ADDIS ABABA UNIVERSITY
FACULTY OF VETERINARY MEDICINE

THE EFFECT OF HERBAL PREPARATIONS ON STAPHYLOCOCCUS AUREUS & STREPTOCOCCUS AGALACTIAE ISOLATED FROM CLINICAL BOVINE MASTITIS

ARAYA MENGISTU KASSA

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A thesis submitted to the Faculty of Veterinary Medicine, Addis Ababa University in partial fulfillment of the requirements for the Degree of Master of Science in Tropical Veterinary Medicine

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DEDICATIONS

I dedicate this work to my wife W/ro Yetmwork Negash who passed away suddenly due to cardiogenic shock on April 6, 2004, aged 32. Yetmwork was the source of my mirror images, strong arm to my sprit, guider to my life and, who was everything to our two children and me. Things go well as she planned but she finished her short distance run as of early thirties. All her memories will remain in my heart as long as God let me live. She passed away by putting double responsibilities on my weak back. Oh! God, how you are great! And no one knows your secret. Our father God! She was so innocent and her departure was so sudden and may you put her soul in heaven. The king of kings, since no one is free from sin, except You, by the name of Jesus Christ and his mother St. Merry, please apologize her and allow to sit at Your right and think her during your resurrection. Yetm! Tears never compensate your loss and I lost everything of mine and hence, my mind would remain in a broken heart and in prison and always regret. Finally, soils be comfortable, termites be kind and stones be light to her, because she was so delicate, polite and kind.

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LIST OF ABBREVIATIONS

CAMP test = Christie, Atkins and Munch-Peterson

Ba = *Brucea antidysentirica*

Cm = *Combertum molle*

Ca = *Cyphostemma adenocaule*

Ps(Per = *Persicaria senegalensis*

N(Neo) =Neomycin

O(Oxy) = Oxytetracycline

P(PeG) =Penicillin G

S(Str) =Streptomycin

EARO =Ethiopian Agricultural Research Organization

FF = Fair-Field

KOH =Potassium Hydroxide

DMSO= Dimethyl Sulphoxide

FVM = Faculty of Veterinary Medicine

AAU = Addis Ababa University

Res. *S.aureus* = Resistant *S.aureus*

Res. S.agalactiae = Resistant S.agalactiae

CPS = Coagulase Positive *S.aureus*

CNS = Coagulase Negative *S.aureus*

ILRI = International Livestock Research Center Institute

ILCA = International Livestock Center for Africa

m.a.s.l. = Meters above sea level

OF = Oxidation fermentation

SCC = Somatic Cell Count

IMVIC = Indole Methylred Vogousproskauer Citrate

MEOH = Aqueous Methanol

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ABSTRACT

A study was conducted in Debre-Zeit town to determine the incidence of bovine clinical mastitis in a purposefully selected two dairy farms and the efficacy of conventional antimicrobial drugs and traditional herbs, from September 2003-March 2004. The objectives of the work were to assess the invitro effect of six herbal preparations; namely, *Brucea antidysentrica, Combertum molle, Cyphostemma adenocale Persicaria senegalensis, Plantago lanceolata* and *Zehneria scabra* on major isolates of clinical bovine mastitis, to compare their efficacy, with conventional antimicrobial agents that are commonly used for the treatment of bovine mastitis, and to investigate the effect of the herbs on the growth inhibition of resistant isolates. The herbs were collected from their natural habitats and processed and extracted with 80% methanol. Both absolute methanol and aqueous extracts of *Combertum molle* were assessed foe antimicrobial property. Milk samples from clinical cases were collected aseptically and causal agents were identified after the severity of the diseases in each cow was categorized into Grade I-Grade III by following standard laboratory procedures and finally sensitivity test was conducted on *Staphylococcus aureus* (n=17) and *Streptococcus agalactiae* (n=14), which were the predominant isolates. The incidence rate was 12.4 new clinical mastitis cases/ 100cows-month and 6.7 new clinical mastitis cases/100cows-month at risk in EARO and Fair-field dairy farms respectively with an overall incidence of 9 new clinical mastitis cases/100cows-month at risk. *Staphylococcus* species (42.3%) and *Streptococcus* species (34.5%) were the major isolates from Grade I (84%) and Grade II (16%) clinical cases. Single infection of 46.8% and mixed 37.5% and contaminated 9.4% infections were recorded with 6.3% negative cultures. *Staphylococcus aureus* was resistant to Oxytetracycline (23.5%) and Penicillin G (64.7%) and *Streptococcus agalactiae* was resistant to Neomycin and Streptomycin (85.7% each) and Oxytetracycline (100%). *Burcae antidysentrica, Combertum molle, Cyphostemma adenocaule* and *Persicaria senigalensis* were effective against susceptible and resistant isolates and among those absolute methanol extract of *Combertum molle* showed a better effect on both test organisms. *Plantago lanceolata* and *Zehneria scabra* were not showing visible inhibitory zone against test organisms. None of the herbal extract preparations showed visible inhibitory effect on *Escherichia coli*. This study indicated that mastitis is a great problem in the two dairy farms and resistant isolates are circulating within farms. For this herbal preparations might be considered as an alternative option for the treatment of resistant isolates of clinical bovine mastitis for the future.

Keywords: Clinical mastitis, herbal preparations, aetiological agents, *Staphylococcus aureus, Streptococcus agalactiae,* sensitivity, effect, conventional antimicrobials and resistant isolates.

1.INTRODUCTION

Mastitis is an inflammation of the udder resulting from the invasion of pathogenic microorganisms, and it is the most costly or expensive disease in dairy cattle industry (Greer and Baker, 1992). Mastitis in dairy cows causes a loss in milk production, which is the most important and the best single food (Woods, 1986). Mastitis reduces milk and milk products in all dairy producing countries of the world (International Dairy Federation, 1999). In addition to heavy losses in milk quality and quantity, it also causes irreversible damage to the udder tissue and less occasional fatalities (Radostits *et al.*, 2000). Mastitis can lead to the reduction of offspring to a given production system due to the insufficient milk production resulting in starvation. In general, mastitis is a complex disease dealing with, the interaction of microorganisms and the cow's anatomy and physiology, dairy husbandry and management, milking equipment and procedures and environment (Woods, 1986).

The cow udder is an ideal environment for microbial growth and under optimum udder conditions, such as temperature, nutrition, and freedom from outside influence, pathogenic organisms multiply astronomically and it is this factor that causes udder damage and triggers the response that is recognized as mastitis (Woods, 1986; Radostits *et al.*, 1994).

Mastitis is a disease of many mammalian species. At least 137 infectious causes of bovine mastitis are known to date and in large animals the commonest pathogens are *Staphylococcus aureus*, *Streptococcus agalactiae*, other *streptococcus* and Coliforms (Fraster, 1986). It may also be associated with many other organisms including *Actinomyces pyogenes*, *Pseudaomonas aeruginosa*, *Nocardia asteroides*, *Clostridium perfringens*, and others like *Mycobacterium*, *Mycoplasma*, *Pastuerella* and *Prototheca* species and *yeasts* (Radostits *et al.*, 2000). The majority of the cases are caused by only a few common bacterial pathogens, namely, *Staphylococcus* species, *Streptococcus* species, Coliforms, and *Actinomyces pyogenes* (Du Preeze, 2000; Quinn *et al.*, 1999).

