major pathogens associated with clinical bovine mastitis (Schroeder, 2009; Schukken et al., 2009; Rahman et al., 2010; Bhatt et al., 2011; Hussein, 2012; Hegde et al., 2013; Najeeb et al., 2013; Kateete et al., 2013; Hawari et al., 2014; Mbindy et al., 2014 and Alekish, 2015).

Among *Staphylococcus* spp. the most predominant bacteria was *Staphylococcus chromogens* in subclinical infection and *Staphylococcus sciuri* was the only one isolated in clinical infection. But among *Streptococcus* spp. in
both clinical and subclinical infection *Streptococcus uberis* represent the most common isolated bacteria followed by *Streptococcus agalactiae*. Gram negative bacteria, *Enterobacteriaceae* spp. was the most predominant in subclinical infection (Zainy and Jeburii, 2015).

The bacterial species isolated recovered from bovine mastitis milk samples around Debrezeit, Ethiopia were coagulase negative *Staphylococci, Micrococcus* species, *Staphylococcus aureus, Staphylococcus epidermis, Mycoplasma* species, *Enterococcus* species, *Streptococcus agalactiae* and *Staphylococcus hycus* (Belachew, 2016).

*Staphylococcus* spp., *Streptococcus agalactiae, Streptococcus dysgalactiae, Archenobacterium pyogenes*, staphylococcal β-lactamase gene, and *Staphylococcus aureus* were the most frequently detected pathogens in Bulk Tank milk samples. In case of bovine clinical mastitis *S. aureus* was the most frequently isolated organism, followed by coagulase negative *Staphylococci, Streptococcus uberis, S. dysgalactiae* and *Escherichia coli*. *Staphylococcus* spp. is more prevalent in mastitis than other pathogens in India (Bi Y et al., 2016; Bhatt et al., 2017 and Krishnamoorthy et al., 2017).

**Disease in cow and buffalo milk produced by pathogenic bacteria**

Pathogenic bacterial disease in cow and buffalo milk is known as Mastitis. Mastitis in cow and buffalo is defined as an inflammation of the mammary gland caused by various pathogenic microorganisms. It is a complex disease involving many factors, which is mainly caused by bacteria and there is no simple model that encompasses all possible facets. Mastitis in cow and buffalo occurs when the udder becomes inflamed and bacteria invade the teat canal and mammary glands. These bacteria multiply and produce toxins that cause injury to the milk secreting tissue, besides, physical trauma and chemical irritants. These cause increase in the number of leukocytes, or somatic cells in the milk, reducing its quantity and adversely affecting the quality of milk and milk byproducts. The fibrous proteins of keratin in the teat canal bind electrostatically to mastitis pathogens, which alter the bacterial cell wall, rendering it more susceptible to osmotic pressure. Inability to maintain osmotic pressure causes lysis and death of invading pathogens. The keratin is a waxy material composed of fatty acids and fibrous proteins in the teat.
The fatty acids are both esterified and non-esterified, representing myristic acid, palmitoleic acid and linolinic acid which are bacteriostatic. During milking, bacteria present near the opening of the teat find opportunity to enter the teat canal, causing trauma and damage to the keratin or mucous membranes lining the teat sinus (Treece et al., 1966; Bramley and Dodd, 1984; Murphy et al., 1988 and Capuco et al., 1992).

According to Keefe (1997) Streptococcus agalactiae continues to be a major reason of subclinical mastitis in dairy cattle and a source of economic loss for the industry. S. agalactiae is an oblique bacterium of the bovine mammary gland and is susceptible to treatment with a variety of antibiotics. Infection with S. agalactiae is associated with elevated somatic cell count and total bacteria count and a decrease in the quantity and quality of milk products produced.

Bovine mastitis, an inflammatory reaction in cow’s udder, is a main contagious disease affecting dairy cattle. It is characterized by physical, chemical and usually bacteriological changes in milk and pathological changes in glandular tissues of the udder and affects the quality and quantity of milk. Incidence of sub clinical mastitis in cows and buffaloes, intensive research and the implementation of various mastitis control strategies over the decades, bovine mastitis has not disappeared (Fetrow et al., 2000; Radostitis et al., 2000; Pyorala, 2002; Maiti et al., 2003; Sharma et al., 2004 and Petrovski et al., 2006).

The canal of a teat may remain partially open for 1-2 hrs after milking and during this period the pathogens may freely enter into the teat canal. Milking may promote the pathogen mastitis elimination and reduce new infections occurrence. The cow susceptibility of contracting infection during the first two weeks of the dry period is 15 to 20 times higher than the rest of the period. The high incidence of bacterial isolates (Escherichia coli, Staphylococcus epidermidis, Corynbacterium bovis, klebsiella spp., Streptococcus uberis, Staphylococcus aureus and Streptococcus agalactiae) were recorded for milk samples collected from sub clinical mastitic group as compared to healthy animals. The diagnosis of clinical mastitis is based on detecting signs of inflammation of the mammary gland and changes and blood present in milk (Jones, 2006, Ahmed et al., 2008; and Ribeiro et al., 2009).
Rahman et al., (2010) checked prevalence of subclinical mastitis (SCM) in the cows of the Sylhet region, Bangladesh and found high prevalence of sub clinical mastitis in cow in the study area. The isolated mastitis causing pathogens were many of which *Staphylococcus*, *Streptococcus*, *Escherichia coli*, and *Bacillus* are the major one.

Cow and buffalo Mastitis presents in two forms clinical and subclinical. The mastitis clinical form shows several signs and symptoms such as mammary gland swelling, pain and stiffening, increased temperature, and inflammatory secretion in the milk. It can be detected by visible changes in milk composition (Clot, wateriness) in the farm environment. Differently, mastitis subclinical form is characterized by not showing visible changes in the milk appearance and is the most prevalent form in dairy herds (Sudhan and Sharma, 2010; Bandoch & Melo, 2011 and Oliveira et al., 2011).

Mastitis is the most common disease in adult dairy cows and buffaloes which mainly caused by pathogenic bacteria, accountable for 38% of all morbidity. Each year, three out of ten dairy cows have clinically apparent inflammation of the mammary gland. In light of findings it can be concluded that the prevalence of mastitis is high and both contagious and environmental pathogens are involved in Fars province (Hashemi et al., 2011 and Peres & Zappa, 2011).

The California Mastitis Test (CMT) is used to determine subclinical mastitis detecting the increase in somatic cell count (SCC) in milk diagnosed. Depending on the diagnosis and the identification of the pathogenic microorganism, a fluid-based therapy may be employed in certain cases (Oliveira et al., 2011; Schvarz & Santos, 2012 and UFLA 2012).