Though mastitis is commonly manifested as subclinical, clinical mastitis is also commonly observed, which could be manifested as per acute, acute or chronic forms (Radostits *et al.*, 1994). Data from the ministry of agriculture's mastitis surveillance scheme in the UK, suggests that 43 clinical cases occurring in every 100 cows each year and 1% of cows exhibiting signs of clinical

mastitis die from the condition (Francis, 1985). An extrapolation of the above result to the national dairy herd in the UK suggests that 3500 new clinical cases occur each day. Study on the management and economics of mastitis carried out by Mungube (2001) in dairy cows in the urban and periurban areas of Addis Ababa indicated an overall clinical mastitis prevalence of 6.6% cow and 2.8% quarter level. He also, indicated that out of the 51 farms studied at least a case of clinical mastitis was registered in 12 farms (24%) and the highest clinical mastitis prevalence was 27.3% in one large farm situated in the periurban production sub-system followed by 14% in another large farm while the highest prevalence in small-scale dairy farms was 33.3%. Radostits *et al*. (2000) suggested that the mammary gland is more susceptible to new infection during the early and late dry periods, which may be due to the absence of udder washing and teat clipping, which in turn may have increased the number of potential pathogens on the skin of the teats.

Subclinical mastitis causes insidious losses of milk production and a reduction in the productivity life of the cow. A national survey in UK showed that 32% of the cows and 14.1% of the quarters were sub-clinically infected with the major mastitis pathogens (Francis, 1985). These sub-clinically infected quarters constitute sources from which infection is easily spread usually at milking times to other uninfected quarters. Study conducted in and around Mekele indicated a subclinical mastitis prevalence of 26.7%, which was 80% of the total cases and non-lactating cows showed a prevalence rate of 54.5% (Temesgen, 1999). From 307 clinically examined cows in Southern Ethiopia, 124 (40.4%) were positive for mastitis, of which 46 (37.6%) were clinical and 78 (62.9%) were subclinical cases (Kerro and Tareke, 2003).

Broadly, cow -to-cow or contagious, environmental, infection in dry cows (example, summer mastitis) and to a lesser extent uncommon causal types of mastitis infection can be recognized. *Staphylococcus aureus* present in the udders of chronically infected cows and also in cuts and chaps on the teat skin, *Streptococcus agalactiae* found only in the udder, though it can survive for 2-3 weeks away from the cow without multiplication and *Streptococcus dysgalactiae* found in the udder and on teat skins are the main pathogenic bacteria that are involved in contagious mastitis (Blowey, 1990; Radostits *et al*., 1994; Radostits *et al*., 2000). *Streptococcus uberis* is found in the mouth, vulva, teats and faeces of the cows as well as the environment. It is probably the common cause of environmental mastitis with less severe clinical signs than *Escherichia coli*. *Escherichia coli* can cause severe even fatal mastitis moreover *E. coli* enormously present in the faeces and

passes out to contaminate the environment and can multiply to greater concentrations away from the cow. Some species like *Pseudomonas*, *Klebsialla* and yeasts are pathogens that are considered as causal agents to environmental mastitis. *Actinomyces pyogenes*, *Streptococcus dysgalactiae* and *Peptococcus indolicus* are bacterial agents that are involved in summer mastitis, which seen especially in pregnant cows and heifers although it can also occur in non-pregnant animals (Blowey, 1990; Radostits *et al.*, 1994; Radostits *et al.*, 2000).

The mammary gland will face few odd infections, which may not fit into the standard disease pattern. Infections due to these organisms are not particularly common and if one has a difficult mastitis problem in his herd it is more likely that environmental organisms or cow –to-cow transmission is involved. *Mycoplasma* species, *Corynebacterium bovis*, *Staphylococcus epidermidis* and Micrococcus are some of the uncommon causes of mastitis (Blowey, 1990).

The emphasis of clinical mastitis treatment has been on antimicrobial therapy and currently there are a number of conventional antibiotics with different degree of spectrums that are used for the treatment of the disease. An important aspect of mastitis therapy is the alleviation of inflammation that can result swelling and subsequent pain associated with clinical mastitis that can cause considerable discomfort to the cow in the udder. Then the purpose of mastitis therapy is to assist the affected quarter to clear infection as rapidly as possible and to enable a quick return of the cow to normal milk production (Fitzpatrick *et al.*, 1998). The conventional antimicrobial agents used in mastitis treatment include penicillin, cloxacillin, erythromycine, cephalosporins, gentamycin, amikacin, trimethoprim-sulfa, ticarcillin,-clavulanic acid, polymyxin B, cephalotin, tetracycline, ampicillin, neomycin, kanamycin, nystatin, miconazole and other drugs with systemic injectable and local intramammary infusion formulations (Rebhun, 1995). Antibiotics have a potential high cure rate when the treatment is well targeted. Antibiotic treatment should be encouraged in the treatment of mastitis caused by *Str. agalactiae* as this pathogen is zoonotic and is easily eradicated from the herd (Sandholm, 1995). Anyhow, use of antibiotics has cost problems and disadvantages in that they impose potential residues in milk, the possibility of resistance development and disruption of symbiotic gut flora of the host when systemic administration is used (Paape *et al.*, 1990; Sandholm and Pyorala, 1995).

The aforementioned disadvantages that were imposed by conventional antibiotics therapy could also lead to the use of an alternative therapy. A multitude of mastitis therapy, which includes the use of frequent striping, herbal udder ointment and oral preparations, massages and diet changes have been used before and after the advent of antibiotic therapy (Fitzpatrick *et al.*, 1998). Due to absence of modern animal health services particularly in rural areas livestock owners frequently visit traditional healers to get solutions for their ill-health animals including mastitis problem (Sahle, 2002; Markos, 2003).

From this point of view plants/herbs that are documented to have effects on the treatment of mastitis and other diseases will have to be tested against a range of existing conventional medicaments and validated. Activities in this area have to be encouraged to develop alternate phytomedicines to avail drug choices in terms of cost and efficacy, to solve problems of emergence of drug resistance. This will support the struggle against poverty, alleviation and initiates further study in the field of ethnoveterinary medicine leading to the exploitation of traditional knowledge.

In China, herbal medicine plays an equally important role along with the use of synthetic drugs and antibiotics and traditional medicine is an alternative to western medicines (Sheng, 1987) and good proportion of modern drugs have been developed from plants used in traditional medicine (Green, 1986).

Many African countries have now begun to recognize the use of herbal remedies as important partners to the conventional healthcare system (MacCorkl and Mathias, 1992). In Ethiopia, Madagascar, and Tanzania practical steps are already being taken to ascertain efficacy and determine optimum dosages for several herbal drugs. Africa is still abundantly rich in herbal medicine practices and as a matter of fact herbal medical care continues to remain the only type of health care available to nearly 80% of the people and animals in Ethiopia while the remaining 20% swing between the modern and the traditional system of healthcare (Abebe, 1987).

In Ethiopia, traditional healers use a number of plants/herbs for the treatment of bovine mastitis and the efficacy of some of these plants/herbs have been tested against a range of causative agents of mastitis. In *vitro* study conducted by Sahle (2002), indicates that *Persicaria senegalensis, Cyphostemma adenocaule* and *Cucumis ficifolius* have shown some degree of

growth inhibitory effects. Markos (2003) screened some herbal preparations against mastitis causing pathogens and observed encouraging results.

The conventional drugs used for the treatment of mastitis are of limited types, especially in Ethiopia, and due to this and other factors causal agents have showed variable degree of resistance. Bacteria like, *Staphylococcus aureus, Streptococcus* species, and *Pseudomonas aeruginosa* developed resistance to certain antibiotics experimentally and practical applications which is confirmed by in *vitro* sensitivity testing (Kerro, 1997). Besides availability, drug cost is another problem, especially in rural areas where high proportion of livestock is reared.

Finding an alternate effective therapy will complement modern animal health services giving efficient animal health delivery system throughout the country especially in the rural areas where modern animal health care is out of the reach due to scarce resources, low level of literacy and poor animal health infrastructures.

OBJECTIVES:

- To determine the extent of clinical mastitis in the two largest dairy farms (EARO and Fair field) in Debre-Zeit and to establish the causal agents of the clinical mastitis.
- To assess in *vitro* antimicrobial effect of six phytopreparations; namely, *Brucea antidysenterica, Combertum molle, Cyphostemma adenocaule Persicaria senegalensis, Plantago lanceolata* and *Zehneria scabra* on *Staphylococcus aureus* and *Streptococcus agalactiae* isolated from bovine clinical mastitis
- To compare the efficacy of 80% methanol crude extract preparations of the plants with commonly used conventional antibiotics for the treatment of bovine mastitis
- To assess the effect of these phytopreparations on resistant strains that are isolated from bovine clinical mastitis

2. LITERATURE REVIEW

2.1. The bovine udder

The mammary glands are transformed dermal glands, in which the secreting tissue is located between the skin and the abdominal wall in a capsule formed by connective tissue. The udder becomes infected when the bacteria penetrates the teat canal and multiply in there (Sandholm *et al.*, 1995). Infection of the mammary gland is almost always via the teat canal. In the cows this often occurs when the teat sphincter is slack, for a period of 20 minutes to 2 hours after milking (Quinn *et al.*, 1999).

2.1.1. Natural resistance mechanism of the mammary gland to microbial infection

Upon introduction of any pathogen into the mammary gland the udder will respond or react to that particular agent in order to defend against the incoming invader and this is manifested as an inflammation.

2.1.1.1. Physical defence mechanisms

The streak canal (teat canal) having a keratin lining provides the most important physical deterrent to the entry of pathogens (Sandholm *et al.*, 1995). Keratin also inhibits pathogens through chemical defense system composed of antimicrobial lipids and proteins Bacteria attached to the keratin in the teat canal may be sealed in this location by tight closure of the sphincter muscle or extruded during milking as keratin desquamates. The udder is very susceptible to new infections during the early dry periods before the teat canal has formed a thick keratin plug (Rebhun, 1995).

2.1.1.2. Cellular defence mechanisms

Macrophages, neutrophils, sloughed alveolar epithelial cells and small fractions of leucocytes compose the majority of somatic cells in the milk. Macrophages may be the most population in non-inflammed glands but neutrophils predominate (90% or more) in inflammed glands (Howard, 1993).

Neutrophils have a relative impairment in milk as compared to blood and a large number of neutrophils are necessary during infections. This is thought to be due to lack of opsonin, energy source and interference by casein and fat (Rebhun, 1995; Howard, 1993).

2.1.1.3. Secretory antibodies

Immunoglobulin G transferred to milk from serum is the major antibody fraction in milk, where as Immunoglobulin A and Immunoglobulin M may be locally synthesized and transferred through the mammary epithelium (Rebhun, 1995).

2.1.1.4. Lactoferrin

Lactoferin is a whey protein, which binds iron in the presence of bicarbonate and therefore makes iron less available for the bacteria requiring iron for their growth. Coliforms and most Staphylococci require iron, where as Streptococci needs very little. Lactoferrin increase greatly in the well involuted dry cow mammary gland and bicarbonate also increases (Rebhun, 1995). As parturition approaches and colostrums is secreted in the udder, lactoferrin is reduced and citrates which compete for iron with lactoferrin are increased (Howard, 1993).

2.1.1.5. Lysozyme and lactoperoxidase

Are other soluble components of the defence mechanism of the mammary gland. Lactoperoxidase produced by mammary epithelial cells may oxidize thiocyanate to hypothiocyanate, which is lethal to some bacteria through cell membrane damage (Rebhun, 1995; Howard, 1993).

2.2. Bovine mastitis

Mastitis is an inflammation of the mammary gland caused by microorganisms, usually bacteria that invade the udder, multiply and produce toxins that are harmful to the gland (Crist *et al.*, 1997). The highest incidence of new intramammary infection in cow occurs during the first week after dry off and it has been estimated that 42% of all new intramammary infections become established during the dry period, although the highest level of exposure occurs during milking. However, many of these infections only become potent in the first 9 weeks of lactation (Howard, 1993).

Clinical classification of mammary gland inflammation differs in location and it can be explained as thelitis (affecting all layers of the gland), cisternitis (when only mucous membrane is involved) galactophoritis (inflammation of the lactiferous ducts) and mastitis (inflammation of the glandular tissue) (Rosenberger, 1979). Table 1 summarizes principal forms of infectious mastitis including their probable causes.

Table 1: Characteristic of the principal forms of infectious mastitis in the cow

Mastitis	Course	Time of occurrence	Probable agents
Catarrhal	Usually chronic Rarely acute	At early stage of lactation & During the dry periods	Micrococcus rarely Coliforms, *A. pyogenes*
Phlegmonous (phlegmnosa)	Peracute or Acute	Early puerperal period and Winter housing	Mainly Coliforms,
Purulent (apostemantosa)	Chronic with acute episodes	Dry grazing cows in summer after udder injury in winter	*A. pyogenes*
Mycotic	Acute to chronic	Usually after intrammamry treatment	Mould, fungi, yeast
Gangrenous	Peracute	At calving or early puerperium	Coliforms, *S. aureus*

Source: (Rosenberger, 1979)

The major immunologic defences of the udder during the dry period involve the teat canal, neutrophils, macrophages, lactiferrins and the lactoperoxidase-thiocyanate systems. To some extent, all of these defences become compromised during the dry period, making the udder more susceptible to new intramammary infections (Howard, 1993). Mastitis could be clinical or subclinacl. Clinical mastitis is characterized by a wide range of findings including, abnormal texture and milk discoloration, flakes or clots in the milk, swelling, increased temperature and pain of the gland. On the bases of clinical manifestations, mastitis could be mild, peracute, acute or chronic (Radostits *et al.*, 1994; Crist *et al.*, 1997).

In the case of subclinical mastitis there is no visible signs of the disease, but somatic cell count will be elevated and bacteria will be detected in the milk sample culture. Subclinical mastitis causes the greatest financial loss to dairy farms through lowered milk production (Crist *et al.*, 1997).

2.2.1. Pathogenesis

Mastitis most commonly begins as a result of penetration by pathogenic microorganisms through the teat canal and establishment into the interior of the mammary gland and it can be explained using three stages, namely, invasion, infection and then inflammation (Radostits *et al.*, 2000). If the interior environment is conducive for the growth and multiplication of invading microorganisms their metabolites irritate the delicate tissues causing an inflammatory response. The characteristic clinical signs developed are an expression of defence with the aim to destroy and eliminate the potential invader and then make way for repair to return the gland to normal. Severity of mastitis is determined to a considerable extent by the nature of the infectious bacteria, natural degree of cow's resistance, and to some extent by stress placed on the mammary glands by milking practices and environmental factors (Schalm *et al.*, 1971).

2.2.2. Aetiology

More than 137 species of microorganisms have been isolated from mastitis. However, the most frequently isolated species are shown in Table 2. Other species like *Mycobacterium*, *Prototecha* species (*trispora* and *zopfiii*), yeasts, and *Candida albicans* could be isolated from mastitic cases (Rebhun, 1995; Radostits *et al.*, 2000).

Causal agents of mastitis could also be classified in to two based on sources of infections, namely, 1. Contagious caused by, *Streptococcus agalactiae* and *Staphylococcus aureus* 2. Environmental mastitis caused by Coliforms- *Eschrichia coli*, *Klebsiella pneumoniae*, *Klebsiella oxytoca* and *Enterobacter aerogenes* and Environmental *Streptococci- Streptococcus uberis*, and *Streptococcus bovis* and *Enterococcus faecium* and *Enterococcus faecalis* (Crist *et al.*, 1997).

Table 2: The most common causes of animals.