Heifer Mastitis is a disease that potentially threatens production and udder health in the first and subsequent lactations. In general, coagulase negative *Staphylococci (CNS)* are the predominant cause of subclinical mastitis in heifers around parturition, whereas *Staphylococcus aureus* and environmental pathogens cause a minority of the cases. Clinical heifer mastitis is typically caused by the major pathogens. The magnitude of the effect of heifer and herd level mastitis is influenced by the form of mastitis (clinical versus subclinical), the virulence of
the causative pathogen (major versus minor pathogens), the ability of the animals to cope with the disease, and the response of the dairy manager to control the disease through management changes. Sub clinical mastitis is prevalent among lactating cows and this is associated with both animal characteristics (age, breed and individual milk quarters) and milking practices (hand washing). Good knowledge of the environment and careful management of the identified risk factors with improved sanitation assist farm managers and veterinarians in implementing preventative programmes to reduce the incidence of subclinical mastitis. Potential risk factors associated with mastitis prevalence and severity includes cow's itself and their surrounding environment particularly farm and milking hygiene procedure (Chavoshni and Husaini, 2012; Federal et al., 2012; Shittu et al., 2012; Vliegher et al., 2012; Amir, 2013; Akram et al., 2013; Elbably et al., 2013; Hamadani et al., 2013 and Deb et al., 2013).

The higher subclinical mastitis (SCM) prevalence was found advancement of age and parity. The prevalence was also high due to increasing of milk production of cows. Hind quarters were more susceptible to suclinical mastitis than fore quarters in lactating cows. It was higher with increases in, amongst other risk factors, teat-end damage severity, cow dirtiness, and level of pure dairy breed genetics. The efficacy of intramammary infections of subclinical mastitis affected cows was found effective treatment with Neomastipra-JR5® intramammary infusion tube (Khanal and Pandit, 2013; Tripura et al., 2014; Iraguha et al., 2015 and Tasegaya et al., 2015).

The prevalence was higher among cross bred cows in comparison to local cows. Subclinical mastitis accounts 40 times more than the clinical mastitis in dairy herds. Although great technological advances have been made, mastitis continues to be a foremost economic issue for dairy producers, necessitating researchers and dairy advisors continue to refine the recommended mastitis control programme (Shaheen et al., 2016 and Gogoi et al., 2017).

**Economic losses due to Mastitis disease**

Mastitis is a global problem as it adversely affects animal health, quality of milk and economics of milk production and every country including developed ones suffer huge financial losses. Mastitis causes heavy economic losses to the
dairy industry worldwide. The first comprehensive report on mastitis caused losses in India published in 1962 indicated annual losses of Rs. 52.9 crore. Mastitis, the most important deadly disease of dairy animals is responsible for heavy economic losses and importance it also carries public health significance due to reduced milk yield, milk discard after treatment, cost of veterinary services and premature culling. The study found that incidence of subclinical mastitis was also related to managemental practices followed by dairy farmers. The subclinical mastitis drastically reduce the milk production of cows and, which was significantly contribute the economic losses among the farmers (Dandha and Sethi, 1962; Janzen, 1970; Kaneene and Hurd, 1990; Miles et al., 1992; Hillerton et al., 1992; Morin et al., 1993; Reinsch and Dempfle, 1997; Ott, 1999; Yalcin et al., 1999; Yalcin and Stott, 2000; Bhikane and Kawitkar, 2000; Dua, 2001; Sharma et al., 2007; Huijps et al., 2008; Sharma, 2010; PD-Admas, 2011; Awale et al., 2012; Sinha et al., 2014; Singh et al., 2014; Sanotharan et al., 2016 and Rathod et al., 2017).

Pathogenic bacteria in cow and buffalo milk

Epidemiological studies on mastitis revealed that mastitic organisms are widespread on different body sites of the cows, milker’s hands, milking cans and in the milk samples. Teat apices have been reported to be the most common site from where these organisms have been isolated. The mastitis causing organism, Staphylococci is the chief udder pathogen, has been isolated from almost all the body site examined and Streptococci from fewer body sites, whereas the prevalence of Escherichia coli has been reported to be widespread (Malhotra and Kapur, 1982; Prabhakar et al., 1990; Joshi et al., 1998 and Itagaki et al., 1999).

More than 140 different types of pathogenic microorganisms may cause cow and buffalo mastitis, and these etiological agents are classified into contagious pathogens Staphylococcus aureus, environmental pathogens Streptococcus uberis, secondary or minor pathogens coagulase negative Staphylococcus spp. and uncommon pathogens Arcanobacterium pyogenes some species of anaerobic bacteria, fungi and yeast (Costerton et al., 1999; Muller, 2002 and Prakash et al., 2003).
Haltia et al., (2006) demonstrated relatively high mastitis prevalence in this study. Contagious bacteria Staphylococcus aureus, Corynbacterium bovis, Streptococcus agalactiae and coagulase negative Staphylococci caused most of the infections. These infections are usually spread from cow to cow at milking if the milking hygiene is not good enough. The mastitis situation could be improved by improving milking procedures and hygiene.

*Staphylococcus aureus* is the main pathogen of cow and buffalo mastitis. This species contributes to mastitis pathogenesis due to the heat-stable toxin production that is active even in pasteurized milk. *Staphylococci*, the ability to produce biofilm are the most important reason for unusual problem with eradication of infection and recurrent infections of mammary glands. Production of slime enables adhesion of bacteria to the epithelium of mammary glands. It also facilitates persistence of micro-organisms in the host tissue by protecting the bacterial cells against the mechanisms of the host defense. Importantly, it causes the evident reduction of susceptibility to antibiotics, due to altered growth rate and delayed penetration of antimicrobial agents within the biofilm structure. Pathogenic bacteria infecting cow and buffalo Mastitis is responsible for huge financial losses to dairy industries and directly affects not only the productivity in both quantity and quality aspects of composition but also alters milk physio-chemical characteristics (Melchior et al., 2006 a, b and 2007; Contreras et al., 2007; Chockalingam et al., 2007; Byarygaba et al., 2008; Krukowski et al., 2008; Dhanawade et al., 2010; Mekonnne and Tesfaye, 2010; Nayak et al., 2011; Peres and Zappa 2011; Silva et al., 2012; Li et al., 2012; Szweda et al., 2012; Saify et al., 2013 and Raza et al., 2013).

Many of bacterial isolated from bovine mastitis are also the causative agent of human diseases Escherichia coli, Staphylococcus aureus and Streptococcus agalactiae and higher incidence rate of *E. coli* may be due to poor hygienic conditions of the environment as *E. coli* infects the udder via teat canal from the environment. Previously researcher reported that spa (X-region) and clfA gene typing in *S. aureus* obtained from milk from cattle and buffalo with clinical mastitis. The study revealed presence of spa and clfA gene with a wide degree of polymorphism. It directly correlates with the pathogenic potential of an organism.
and their association with clinical manifestations in mastitis among cattle and buffalo. The number of repeats along the X region of the spa gene correlates with the virulence level of the strains (Mir et al., 2014 and Yadav et al., 2015).

The poor management and udder health practices, inadequate milking procedures would depiction the cows to subclinical mastitis caused by environmental and contagious bacteria during milking by miller’s hands. They included coagulase negative *Staphylococci, Staphylococcus aureus, Streptococcus* species, *Bacillus* species, *Streptococcus agalactiae* and *Escherichia coli*. Environmental *Streptococci* are ubiquitous in the farm environment and are a frequent cause of mastitis in dairy cows. On the other hand, nonuse of teat dips and other mastitis control techniques due to lack of knowledge should have greatly contributed to the high prevalence of subclinical mastitis. Farmers need to be educated and encouraged to practice good farming, animal health management practices, and milking practices (Cameron et al., 2016, Hossian et al., 2017 and Mpatswenumugabo et al., 2017).

**Antimicrobial sensitivity of antibiotics**

Bovine mastitis is the most important and serious threat to the dairy industry, which not only affecting the milk yield but also destroying one or more quarter of the udder. The main obstacle in the effective control of bovine mastitis is the emergence of drug resistant bacterial strains that might be due to indiscriminate use of wide variety of antibiotics. Antimicrobial resistant pathogens in animals have also been considered as a potential health risk to humans from the possible pathogens. The emergence of antibiotic resistance has been a major problem in human and animal medicine involving spontaneous mutation or recombination of genes. Mastitis is the single largest cause of antimicrobial use in dairy. In fact, the widespread use of antibiotics against mastitic pathogenic bacteria could lead to the selection and emergence of resistant strains (Owens et al., 1997; Mota et al., 2005; Vengadabady et al., 2005 and Walther et al., 2006).
Mastitis is one of the most costly diseases of dairy animals. Bacteria constitute the most common etiological agents of mastitis. *Staphylococcus aureus* was the most frequently isolated pathogen followed by *Streptococcus agalactiae*, *Escherichia coli*, *Bacillus* spp. and mixed growth. Norfloxacin, Gentamycine and Choramphenocol were found most effective drugs among the antibiotics tested in vitro (Farooq *et al.*, 2008).

Sumathi *et al.*, (2008) investigated the current status of clinical mastitis among dairy cattle in and around Bangalore. The prevalence of major pathogens isolated was *Staphylococcus aureus*, *Escherichia coli*, *Staphylococcus epidermidis*, *Streptococcus* spp and *Klebsiella* spp. Antibiogram studies were also performed for these isolates and Gentamicin was found to be the most effective drug.

Ribeiro *et al.*, (2009) observed high rates of bacterial resistance to penicillin, ampicillin and neomycin were found in the milk of cows infected with mastitis and created in organic dairy farms.

Mastitis causing coagulase negative *Staphylococci* can serve as a reservoir of resistance genes that can be transferred to other bacteria. This phenomenon increases the difficulties of controlling and treating against Mastitis. Mastitis in bovines has become extremely complex and costliest disease so antibacterial therapy treating mastitis is the most common cause for antibacterial use in dairy cattle. *In vitro* antimicrobial sensitivity test of bovine mastitis pathogens are frequently used by bovine practitioners to guide cow or herd level treatment decisions (Leclercq, 2009; Kasravi *et al.*, 2010 and Ranjan *et al.*, 2010).

The continuous use of antibiotics for a long period may lead to multidrug resistance in causative organisms which has resulted in the use of high doses of antibiotics and leads to the danger of increasing amounts of antibiotics residues in milk, a potential hazard. For suitable antibiotic therapy, bacterial isolation and antibiotic sensitivity studies are always essential (Gopinath *et al.*, 2011 a, b & c and Kujrogi *et al.*, 2011).
The antibiotics are widely used in veterinary medicine and the indiscriminate use of these drugs contributes to the presence of antibiotic residues in milk that currently represents the main chemical contamination in dairy products. The main antibiotics are becoming ineffective, including against mastitis, due also to the transferring of the resistance bacteria-to-bacteria even among different species. The coagulase negative *Staphylococci* have become the most common bovine mastitis isolates in many countries. Therefore, they are considered now an important emerging mastitis pathogen. Coagulase-negative *Staphylococcus* sp. resistant to five antibiotics (amoxicillin + clavulanic acid, enrofloxacin, gentamicin, vancomycin and penicillin G) were detected (Bhatt *et al.*, 2011; Laport *et al.*, 2012; Miguel *et al.*, 2012; Silva *et al.*, 2012 and Suleiman *et al.*, 2012).

The infections caused by *Staphylococcus aureus* showed main causative agent in mastitis disease. This bacterium specie stands out as the cause of the higher incidence of mastitis due to its high resistance to antibiotics as a result of the indiscriminate use of these therapeutic agents. *Streptococcus agalactiae*, *Streptococcus dysgalactiae* and *Streptococcus uberis* are important mastitis environmental pathogens and the majority of the isolates were susceptible to all drugs except for aminoglycoside, macrolide, lincosamide and tetracycline (Nunes *et al.*, 2013; Rato *et al.*, 2013 and Idriss *et al.*, 2014).

Bansal *et al.*, (2015) examined Comparative antibiogram of coagulase negative *Staphylococci* (CNS) associated with subclinical and clinical mastitis in dairy cows. Results showed CNS isolates were susceptible to chloramphenicol, gentamicin and streptomycin, while higher resistance was recorded against routinely used penicillin group.