Bacteria	Species of animals						
	Cow & goats	Sheep	Mare	Rabbit	Bitch & Queen	Sow	Camel
Coagulase Neg. *Staphylococcus*	+	+					+
Staphylococcus aureus	+	+				+	+
Streptococcus agalactiae	+	+					+
Streptococcus dysgalactiae	+	+					+
Streptococcus uberis	+	+					
Other lancifield Str. Groups	+	+					
Coliforms- *E.coli*	+	+	*Strept.* and *Staphy. Species*	*Strept.* and *Staphy. Species* and others	*Strept.* & *Staphy. Species*	+	+
Enterobacter aerogenes	+					+	
Klebsella species	+					+	
Pseudomonas aeruginosa	+	+				*Pseud.*spec	
Actinomyces pyogenes	+	+				+	Species
Anaerobic micrococcus (Peptococcus asccharolyticus)	+						+
Mycobacterium species	+						
Nocaradia asteriodes	+						
Mannheimia hemolyitica	+	+					+
Actinobacillus lignieressi	+					+	
Actinomyces bovis	+					+	
Spherophorus necrophorus						+	
Bacillus species	+						+

2.2.2.1. Contagious mastitis

Streptococcus agalactiae

Streptococcus agalactiae is the classic example of contagious mastitis, because it is highly contagious and an obligate inhabitant of the mammary gland. The agent can survive for 2-3 weeks away from the cow but multiplication occurs only in the udder (Blowey, 1990). The bacterium does not invade the glandular tissue (Quinn *et al.*, 1999) and hence doesn't cause fibrosis and abscess. Streptococcal mastitis is largely subclinical with occasional acute flare-ups. It will permanently decrease productivity in the affected gland in chronic infections (Rebhun, 1995).

Streptococcus dysgalactiae

Streptococcus dysgalactiae found in the udder and teat lesions (Blowey, 1990) and tends to have a lower prevalence than *Streptococcus agalactiae* and may become overtly clinical (Rebhun, 1995).

Staphylococcus aureus

Staphylococcus aureus is found in the udders of chronically infected cows and also in cuts and chaps on the teat skin (Blowey, 1990). About 10% of the cows may have clinical mastitis but another 50% can have subclinical mastitis and act as a source of infection for further clinical cases (Quinn *et al.*, 1999). It is not an obligate inhabitant of the mammary gland and is the worst of the contagious bacterial organisms causing chronic deep infection of the mammary gland causing fibrosis and abscess, which is extremely difficult to cure. It is very difficult to cure the infections once it is established and chronic infections are resistance to antibiotics (Rebhun, 1995). Many cases are characterized by slowly developing induration, atrophy with the occasional appearance of clots in the milk or wateriness of the first streams (Radostits *et al.*, 1994). The type of mastitis ranges from subclinical to the peracute life threatening form, one of which is gangrenous mastitis caused by the action of Alpha toxin that damage the blood vessels resulting in ischemic coagulative necrosis of the adjacent tissue (Quinn *et al.*, 1999). The source of infection is secretion from the infected quarter (Rebhune, 1995).

Mycoplasma species

Cows of all ages and all stages of lactation can be affected by mycoplasmal mastitis; however, those that have recently calved show the most severe signs. These can be due to the long-term persistence of the organisms in the udders (up to 13 months) and some cows may become shedders of mycoplasmas without severe clinical signs (Qiunn *et al.*, 1999).

Mycoplasma bovis is the most common cause and *Mycoplasma californicum* and other species have been isolated from the milk. *Mycoplasmal* species cause herd endemics of acute mastitis that subsequently evolve into chronic mastitis. Following acute attack cows may show chronic

mastitis, intermittent acute flare-ups or have subclinical infection requiring culture confirmation. (Rebhun, 1995).

2.2.2.2. Environmental mastitis

Streptococcus uberis and non-agalactiae *Streptococcus* species

Streptococcus uberis may be associated with an acute mastitis, which later becomes chronic. Its course is more transient with fewer tendencies to permanent infections. The organism also has been found in the udder of the cows not showing any obvious evidence of infection and less than 10% of the cases of *Streptococci* mastitis are found to be caused by this organism (Merchant and Packer, 1983).

Streptococcus uberis is ubiquitous throughout the farm environment because of faecal contamination by cows harbouring the organism in the rumen. Most infection occurs in early lactation or late dry period (Rebhun, 1995).

Coagulase negative Staphylococcus

Coagulase negative *Staphylococcus* is normal flora of the skin including the skin of the teat and external orifice of the streak canal. These organisms cause subclinical mastitis resulting in decreased milk production and elevated somatic cell count. Mastitis due to this group of organisms is common and infection may occur during lactation or late in the dry periods (Rebhun, 1995).

Actinomyces pyogenes

Actinomyces pyogenes causes dry cow or summer mastitis and infection is extremely purulent. The incidence of infection for the dry cow is increased by filthy, wet or muddy environment. Most of the infection begins after the udder has been dry for 2 weeks or more and muddy or wet, dirty environment usually are present. Epidemics are also possible with up to 25% of the dry cows being affected. The degree of damage to the affected glands based on future productivity was not reported (Rebhun, 1995).

Coliforms

Lactose fermenting gram-negative rods, such as *E.coli, Klebsiella* species *and Enterobacter* species are the causative agents of coliform mastitis. Which are classic examples of environmental causes of mastitis. Summer heat and humidity contributes to multiplication and persistence of coliforms in the environment. Cows in herds with low somatic cell counts had the highest incidence of clinical mastitis within 30 days of lactation (Rebhun, 1995). Inflammatory reactions destroy a large proportion of gram negative bacterial populations and their lysis results in release of endotoxin that causes a severe, life threatening toxemia. A unique feature of coliform infections is that, in the cows that recover, the udder tissue gradually returns to normal without fibrosis and in its subsequent lactations the gland produces to its optimal capacity (Quinn *et al.*, 1999). Dry cows are at greater risk of infection just after drying off and just before calving. These organisms in the gland release lipopolysaccharide endotoxin through destruction or rapid multiplications of the organisms, which creates local and systemic signs associated with coliform mastitis. Chronic infections following were once thought to be rare but now have been routinely confirmed in at least 10% of infected quarters. Chronically infected quarters may be non-productive or may cause subclinical mastitis with intermittent flare-ups that mimic cases of acute mastitis (Rebhun, 1995).

Other organisms

Pseudomonas species and *Serratia* species

Occasional causal agents of mastitis and infection may be epidemic, sporadic or endemic within the herd. *Pseudomonas* causes an acute mastitis, which may be necrotizing. Following initial infection, the quarter may remain hard and agalactic or may improve somewhat but remain clinical with clots or pus in the secretion (Rebhun, 1995). Infection by *Pseudomonas aeruginosa* can have a pathogenesis similar to coliform mastitis and a severe endotoxmeia can occur. The infection may result in a subclinical mastitis with the pathogen persisting in the mammary gland (Quinn *et al.*, 1999). *Serratia liquefaciens or Serratia marcescens* cause a chronic subclincal or clinical mastitis that has no unique sign (Rebhun, 1995).

Yeasts

Yeast mastitis usually caused by *Candida* species is almost always secondary to acute bacterial mastitis especially because of contaminated infusion canals, syringes or multi-dose mammary infusion solutions. There is persistent swelling of the gland with abnormal secretions. Yeasts can grow well in the presence of some antibiotics; therefore, continued use of antibiotics or combinations of antibiotics to cure "resistant" infections only supports a yeast infection and corticosteroids worsen the condition. They don't invade tissue but resides on the mucosal lining and cause local inflammation (Rebhun, 1995).

Fungus

Aspergillus species are opportunistic fungi and rarely gain access to the udder through the same mechanism as yeasts. *Cryptococcus neoformans* has been reported to cause mastitis and present a public health hazard if contaminated raw milk is consumed (Rebhun, 1995).

Algal mastitis (*Prototheca* species)

Prototheca zopfii is most common but *Prototheca wickerhami* and *Prototheca trispora* also have been identified from infected glands. These organisms are widely spread in the environment and are found routinely in mud, faeces, water, stagnant ponds and other locations. Public health concerns exist to some degree as human cutaneous or systemic; usually in immunocompromised subjects, infections have been reported (Rebhun, 1995).

2.2.3. Prevalence of mastitis in Ethiopia

In Ethiopia, there have been reports that both clinical and subclinical mastitis have affected a high proportion of dairy cows in central high lands (Kassa *et al.*, 2000). Mungube (2001) reported an overall prevalence of subclinical mastitis in urban and peri-urban areas of Addis Ababa at cow level 46.6% and at quarter level 27.8%. In his study the urban production system had a higher prevalence of subclinical mastitis. According to the production system the prevalence was 60% and 46.9% at cow level in urban and peri-urban areas, respectively.

Besides, studies in Ethiopia indicated the prevalence of subclinical and clinical mastitis in different parts of the country. Yigezu (1990) indicated a mastitis prevalence of 44.6% in six different dairy sites, and 3.8% clinical and 40.8% of subclinical mastitis, respectively in the southern region of Ethiopia. Kerro (1997) in selected areas of southern Ethiopia reported a prevalence of mastitis to be 40.39% of which 41.93% clinical and 58.06% subclinical infection. Dagne and Abdicho (2001) reported a subclinical mastitis prevalence rate of 34.6%, 45.15% and 38.7% in Repi, Debre Zeit and Modjo, respectively.

2.2.4. Incidence and severity of mastitis

In the UK during the recent 12-month monitoring period the mean incidence was 41.6 cases per 100 cows per year with a value ranging from 13-75 new cases (Bradley and Green, 2001). Study of incidence of clinical mastitis in Debre Zeit ILCA research center (the current ILRI) by Argaw (1992), indicated that suckling cows were affected 42.50% once for the first time and 15.50% cows twice. The pathogenic agents isolated in his study were *Staphylococcus aureus* (35.64%), S. *epidermidis* (18.81%), *E. coli* (7.92%), *Klebsiella* species (4.95%), *Actinomyces pyogenes* (11.88%) and *Corynebacterium bovis* (3.96%).

The severity of mastitis could be categorized as peracute, acute, subacute and chronic. Bradley and Green (2001) defined the degree of severity on the basis of the presence of an abnormal foremilk secretion, and/or under changes with cardinal signs such as pain, heat and udder swelling and they were graded into three. These were: grade 1(cases having milk changes only, like, clots), grade 2 (milk and udder changes, like, swelling and heat) and grade 3 (systemic signs, like, depression and anorexia). As shown in Table 3, the study showed 67.4%, 26.4% and 6.2% of the cases were being grade I, grade II and grade III, respectively. In their study, among the aetiological agents *Escerichia coli* (the environmental one) was significantly more likely to result in systemic signs.

Table 3: The proportion of different grades of mastitis associated with each pathogen

Pathogen	Mastitis grade			Remark
	I	II	III	
All Enterobacteriacea	54.3	35.5	10.2	
Escerichia coli	48.7	41.0	10.3	
Streptococcus uberis	67.4	30.2	2.4	
Coagulase positive *Staphylococci*	83.3	16.7	0	
Streptococcus dysgalactiae	75.0	25.0	0	
Coagulase negative *Staphylococci*	90.9	9.1	0	
Corynebactrium species	77.8	22.2	0	
Arcanobacter pyogenes	25.0	25.0	50.0	
Other species	90.6	4.7	4.7	
Mixed growth	68.2	27.3	4.5	If two known mastitis pathogen isolated
Contaminated	100.0	0	0	If three or more organisms are isolated
All pathogens	67.4	26.4	6.2	
No growth	76.5	17.6	5.9	

Source:(Bradley and Green, 2001)

2.3. Significance of mastitis

2.3.1. Economic significance

Assessing a dollar value to losses due to mastitis is nearly impossible, although several attempts have been made. From the literature 140-200 USD/cow/year is lost due to mastitis with approximately 8% being due to discarded milk, 8% treatment costs, 14% to death and premature culling, and 70% to reduced milk production (Woods, 1986). Investigation showed that annual milk sales from herds with high levels of subclinical udder infection were 20% less than similar herds with low levels of sub-clinical mastitis (Francis, 1985).

Various reports indicate substantial production losses (5% or more) begin when the somatic cell count/SCC/ increases above 300,000. The higher the cell count, the greater the loss and with a count of around $1x\ 10^6$ creating a loss of approximately 20% or more (Woods, 1986). Subclinical mastitis is the greatest source of economic losses. On average about 40% of all cows in the

United States are infected in one or more quarters of the udder at any one time. The estimated cost in lost production is USD 200/cow/year (Howard, 1993). Annual losses of 28 million USD in Kentucky and 1.7 billion USD in United States of America have been recorded from an extrapolation of the result of a loss of 18,400 USD per 100-cow herd.

Study conducted in New York and Pennsylvania in 1997, indicated a financial milk loss per lactation as a result of specific pathogen based on 305-day mean estimate with a milk value of USD 13.00/cwt. Losses due to *Pasteurella* species USD 500.12, *Mycoplasm* species USD 451.63, *Streptococcus agalactiae* USD 388.19, *Arcanobacter pyogenes* USD 348.15 and *Staphylococcus aureus* USD 185.51were recorded (Wilson *et al.*, 1997).

Management and economics study of dairy cow mastitis in the urban and peri-urban areas of Addis Ababa indicated a total loss from mastitis 73241.24 Birr in a single lactation for 363 cows (Mungube, 2001). Annual loss caused by mastitis in the central highlands of Ethiopia is summarized in Table 4.

Table 4: Estimated annual losses due to mastitis in central highland of Ethiopia

Source of loss	Loss in Birr	%
Milk production loss	29,356.17	25.4
Treatment cost	7122.60	6.2
Withdrawal loss	2534.40	2.2
Loss due to culling	76513.17	66.2
Total	**115,526.34**	**100.0**

Source: (Mungube, 2001)

2.3.2. Public health importance

Milk from mastitic cows may contain harmful pathogenic microorganisms to human beings. Bad milk would be responsible for more sickness and deaths (Howard, 1993). Although, pasteurisation has eliminated the gross public health significance of milk, there are still enough consumers of raw milk to mention the various mastitis or milk related factors affecting human health. In recent years a human group B streptococcus, not dissimilar to *Streptococcus agalactiae*

has been reported as a causes of meningitis and death in newborn infants and also urinogenital tract infection in adults (Woods, 1986). There has also been reported of individuals taken ill after consuming milk products high in toxins produced by *Staphylococcus aureus* that pasteurisation did not eliminate. Besides *Escherdichia coli* can cause enteritis, diarrhoea and vomiting (Woods, 1986). Diseases like Tuberculosis, Brucellosis, Listeriosis, and Q-fever may be transmitted through milk to human beings (Hugh-Jones *et al.*, 1995) and *Cryptococccus neoformans* and *prototheca* species also have zoonotic importance (Rebhun, 1995).

2.4. Treatment of mastitis

2.4.1. Treatment using conventional drugs

Since mastitis results in the destruction and disturbances of the mammary gland and affects milk production and productivity, it needs serious and immediate action as soon as possible. Among the many actions that could be taken as treatment, the administrastion of antimicrobial agents is the most commonly used method. Pathogenic microorganims are sensitive to one or more antimicrobial agents and at the same time are resistant to one or a number of conventional drugs (Delaat, 1975). Mastitis could be grouped, according to how the various infections respond to antibiotic therapy, into three groups.

Group I- Organisms that respond well to treatment (*Streptococcus agalactiae*)

Group II- Organisms that have variable responses (other *Streptococci*, *Staphylococci* and all Gram negatives.