Bacterial isolates were resistant to penicillin, clindamycin, ampicillin, and cefotaxime. Bacteriocins synthesized by *Bacillus thuringiensis* inhibited the growth of multiantibiotic resistance bacteria such as *Staphylococcus agnetis*, *Staphylococcus equorum*, *Streptococcus uberis*, *Brevibacterium stationis*, and *Brachybacterium conglomeratum*, but they were not active against *Staphylococcus sciuri*, a microorganism that showed an 84% resistance to antibiotics tested (Galvan *et al.*, 2015).
The incidence of *Staphylococcus aureus* in clinical as well as sub-clinical mastitis was higher in cattle in comparison to buffaloes. Identification of mastitis pathogens and antimicrobial susceptibility testing are essential to control the disease, guiding antimicrobial treatment decisions and for the detection of emerging resistance. The antimicrobial susceptibility tests shows higher susceptibility of *S. aureus*, coagulase negative *Staphylococci*, *Escherichia coli*, *Streptococcus agalactiae*, *Streptococcus dysgalactiae*, *Streptococcus uberis*, *Corynebacterium* spp., and *Bacillus* spp., to amoxicillin/ clavulanic, oxytetracycline and trimethoprim. Environmental *Streptococci* are ubiquitous in the farm environment and susceptible to beta-lactam antimicrobials. A departure from the expected susceptibility to beta-lactams was the apparent reduced susceptibility of *S. uberis* to penicillin (Sharma *et al.*, 2015; Cameron *et al.*, 2016; Sylejmani *et al.*, 2016; Vasquez-Garcia *et al.*, 2017 and Seyoum *et al.*, 2018).

**Medicinal plants and their antimicrobial activity**

The leaves of trees and shrubs are a component of most natural pastures for ruminant diets. Many tree leaves have antimicrobial factors, like tannins, essential oils, or other aromatic compounds. Essential oils consist in complex mixtures including volatile substances whose components are related to different chemical groups: terpene hydrocarbons, simple alcohols, aldehydes, ketones, phenols, esters, and organic acids in different concentrations. In addition, many biological activities and antibacterial promoting effects have been reported for plant tannins and flavonoids, and their investigation is now increasingly relevant (Hasalam, 1989; Kumar and Vaithiyanathan, 1990; Chung *et al.*, 1998; Simoes and Spitzer, 2000; Nascimento *et al.*, 2000; Wynn, 2001 and Rojas *et al.*, 2006).

Scientific experiments on the antimicrobial properties of plant components were first documented in the late 19th century. In India, from ancient times, different parts of medicinal plants have been used to cure specific ailments. This interest primarily stems from the belief that green medicine is safe and dependable, compared with costly synthetic drugs that have adverse effects. Natural antimicrobials can be derived from plants, animal tissues, or
microorganisms. Medicinal plants are rich in a wide variety of secondary metabolites such as tannins, alkaloids and flavonoids, which have been found in vitro to have antimicrobial properties (Nair and Chanda, 2006; Lewis & Ausubel, 2006 and Ahmad & Aqil, 2007).

According to World Health Organization (WHO), 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. *Alternanthera brasiliiana*, *Achillea millefolium*, *Baccharis trimera* and *Solidago chilensis* extracts were active against *Staphylococcus aureus* while *Symphythum officinale*, *Sambucus nigra*, *Mentha spp.*, *Ocimum basilicum*, *Parapiptadenia rigida* and *Cuphea carthagenensis* extracts were active against *S. aureus* and *Salmonella choleraesuis* microorganisms. *Phyllanthus acidus* showed antimicrobial inhibitory activity and study suggests that the ethanol extracts of *Phyllanthus acidus* can be used as herbal medicines in the control of *Escherichia coli* and *S. aureus* following clinical trials. Crude extracts of Asiatic Pennywort (*Centella asiatica* (Linn.) Urban) extracted with ethanol and water showed antimicrobial activity against *S. aureus*. The ethanol extracts had more potential antibacterial activity than the water extracts (Avancini et al., 2008; Jagessar et al., 2008; Taemchuay et al., 2009; Baskaran et al., 2009 and Kamali & Karim, 2009).

Ayyappadas et al., (2009) and Kowti et al., (2010) examined that Methanol extracts of *Tridax procumbens* and *Spathodea campanulata* showed significant activity against coagulase positive *Staphylococcus aureus* and *Streptococcus agalactiae*. The dose dependent study was observed that the ethanol flower extract was more potentent than leaf extract. Flavonoids and tannins present in the both ethanol extract may be responsible for the antimicrobial activity.

The ethanolic extracts of *Stevia rebaudiana*, *Murraya koenigii*, *Psidium guajava* and *Hibiscus roasanensis* obtain new and effective herbal medicines to treat infections caused by multi-drug resistant strains of microorganisms from community as well as hospital settings (Singh et al., 2010).
Mathur et al., (2010) and Pozzo et al., (2011) observed the susceptibilities of some of the common aerobic bacterial isolates from milk samples collected from different cows with udder inflammation and result showed that methanolic extract of leaves of Murraya koenigii inhibited Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus uberis, Pseudomonas aeruginosa, Escherichia coli, Corynebacterium gravis and Bacillus cereus. The activity of essential oils from spices showed antimicrobial activity against Staphylococcus spp. isolated from bovine mastitis.

Gopinath et al., (2011 a, b and c) reported that the antibacterial activity of aqueous and methanol extracts of Punica granatum and Tabernaemontana divaricata was treating mastitis causing organism. The phytochemical screening of the plant revealed the presence of alkaloids, tannins, carbohydrates, flavanoids, phytosterols, phenols, sterols, terpenes, and volatile oils.

Alternative treatments to bovine mastitis with bacteriocins and the antibacterial activity of plant derived compounds against mastitis pathogens have been described aqueous and methanolic extracts of Acacia nilotica, Acacia leucophloea, Acyranthus aspera and Acalypha indica possess potential antibacterial activity against mastitis isolates Staphylococcus aureus, Escherichia coli, Streptococcus agalactiae and Klebsiella pneumoniae (Mubarack et al.,2011 a, b and c).

In vitro and in vivo study of the antibacterial effects of Nigella sativa methanol extract and dried leaves extract of Tabernaemontana divaricata in dairy cow mastitis. Results of in vitro experiments also showed significant activity of the extract against Staphylococcus aureus and Escherichia coli. The extract showed significant in vitro and in vivo inhibitory effects on causative organisms compared to standard drugs and also induced healing of the disease. Interesting both extracts were active against Streptococcus agalactiae, E. coli, S. aureus and Klebsiella pneumoniae (Gopinath et al., 2011 b and c; Rakhshandeh et al., 2011 and Doss et al., 2012.)

Shekhan and Hussaini, (2012) and Kalayou et al., (2012) reported the ethanolic extract of Coriandrum sativum, Vitis vinifera, Zingiber officinale and Calpurinia aurea showed strong antibacterial activity against clinical bacterial isolates of mastitis and wound.
Mushrooms are rich sources of natural antimicrobials, as they produce antibacterial and antifungal compounds to survive in their natural environment and mushrooms extracts emerge as interesting possibilities to be explored as antimicrobial drugs. Essential oils extracted from cinnamon, clove and lemon grass exhibited broad spectrum in vitro inhibitory activity against yeast and yeast like fungal organisms (Alves et al., 2012a; Rathee et al., 2012; Sukumar & James, 2012 and Alves et al., 2013).

The assessment of the effect of medicinal plants and alternative medicine will perform against milk pathogens and trying to detect an antimicrobial profile directly against them. The consolidation of new research lines is important if they have the goal of synthesizing or identifying new therapeutic options in medicinal plants and pharmacological evaluation of new active molecules against bovine diseases of economic importance. Scientists have been found in vitro antibacterial effective activity of medicinal plants against bovine udder isolated bacterial pathogens from dairy herds and substances against a wide array of infectious agents (Gulten et al., 2013; Laham and Fadel, 2013 and Motlagh et al., 2013).

Okmen and Turkcan (2013) reported antibacterial activity of Elaeagnus angustifolia against Staphylococcus aureus and coagulase negative Staphylococci. Result revealed that methanolic extract showed a maximum zone of inhibition against Staphylococcus aureus and E. angustifolia leaf extract showed lowest antibacterial activity against S. aureus and Coagulase negative Staphylococcus.

Cyclamen mirabile tuber extracts has significant antibacterial activity and it could be very useful in the discovery of novel antibacterial agents against S. aureus and coagulase negative Staphylococcus pathogens isolated from subclinical cow mastitis. Aqueous and methanolic root extract of Glycyrrhiza glabra and Carum copticum showed potent antimicrobial activity against gram positive (Staphylococcus aureus and Staphylococcus agalactiae) and gram negative (Escherichia coli) organisms (Kazemi, 2014; Okmen et al., 2014 and Mahto et al., 2014).

Essential oils make up as an alternative to control mastitis pathogens in dairy cows because they have antibacterial action against gram-positive and gram-negative bacteria and no diverse effects on human health. Cinnamomum cassia, Thymus vulgaris, Cymbopogon flexuosus, Eugenia caryophyllata and Cymbopogon winterianus essential oils presented high antibacterial action against
mastitis pathogens. The results indicated the prospect of using essential oils to control bovine mastitis caused by *Staphylococcus aureus* and *Streptococcus agalactiae*. The hydroalcoholic extract of pomegranate peel showed antimicrobial activity against mastitis pathogens (Perini *et al.*, 2014; Zeedan *et al.*, 2014; Motlagh *et al.*, 2014; Moreira *et al.*, 2015 and Tawab *et al.*, 2015).

Plant extract are supplemented in the treatment of subclinical cow mastitis and spice extracts have the potential to used as antibacterial agents for searching new medicines and remedies. *Crocus sativus*, Garlic and neem plant extract found potential antibacterial activities against *Staphylococcus aureus*, *Escherichia coli* and coagulase negative *Staphylococcus* pathogens isolated from subclinical cow mastitis. The study of *Crocus sativus*, showed that the plant extracts have great importance as antioxidant activities. The anti-infective properties of a Thai traditional polyherbal formula, namely Ya-Sa-Marn-Phlae (YSMP), its herbal components (*Curcuma longa*, *Areca catechu*, *Oryza sativa*, and *Garcinia mangostana*), and representative chemical constituents (catechin, α-mangostin, and curcumins) showed antibacterial activity against bovine mastitis isolates *Staphylococci* (Okmen *et al.*, 2016; Chusri *et al.*, 2017 and Edward *et al.*, 2017).

*Allium sativum*, *Bunium persicum*, *Oryza sativa* and *Triticum aestivum* plant extract showed significant inhibitory activity against *Staphylococcus aureus*, *Escherichia coli* and *Klebsiella pneumonia* mastitis livestocks in northwest Pakistan. Among phytochemicals, alkaloids of all tested antimastitis plants produced significantly higher inhibition zones against bacteria. Medicinal plants traditionally used against mastitis are therapeutically active against bacterial pathogens. *Allium sativum* and *Bunium persicum* were posses to potential candidate species for the development of novel veterinary drugs with low cost (Amber *et al.*, 2018).

**Following three medicinal plants have been selected for antimicrobial activity against pathogenic bacteria in milk of cow and buffalo milk:-**

1. *Trigonella foenum-graecum* (Methi)
2. *Balanties aegyptiaca* L. (Hingot)
3. *Trachyspermum ammi* L. (Ajwain)
1. **Trigonella foenum-graecum (Methi)**

   Kingdom : Plantae
   Order : Fabales (or Leguminales)
   Family : Fabaceae
   Genus : *Trigonella*
   Species : *foenum graecum*

*Trigonella foenum-graecum* is commonly known as methi and Fenugreek is used both as an herb (the leaves) and as a spice (the seed, often called *methi*). The plant is cultivated worldwide as a semi-arid crop and is a common ingredient in many curries. The name *fenugreek* or *T. foenum-graecum* is from Latin for "Greek hay". The important chemical constituents are saponins, coumarin, fenugreekine, nicotinic acid, phytic acid, scopoletin and trigonelline. The plant contains a number of steroidal sapogenins. The alkaloid trigonelline, trigocoumarin, trimethyl coumarin and nicotinic acid are also present. Mucilage is a prominent constituent in the seeds (Hardman *et al.*, 1980).

The main chemical constituents of *Trigonella foenum-graecum* are fibers, flavonoids, polysaccharides, saponins, flavonoids and polysaccharides fixed oils and some identified alkaloids *viz*., trigonelline and choline (Jayaweera, 1981).