Group III- Organism that are refractory to treatment (*Mycoplasma*, *Prototheca*, *Nocardia* and *Pasteurella*) (Woods, 1986).

Treatment of a cow acutely sick from mastitis must be directed towards saving the cow's life. All clinical cases should be treated as they occur, otherwise a permanent loss could commence. Before any attempt made to treat mastitis, selection of the most likely effective antibiotic for the treatment is essential. Antibiotics are selected according to the identified pathogen and sensitivity of the organism cultured from a milk sample. Sensitivity testing has advantages over blind treatment, in that, it helps to cure animals within short period of time and return to production, reduce further disease spread and serving as a source of infection, avoids the risk of bacteria developing resistance and is more of economical.

Treatment of sick animals without sensitivity testing and indiscriminate drug usage by many non-professionals leads to the development of drugs resistance. Conventional antibiotics like, penicillin, cloxacillin, erythromycin, and cephalosporins have excellent successes against mastitis caused by *Streptococcus agalactiae* and *Str.dysgalactiae*. Before treating *Staphylococcus aureus* cases susceptibility testing is recommended. Systemic treatment with penicillin, ceftiofur or pirlimycin result greater cure when combined with local intramammary infusion containing cloxacillin and cephalosporin. Drugs like gentamycin, amikacin, trimethoprim-sulfa, and ticarcillin-clavulanic acid work against most coliforms, polymyxin B and cephalotin and tetracycline, ampicillin, neomycine and kanamycin work against 60-80% and 40-60% in *vitro*, respectively (Rebhun, 1995).

Treatment of *Pseudomonas* species with conventional antibiotics is rarely successful, while *Serratia* species treatment can be done based on culture and sensitivity. Yeast infections can be cured spontaneously if all antibiotic therapy is stopped and the affected quarters are milked out four or more times per day. Miconazole, nystatin, and iodine have been used for the treatment of mastitis caused by yeasts. Antifungal drugs like miconazole, clotrimazole and ketaconazole can be used for the treatment of fungal mastitis. Alagal species have no successful treatments (Rebhun, 1995).

Hence, treatment of mastitis effectively with conventional antibiotics may not be efficient enough because of the possibility of resistance problems and lack of effective animal health services in Ethiopia. Most livestock owners are incapable to pay the fees for existing medicaments. Many of them do not have access to animal health posts. According to Mungube (2001) for a one-time treatment of a case of mastitis 74.10 Birr per cow was required, which is not affordable by many farmers. As a result of the aforementioned points and other factors livestock owners' use as an option or depend on herbal medical preparations that are available in their vicinity for the treatment of different ailments

Ethnoveterinary medicinal practices are deep rooted in Ethiopia like other countries in the world. The people of Kenya have traditionally relied on a whole range of indigenous practices to keep their livestock healthy and to treat them when they are sick (ITDG and IIRR, 1996).

Traditional healthcare (ethnoveterinrary medicine) include the use of medicinal plants/herbs, surgical techniques and management practices to prevent and treat a range of diseases and problems encountered by livestock holders. Research has shown that many of the plants used to prepare indigenous medicines do in fact contain valuable active ingredients, though much research remains to be done in this area (ITDG and IIRR, 1996; MacCorckle and Mathias, 1996). In India where 60% of the population lives below poverty line, herbal medicine is the only hope. The World Health Organization has recognized its full value and is helping in a big way. Traditional medicine weather in Africa or Asia has a bright future and an immense potential to extend medical relief to millions who for lack of resources remained deprived of it (Sahab, 1990).

2.4.2. Drug resistance

Due to one or other reasons bacterial agents that cause mastitis develop resistance of variable degree to different antibiotics. The emergence of bacteria resistance to antimicrobial agents within animal population or during therapy is a matter of great concern (Fraster, 1986). Drug resistance isolated from domestic animals is important in limiting the use of antimicrobial agents in animals and potentially in humans (Prescott and Baggot, 1988). Among the main pathogenic organisms causing mastitis, some *Streptococcus* species and *S. aureus* develop resistance to antibiotics like penicillin, streptomycin and oxytetracyclines (Ak, 2000). Some of the bacterial agents isolated from a case of mastitis that develop resistance for in *vitro* trial in different places are summarized in Table5.

Table 5: Some of bacterial isolates that develop resistance to some antibiotics

Bacteria	Type of drug	% of resistance
Streptococcus dysgalactiae	penicillin, oxacillin, chloramphenicol	3.7
Stre. dysgalactiae	erythromycin and oxyteteracycline	7.4
Streptococcus uberis	penicillin, oxacillin, oxyteteracycline	2.6
Beta haemolytic streptococcus	oxyteteracyclin	1.9
Beta haemolytic streptococcus	penicillin, oxacillin, chloramphenicol, erythromycin	3.7
Staphylococcus aureus	penicillin	31.3
Eschrichia coli	oxytetracycline	26.1
Pseudomonas aeruginosa	oxytetracyclin	30.4
Corynebacterium isolates	penicillin, erythromycin	15
Corynebacterium isolates	chloramphenicol	20
Staphylococcus aureus	penicillin	83
Staphylococcus aureus	streptomycin	60

Source: (Ak, 2000; Heras *et al.*, 1999; Mallikarjunaswmy *et al.*, 1997; Woods, 1986; Kerro, 1997).

2.4.3. Herbs used for the treatment of bovine mastitis

2.4.3.1. Plants/herbs used for the treatment of bovine mastitis

In Ethiopia, ethnoveterinary medicine has a long history and reached to this generation verbally, though documentation may exist in churches and monasteries. Since the life of farmers is linked to their livestock, they have developed their own way of keeping animal health. They treat animals whenever they get sick or health is compromised and prevent when there is any disease problem circulating in and around their locality.

Sahle (2002) conducted a study on medicinal plants that are used for the treatment of bovine mastitis in selected central high lands of Ethiopia and identified about 21 different species of plants, namely, *Achyranthus aspera, Ajuga integrifolia, carissa edulis, Clerodendron myricoides, Commicarpus podunculosis, Cucumis ficifolis, Croton macrostachys, Cyphostemma adenocaule, Dutra stramonium, Eucalyptus globules, Haginea abyssinica, Lageneria siseria, Lapidium sativum, Launaea intybacea, Nicotina tobacum, Olea africana, Ocimum urticifolium, Persicaria senegalensis, Phytolaca dodecandra, Rumex abyssinicus* and *Witania somnifera* used in different forms of preparations and parts of the plant were used.

Ethnoveterinary practice of the Borana rangeland pastoral system indicated that *Acacia busei* (bark, burnt, powdered and mixed with butter to make paste, topical), *Carissa edu*lis (leaf/root, paste, topical), *Rossa abyssinica* (root, paste/poultice, topical) and *Sasbania sasban* (root/bark/leaf, decoction, topical) have been used for the treatment of mastitis (Sory, 1999). Similarly, Chekol (2002) in his survey on ethnoveterinary knowledge and practices showed that farmers in North Gondar reported the use of *Thalicrum rynchocarpum* (root, juice) for the treatment of bovine mastitis.

In Kenya, a handful of *Sasbania sesban* leaves mixed with 125gm of cream or butter for 5 minutes, and rub the mixture onto the affected area until the swelling disappears for the treatment of mastitis. This practice is similar with the practice of the Borana pastoralists (Hyato, 2003). A handful of *Ajuga remota* leaves and stems were chewed and 2 mouthfuls of the juice and saliva directly spited onto the swollen udder once a day for 7 days for treatment of bovine mastitis (ITDG and IIRR, 1996).

2.4.3.2. Scientifically validated phytoremedies for bovine mastitis

In India, cows with subclinical mastitis were treated by intramammary application of Tilox (Ampicillin and Cloxacillin) and other groups by topical application of root of *Withania somnifera, Asparagus racemosus*, and *Curcuma ameda* and leaves of *Ocimum sanctum*. Both preparations were effective as assessed by a return to normal biochemical milk profile, but the plant preparations acted more slowly (Kolte *et al*., 1999).