Fenugreek (*Trigonella foenum-graecum*) found in nature and is cultivated in India and Pakistan is a well known medicinal plant. Fenugreek is a good source of dietary protein for consumption by human and animals. Also it contains many carbohydrates, minerals and vitamins. In recent years, laboratory studies and clinical trials have focused on fenugreek as a potential nutraceutical. The pharmacological activities of *T. foenum-graecum* includes anthelmintic, antibacterial, antidiabetic, anti-inflammatory, antifertility, antifungal, analgesic, anti-inflammatory, antipyretic, immunomodulatory activities, antipyretic, antimicrobial properties, hypcholesterolaemic, immunomodulatory, hypoglycaemic, gastro and hepatoprotective and antioxidative properties (Sharma, 1986; Stark and Madar, 1993; Anuradha and Ravikumar, 2001; Choudhary *et al.*, 2001; Al-Habori & Raman, 2002; Puri *et al.*, 2002; Schryver, 2002; Bash *et al.*, 2003; Bin-Hafeez *et al.*, 2003 and Thirunavukkarasu *et al.*, 2003).
Trigonella foenum-graecum contains lecithin and choline that helps to
dissolve cholesterol and fatty substances, minerals, B. Complex, iron, Phosphates,
PABA (Para-Amino Benzoic Acid) and vitamins A and D. It also contains neurin,
biotin, trimethylamine which tends to stimulate the appetite by their action on the
nervous system. The seeds are hot, with a sharp bitter taste, tonic, antipyretic,
anthelmentic, increase the appetite, astringent to the bowels, cure leprosy, “vata”,
vomiting, bronchitis, piles; remove bad taste from the mouth, useful in heart
disease. The antioxidant effect was evaluated by estimating thiobarbituric acid-
reactive substances and reduced glutathione and measuring the activities of
catalase and superoxide dismutase in liver, heart, and kidney in diabetic rats
(Michael and Kumawat, 2003; Prajapati et al., 2003; Annida & Stanely, 2005 and
Srinivasan et al., 2006).

Hypoglycemic effect of Trigonella foenum-graecum on rats, these are
Alloxan induced diabetic mice with 2 active ingredients 4-hydroxyisoleucine and
Trigonelline, both of these substances has shown hypoglycemic effects on rats. T.
foenum-graecum plant has been used for blood lipids and sugar decreasing in
diabetic and non diabetic peoples and has antioxidant and antibacterial activity.
The research showed that fenugreek significantly lowers the total blood
cholesterol (Shah and Bodhankar, 2006; Joshi & Rajni, 2007 and Nandini et al.,
2007).

Trigonella foenum-graecum (fenugreek) is commonly consumed as a
condiment and used medicinally as a galactagogue by nursing mothers to increase
inadequate breast milk supply. Several studies have also shown the anticancer
properties of this herbal plant. The chemopreventive aspects and the potential
protective effect of fenugreek seeds against 7, 12-dimethylbenz anthracene
(DMBA) in rats have been reported. Crude extracts of T. foenum-graecum have
shown good antibacterial activities towards the Bacillus cereus, Lactobacillus
acidophilus, and Streptococcus pneumoniae. Multidrug resistance bacterial strains
were remarkably inhibited by the acetone and chloroform extract of T. foenum-
graecum. The methanol extracts of leaves and seeds of fenugreek showed highest

The aqueous extract of *Trigonella foenum-graecum* seeds shows various degrees of bacteriostatic activity against gram positive and gram negative bacteria. Seeds are found to be effective against bacteria like *Escherichia coli*, *Salmonella typhi* and *Staphylococcus aureus*. *T. foenum-graecum* is a mixture of bioactive compounds include alkaloids, saponins, amino acids (act as insulin secretogogues 4-OH Ile, arginine), flavonoids, coumarins, mucilaginous fibers (galactomannan), nicotinic acid and other vitamins and minerals (Fe, Mn, Mg, Zn etc.). Flavonoids show remarkable biological activities inhibitory effects on enzymes, modulatory effect on some cell types, antioxidant, anticarcinogenic property, antiallergic, antibacterial, antifungal, antiviral, anti-malarial and anti-inflammatory properties (Mehrafarin *et al.*, 2010; Priya *et al.*, 2011; Yadav *et al.*, 2011; Bhatia & Sharma, 2012; Tejswini *et al.*, 2012; Mahmoud *et al.*, 2012 and Sudha & Mathangi, 2013).

The antibacterial study of the medicinal plant *Trigonella foenum-graecum* crude extract and its subsequent solvent soluble fractions against six bacterial strains like *Escherichia coli*, *Pseudomonas aeuroginosa*, *Staphylococcus aureus*, *Salmonella typhi*, *Erwinia carotovora* and *Agrobacterium tumifaciens* showed that all fractions except water fraction showed promising inhibitory activity against *Escherichia coli* and *Staphylococcus aureus*. The chloroform and ethyl acetate fractions were active against all bacterial strains and show excellent activity. Hydroalcoholic extracts of *T. foenum-graecum* seeds showed antioxidant activity and good adjuvant in prevention and management of various diseases including cancer. In India, fenugreek powder is also used as a lactation stimulant and protective against ethanol toxicity (Rehman *et al.*, 2013; Saranya *et al.*, 2014; Yadav & Baquer, 2014 and Singh *et al.*, 2014).
Swarnakar et al., (2014) studied the morphology of control *Gastrothylax crumenifer* compared with treated animal by light microscopy. The effect of aqueous extracts of *Trigonella foenum-graecum* showed detachment, discontinuation and blebbing in tegument surface, rupture the parenchyma cells, tegument cells and also observed damaged the musculature cells in sucker of worm.

Laila and Murtaza, (2015) examined Diabetes is a major health problem predisposing to markedly increased complications. Plant secondary metabolites have long been known to have health benefits against various oxidative stress related diseases including diabetes. One of the most promising vegetable providing treasures of such secondary metabolites is fenugreek. The herb have an enormous potential to prevent or cure diabetes more than other plant species especially due to the presence of unique chemical constituents including quercetin, diosgenin, trignolline, galactomanin and unusual amino acid 4 hydroxy isolucine.

Phytochemical screening for *Trigonella foenum-graecum* seeds and callus extracts indicated the presence of various Secondary metabolites like alkaloids, flavonoids, tannins, phenols, saponins and terpenoids. The petroleum ether, methanol, Acetone and aqueous extract of *T. foenum-graecum* seed showed highest antimicrobial activity against mastitis pathogens. The plant extract showed antifungal activity against Multi drugs resistant Candida spp. The plant containing various phytochemicals may be exploited in the treatment of infectious diseases caused by drug-resistant microorganisms. The plant extract may be the potential alternatives of antibiotics to avoid their overuse and side effects on human health and environment (Chalghoumi et al., 2016; Sharma et al., 2016 and Khan et al., 2017).
2. **Balanites aegyptica** (Hingot)

Kingdom : Plantae  
Order : Zygophyllales  
Family : Zygophyllaceae  
Genus : Balanites  
Species : aegyptiaca

*Balanites aegyptiaca* (Zygophyllaceae), known as 'desert date,' is spiny shrub or tree up to 10 m tall, widely distributed in dry land areas of Africa and South Asia. The fruit of *B. aegyptiaca* consists of an epicarp, a mesocarp, an endocarp and a kernel. The total saponin content has been found to be 7.2% in the mesocarp and 6.7% in the kernel. The oil extracted from the kernel constituted 44-51% w/w and is composed of mainly triglycerides, with small quantities of diglycerides, phytosterols, sterol esters and tocopherols. Besides, a known spirostanol glycoside, balanitin-3, and a new sapogenol, 6-methyl-9,11-seco-diosgenin, a new furostanol saponin, balanitoside and two pregnane glycosides have been isolated from the fruits of *B. aegyptiaca* (Kamel *et al.*, 1991; Hall, 1992; Honsy *et al.*, 1992, Kamel, 1998 and Mohmed *et al.*, 2002).