Another study indicated that herbal mix prepared from *Glycerriza glabra* (5gm), *Curcuma longa* 2gm), *Cadrus deodara* (10gm), *Paederia foetida* (5gm), and sulfur (10gm) in gel base (named as *"Mastilep")*, topical and intramammary infusion was given to one group and the other group was treated with intramammary infusion and the recovery rate in combined treated groups was 40%, while intramammary infusion only was 30%. It was concluded that topical administration of "Mastilep" gel is useful supportive therapy in treatment of bovine clinical mastitis (Rahaman and Sharma, 2000).

In China injection of medicinal herb preparations containing the Honey suckle flower, *Chrysanthemum indicum, Voila yedoensis* and *Citrus reticulata* was effective for preventing clinical mastitis during the dry period (Jaing *et al*., 1994).

Sahle (2002) carried out test on *Persicaria senegalensis, Cyphostemma adenocaule* and *Cucumis ficifolius* against their effects on most pathogenic bacterial causative agents of mastitis. *E.coli, A. pyogenes, K. pneumoniaae, S. aureus, Str. agalactiae* and *Str. dysagalactiae* were sensitive to *Persicaria senegalensis* at different concentrations, and has shown a very good inhibitory effect in all concentrations against *S. aureus, Str. agalactiae* and *Str. dysagalactiae*. The in *vitro* test of *Persicaria senegalensis* showed that isolates of *S. aureus, Candida albicans and Corynebacterium bovis* from subclinical cases and isolates of *P. aeruginosa* from clinical cases of mastitis were all inhibited. In vivo trial of 0.77 kg of leaf powder (equivalent to 3 kg of wet leaf) fed daily for 5 days resulted in an apparent cure rate of 92.8%, in contrast to 80% of in positive control group treated with an intramammary antibiotic preparations (Dagne and Abdicho, 1999).

3. METHODS AND MATERIALS

3.1. General Information

Study of incidence of bovine clinical mastitis and the experiment on bacterial isolates was carried out in Debre-Zeit. Debre Zeit is located at about 47kms (south east) from the capital city Addis Ababa. It has about 95,000 human populations. The altitude is 1850 meters above sea level. The soils and climates are similar to those of many highland areas in Africa. The main rainy season extends from the month of June to September with an average rainfall of 866mm. A short rainy season, from March to May, is not uncommon in this area. The annual average temperature ranges from 11^0C to 26^0C, peak on May, with an overall average of 18.7^0C. Day length is fairly constant throughout the year (12-13hrs) with about 6hrs of sunshine during the rainy season and 8-10hrs for the rest of the year. The humidity is about 50.9% (Mungube, 2001).

The livestock population of Adaa Liben District includes 89,057 oxen, 31,781 bulls, 73,145 cows, 40,629 heifers, 31,074 calves, 64,579 caprine, 39,126 ovine, 48,366 donkeys, 2,115 horses, and 2,025 mules.

3.2. Herbal materials used for the study

3.2.1. *Brucea antidysenterica* ("Kakero, Avalo, Waginos" in Amharic, "Dadatu, Tollu, Tamijja" in Oromiffa).

It is an evergreen, erect shrub or small tree belonging to the family Simaroubaceae and growing to 7 meters high. Leaves with ovate to oblong or elliptic leaflets, usually crowded at the end of branches. It is flowering all year round and flowers are pale and yellow in erect racemes from the leaf axils. The plant is found at an altitude from 1000-3700 meters in bush land, at forest edges or in re-growth of deforested areas. The roots and leaves were used to assess efficacy against bacteria isolated from mastitic cases.

3.2.2. *Combertum molle* ("Agalo, Abalo" in Amharic, "Bika, Dadamata" in Oromiffa)

This is a member of the family Combertaceae which is small deciduous tree growing up to 15 meters high with an often-crooked trunk, commonly branching to the base. The bark is dark brown to black and deeply grooved in squares. The leaves are oppositely arranged, elliptic to lanceolate, large that overed with soft hairs, rounded at the base. Flowers sweetly scanted, many crowded into greenish. The flowers generally appear before the leaves and the fruits yellowish, four-sided with wings. The leaves were used to assess the antimicrobial effect on bacteria isolated from mastitic cases.

3.2.3. *Cyphostemma adenocaule* ("Aserkush Tebetebkush" in Amharic)

It is a member of Vitaceae family, which is herbaceous climber or scrambler with stems growing to 6 meters long. Leaves 5-11 foliate leaflets elliptic or ovate with crenate or toothed margins having pale yellow flowers. It grows between 850-2650m.a.s.l. and is found on wooden grassland, reverine forest and clearings of forest. The root of the herb is used as a medicine traditionally. The roots were employed as a test material to assess antimicrobial effects.

3.2.4. *Persicaria senegalensis* ("Gosh" in Amharic)

Persicaria senegalensis belongs to the Polygonaceae family, which is a perennial erect or semi-decumbent herb with 4-5ft high or more. Leaves distinctly petiolate oblong lanceolate and flowers are light yellow. The fruit is light brown with deep brown seed. It is almost glabrous in riverbeds and swampy areas. Traditional herbalists feed the herb freshly to mastitic cows. The leaves were used to investigate the antimicrobial effect on bacterial isolates.

3.2.5. *Plantago lanceolata* ("Gorteb" in Amharic, "Korrisa" in Oromiffa)

Plantago lanceolata is a member of the family Plantaginaceae and is a cosmopolitan weed and herb with flowering stems growing to 40 centimeters high from a rosette of leaves. Leaves lanceolate, mostly erect, but sometimes also flat on the ground. Flower heads are cylindrical spikes with small green flowers and creamy white anthers and filaments. It grows along paths and

roadsides, in pasture and cultivated fields at altitudes between 1200-3200masl. This herb flowers throughout the year and profusely during the rains. The seeds and leaves were used separately to assess antimicrobial activities.

3.2.6. *Zehneria scabra* ("Hareg ressa" in Amharic)

Zehneria scabra is a climbing or trailing herb to 10 meters belonging to the family Cucritaceae, old stems becoming woody with corky-ridged bark. Leaf ovate, broadly or pentagonal. Flowers dioecious, seeds ovate or elliptic in outline. It is found with 100-3500 meters-altitude range in upland forests and woodland, wooded grassland, river and lake margins and also in secondary vegetation. The root part was used as a test material.

3.3. Study Design

Two dairy herds; namely, Debre Zeit EARO and Fairfield were selected for longitudinal investigation of clinical mastitis. Milk samples were collected from each clinical cases and isolates identified *S. aureus* and *Str. agalactiae* were tested with conventional antimicrobials and plant extracts to determine sensitivity profiles.

3.3.1. Investigation of clinical mastitis

It was determination of incidence of clinical bovine mastitis in 89 lactating cows over 6 months period in two dairy farms.

3.3.2. Study methodology

Milking cows were clinically examined every week for six months. All milking cows were checked for the presence of clinical mastitis. Milk sample was collected from clinically mastitic quarters. Clinical mastitis was defined as the presence of an abnormal foremilk secretion and/or udder changes, for example, pain, heat and swelling and/or involvement of systemic signs. Cases, which occurred 14 days after a previous episode in the same quarter was defined as a new quarter case (Miltenburg *et al.*, 1996). Then the severity of the disease was determined as described by Bradley and Green (2001) as Grade I-(milk changes only, for example, clots), Grade II- (milk and

udder changes, for example, swelling and heat) and Grade III- (systemic signs, for example, depression and pyrexia were involved).