The extract of *Balanites aegyptiaca* fruit mesocarp, root bark, leaves and seeds kernels shows larvicidal, vermicidal, antibacterial, insecticidal, antihelminthic, antifeedant, molluscicidal, antibacterial, antifungal, hepatoprotective, anticancerous, antiparasitic, antidiabetic, anti-inflammatory, contraceptive activities and wound healing activity and use as an alternative protein source in animal feeding. Methanolic and aqueous extracts of whole plant extract showed 4 mm inhibition zone in *Staphylococcus aureus* and 11 mm zone of inhibition in case of *Staphylococcus epidermidis* (Iqbal *et al.*, 2004; Chapagain and Weisman, 2005; Doughari *et al.*, 2007; Parekh and Chanda, 2007; Hena *et al.*, 2010 and Yadav & Panghal, 2010).

*Balanites aegyptiaca* fruit is a rather long, narrow drupe, 2.5 to 7 cm long, 1.5 to 4 cm in diameter. Young fruits are green and tormentose, turning yellow and glabrous when mature. The aerial part of *B. aegyptiaca* ethanolic extracts effect similar to the reference drug indomethacin, which showed inhibition (Chothani & Vaghasiya, 2011 and Suky *et al.*, 2011).
The fruits, leaves, roots, stem bark, stem wood and seeds of hingot are known for their traditional medicinal value. Flavonoid extracts of callus tissue of *Balanites aegyptiaca* were screened against *Escherichia coli*, *Proteus vulgaris*, *Pseudomonas aureginosa*, *Citrobacter amalonaticus*, *Staphylococcus aureus*, *Micrococcus lylae* and *Bacillus subtilis*. Maximum activity was observed in free flavonoid fraction of callus tissue (Bidawat *et al.*, 2011; Dubey *et al.*, 2011 and Kumawat *et al.*, 2012).

The methanol extract of the fruit (edible mesocarp) of *Balanites aegyptiaca*, from Sudan, widely employed in Sudanese folk medicine showed the presence of saponin, terpenoids, phenolic compounds and alkaloids. The methanolic extract of the fruits extracted from *B. aegyptiaca* exhibited a strong antioxidant activity in the DPPH assay and a potent capacity in preventing linoleic acid oxidation. These findings support some of the traditional applications of the fruit of *B. aegyptiaca* against microbial ailments (Abdallah *et al.*, 2012).

Physicochemical investigation and preliminary phytochemical screening of powdered leaves of *Balanites aegyptiaca* provide useful information in regard to its correct identity and evaluation. *B. aegyptiaca* possess many biological activities such as antimicrobial, antioxidant, anti diabetic, antiasthmatic, xanthine oxidase and acetylcholinesterase inhibitory activities, antinociceptive, antiviral activity, wound healing activity, hypocholesterolemic activity and diuretic activity. They were found to be toxic to pests, molluscs and larvae. *B. aegyptiaca* possessed all the phytochemical components tested except anthroquinones and alkaloids, while root bark lack anthroquinones, cardiac glycosides and phlobatannins and stem bark possessed only flavonoids and polyphenols. Their anti-inflammatory activity has been known from the ancient period. The plant possesses promising applications for the drug development and research purposes. *Balanites aegyptiaca* leaves extract shows significant wound healing activity. The alcoholic extract of fruit of *B. aegyptiaca* showed excellent antibacterial activity against gram positive, gram negative bacteria as well as resistant bacteria harbouring bla genes (Kumawat *et al.*, 2012; Gajalakshmi *et al.*, 2013; Kommu *et al.*, 2013; Jahan *et al.*, 2013; Saboo *et al.* 2014 and Kumar *et al.*, 2014).
Swarnakar *et al.*, (2015) investigated the plant *Balanites aegyptica* shows anthelminthic activity. Treated with *B. aegyptiaca* and control *Paramphistomum cervi* was observe and compared by Light microscopy. Hingot showed discontinuous, damaging tegument, vacuolization and breakage in sucker.

The hydro ethanolic extract of the bark inhibited *in vitro* the growth of *Pseudomonas aeruginosa* and *Staphylococcus aureus* in a dose-dependent manner. Bacteriostatic effects were observed on 15 (33%) and 21 (47%) strains of *S. aureus* and *P. aeruginosa* respectively. This study indicated that the bark extract of *Balanites aegyptiaca* possess bioactive compounds implicated in the free radical scavenging and antibacterial activities, justifying the use of the plant in the traditional medicines *(Anani et al., 2015).*

The *Balanites aegyptiaca* plant possess the presence of saponins, flavonoids, cardiac glycosides, alkaloids and tannins in both the aqueous and methanolic leave extracts of *B. aegyptiaca*. Phenols were only detected in the aqueous leave extract, while terpenoids were only detected in the methanolic leave extract of *Balanites aegyptiaca*. The results also revealed that the two extracts possess wider antimicrobial activities against *Escherichia coli*, *Pseudomonas aeruginosa*, *Samonella typhi*, *Staphylococcus aureus*, *Aspergillus flavus* and *Aspergillus niger* the tested organisms at different concentrations. Therefore, both the aqueous and methanolic leave extracts of *Balanites aegyptiaca* may provide a target for drug discovery *(Abdulhamid and Sani, 2016).*

*Balanites aegyptiaca* stem is an important woody, true xerophytic tree of tremendous importance and used in a variety of folk medicines. Stem bark of *B. aegyptiaca* possess secondary metabolisms, which indicates the presence of Alkaloids, Tannins, Triterpenoids, Saponins. The microbial activities were provide that most of the extracts methanol, ethyl acetate, chloroform and aqueous extract showed antibacterial activities with high to moderate inhibitory effect against *Escherichia coli* and *Klebsiella pneumoniae* respectively. The chloroform and the aqueous extracts of the plant organ showed positive antifungal activity *(Mutwali and Abdelgadir, 2016).*
Balanites aegyptiaca has been reported to be an anti-helminthic, a purgative, febrifuge, emetic and can also cure other types of ailments like skin boils, malaria, wounds, colds, and syphilis, liver and spleen disorders. The fruit and leaf extract of B. aegyptiaca have promising antioxidant indicates and promising agent in scavenging free radicals and antimicrobial activity against bacteria (Koko et al., 2017).

3. **Trachyspermum ammi L. (Ajwain)**

Kingdom: Plantae
Division: Magnoliophyta
Class: Magnoliopsida
Order: Apiales
Family: Apiaceae
Genus: Trachyspermum
Species: ammi

Trachyspermum ammi commonly known as ‘Ajwain’ belonging to family Apiaceae is distributed throughout India and it is mostly cultivated in many parts of Pakistan, India, Afghanistan, Egypt and Europe. The roots are diuretic in nature and the seeds possess excellent aphrodisiac properties. The seeds contain 2–4.4% brown colored oil known as ajwain oil. The main component of this oil is thymol, which is used in the treatment of gastro-intestinal ailments, lack of appetite and bronchial problems. The oil exhibits fungicidal, antimicrobial and anti-aggregatory effects on humans. Ajwain is traditional potential herbs, is widely used for curing various diseases in humans and animals (Chopra, 1982 and Saxena & Vyas, 1986).

Ajwain oil has also been reported to posses to a broad spectrum of fungitoxic behavior against fungi such as Aspergillus niger, Aspergillus flavus, Aspergillus oryzae, Aspergillus orrhaceus, Aspergillus fumigates, Fusarium monoliforme, Fusarium graminearum, Penicillium citrium, Penicillium viridicatum, Penicillium madriti and Curvularia lunata (Sridhar et al., 2003; Singh et al., 2004; Shimelis et al., 2006 and Bansod & Rai, 2008).
Antimicrobial activity of essential oil of ajwain (*Trachyspermum ammi*) were found susceptible to both Gram positive bacteria such as *Lactobacillus acidophilus*, *Streptococcus*, *Staphylococcus aureus*, *Micrococcus luteus*, *Bacillus cereus* and Gram negative bacteria such as *Klebsiella pneumoniae*, *Escherichia coli*. *Trachyspermum ammi* also reported activity against *Enterobacter sakazakii* (Kaur and Arora, 2009 and Upadhya et al., 2010).

*Trachyspermum ammi* fruits yielded brownish essential oil, with thymol as the major constituent. Studied reveal the presence of various phytochemical constituents mainly carbohydrates, glycosides, saponins, phenolic compounds, volatile oil (thymol, γ-terpinene, para-cymene, and α- and β-pinene), protein, fat, fiber and mineral matter containing calcium, phosphorous, iron and nicotinic acid. It also contains monoterpenoids and reported some new constituents. The plant is used traditionally as a stimulant, carminative, flatulence, atonic dyspepsia, diarrhoea, abdominal tumours, abdominal pains, piles, and bronchial problems, lack of appetite, galactogogue, asthma and amenorrhoea. It possess various pharmacological activities like antifungal, antioxidant, antimicrobial, antinociceptive, cytotoxic activity, hypolipidaemic, antihypertensive, antispasmodic, broncho-dilating actions, antilithiasis, diuretic, abortifacient, antitussive, nematicidal, anthelmintic and antifilarial activity (Bairwa et al., 2012; Chauhan et al., 2012 and Javed et al., 2012).

Usha et al., (2012) reported that ethanol extract of cinnamon and ajwain revealed an antibacterial activity against *Pseudomonas* spp., whereas acetone extract of spices exhibited highest activity against *Escherichia coli*. The results obtained in the study suggest that the ethanol extract of *Cinnamnum zeylanicum* and *Trachyspermum ammi* revealed a significant scope to develop a novel broad spectrum of antibacterial herbal formulation and can be used for cooked food preservation.

The plant *Trachyspermum ammi* is used traditionally as a stimulant, carminative, flatulence, atonic dyspepsia, diarrhoea, abdominal tumors, abdominal pains, piles, and bronchial problems, lack of appetite, galactogogue, asthma and amenorrhoea. *T. ammi* roots are diuretic in nature and the seeds possess excellent aphrodisiac properties. Medicinally, it has been proven to possess various
It contains many phytoconstituents including carbohydrates, glycosides, saponins, phenolic compounds, volatile oil (thymol, \(\gamma\)-terpinene, para-cymene, and \(\alpha\) and \(\beta\)-pinene), protein, fat, fibre and mineral matter containing calcium, phosphorous, iron and nicotinic acid. \(T.\ ammi\) essential oil inhibits the growth of all of the tested bacteria. The ethanol extract of \(T.\ ammi\) was found sensitive to \textit{Escherichia coli}, \textit{Proteus mirabilis} and \textit{Klebsiella pneumoniae}. The acetone extract of \(T.\ ammi\) produced antimicrobial activity against \textit{K.pneumoniae} (Moazeni \textit{et al.}, 2012; Jeet \textit{et al.}, 2012; Hassanshahian \textit{et al.}, 2014; Tariq \textit{et al.}, 2014; Zarshenas \textit{et al.}, 2014 and Reji & Rajasekaran, 2015).

\textit{Trachyspermum ammi} seeds are an excellent source of vitamins which are essential in the human diet and contain significant amounts of proteins, dietary fibers and a number of useful phytochemicals such as alkaloids, flavonoids, glycosides, phenolic compounds and tannins. The plant found antioxidant activity and acetonic extract of plant also exhibited the strong antimicrobial activity confirming the traditional use of this plant in the treatment of various bacterial infections such as diarrhea and skin diseases (Hassan \textit{et al.}, 2016).

\textit{Trachyspermum ammi} (Apiaceae) commonly known as ajwain is a significant medicinal, aromatic and spice plant. It was originated in Egypt and generally distributed throughout the World. Ajwain seeds yield 2-5% brownish essential oil, with thymol as the major constituent along with p-cymene, \(\gamma\)-terpinene, \(\alpha\)-pinene, \(\beta\)-pinene and \(\alpha\)-terpinene. Due to presence of various chemical constituents in ajwain, various biological and pharmacological properties have been reported. Chemical composition of essential oil and presence of variety of diverse constituents in it are responsible for a wide range of biological properties (Chahal \textit{et al.}, 2017)

In view of the above review it is pertinent to attain the present research work on “Studies on antimicrobial properties of fruit of \textit{Balanites aegyptiaca} (Hingot), seeds of \textit{Trachyspermum ammi} (Ajwain), seeds of \textit{Trigonella foenum-graecum} (Methi) medicinal plants against pathogenic bacteria infecting cow and buffalo milk”.