3.3.3. Milk collection from the affected quarters

In the first place teats were wiped to remove gross contaminations by cotton or clean gauze. They were dipped and scrubbed with 70% ethanol and allowed to dry for at least 30 seconds. Then the teat ends were scrubbed with a cotton swab soaked in 70% ethanol before the milk sample is collected and allowed to dry. Fifteen-milliliter of milk sample was collected in a test tube held as nearly as possible horizontal. In order to avoid contamination disposable gloves were worn through out the sampling process. Samples were identified by farm, cows, quarter, and samples date and disease severity. The samples were kept in a container containing an ice pack to transport to the laboratory and kept at 4^0C until cultured (Bradley and Green, 2001).

3.3.4. Microbilological examination (Isolation and identification of mastitic agents)

From each sample 10 micro litres was inoculated with a loop on to 5% sheep blood agar, Edwards's agar, and onto MacConkey agar plates (Smith *et al.*, 1985; Bradley and Green 2001). Plates were incubated at 37^0C and reading was made after 24 and 48hrs. The organisms were identified by standard laboratory techniques (Carter, 1984; Quinn *et al.*, 1999). Gram negative bacteria or Coliforms were identified by colony morphology, oxidase and IMVIC test.

Biochemical and related tests. The laboratory diagnosis procedure followed is summarized in appendix 8.4.

Gram positive cocci were identified by catalase test. Those catalase positive cocci were inoculated onto Oxidation-fermentation medium and those fermentatives were considered as Staphylococci (coagulase positive and coagulase negative) and caoagulase test were carried out for fermentative cocci. *S.aureus*, *S.hyicus* and *S.intermidius* were considered as coagulase positive. Further identification of Staphylococci was made by carbohydrate tests. Catalase negative cocci were transformed to Edward's media for the detection of esculin hydrolysis and growth. CAMP test was conducted for esculin hydrolysis negative bacteria and CAMP test positives were considered as *Streptococcus agalactiae*, where as CAMP test negatives considered as other *Streptococci* and further identification was made by carbohydrate tests. Esculin hydrolysis positive cocci were transferred to MacConkey agar to detect growth and Bacteria

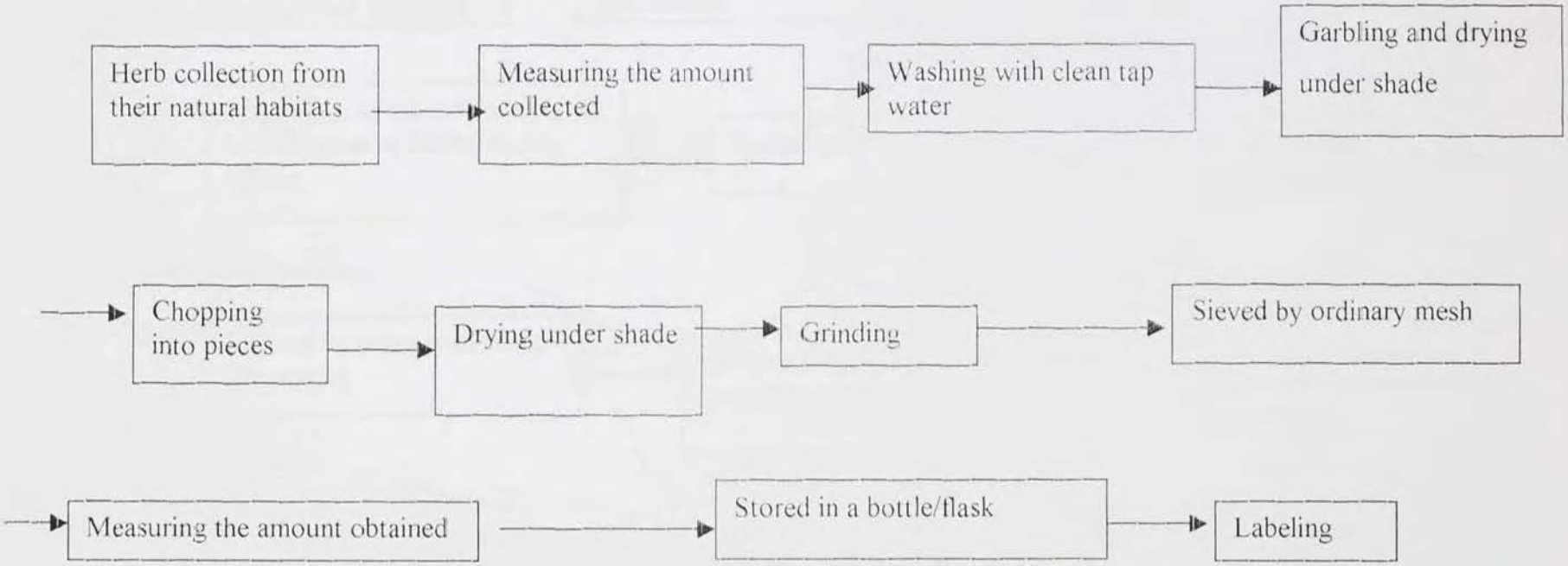
grown on MacConkey agar was considered as *Enterococcus fecalis* (Carter, 1984; National mastitis council, 1990; Quinn *et al.*, 1999). Those, which were not grown on MacConkey were considered as *Streptococcus uberis*. For *Corynebacterium* species colony morphology, catalase and carbohydrate tests were used. While *Bacilleus* species were identified by colony morphology, hemolytic patterns, catalase, motility and carbohydrate tests.

3.4. Herb selection and collection

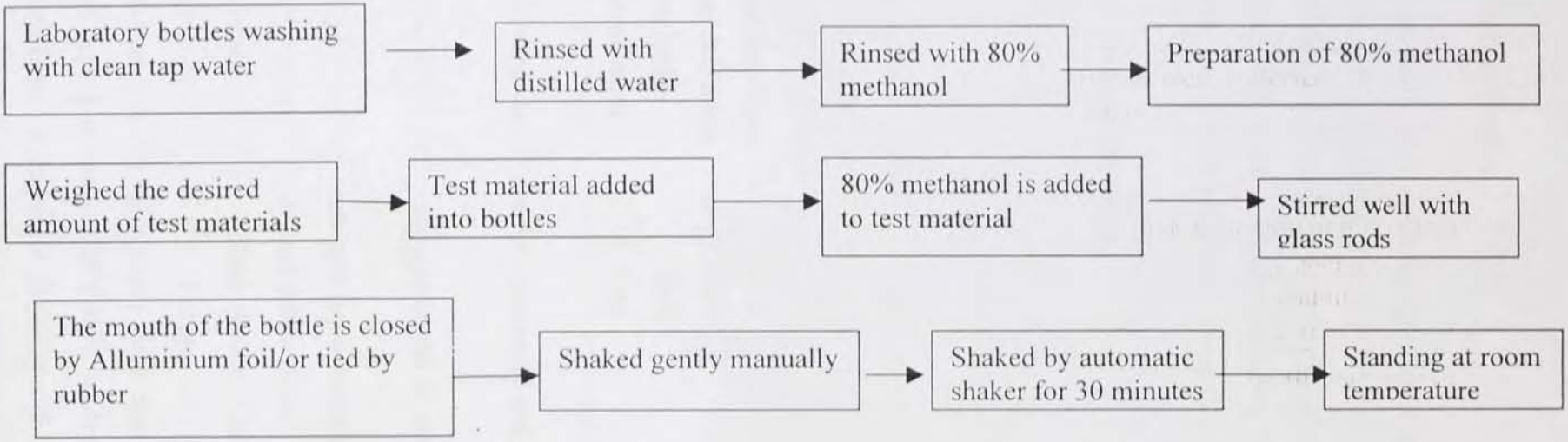
3.4.1. Herb selection

Study herbs were selected according the results they showed by previous workers and one plant (*Combertum molle)* was selected from the traditional healers knowledge and was tested deliberately. The flow chart from collection to extraction is shown below.

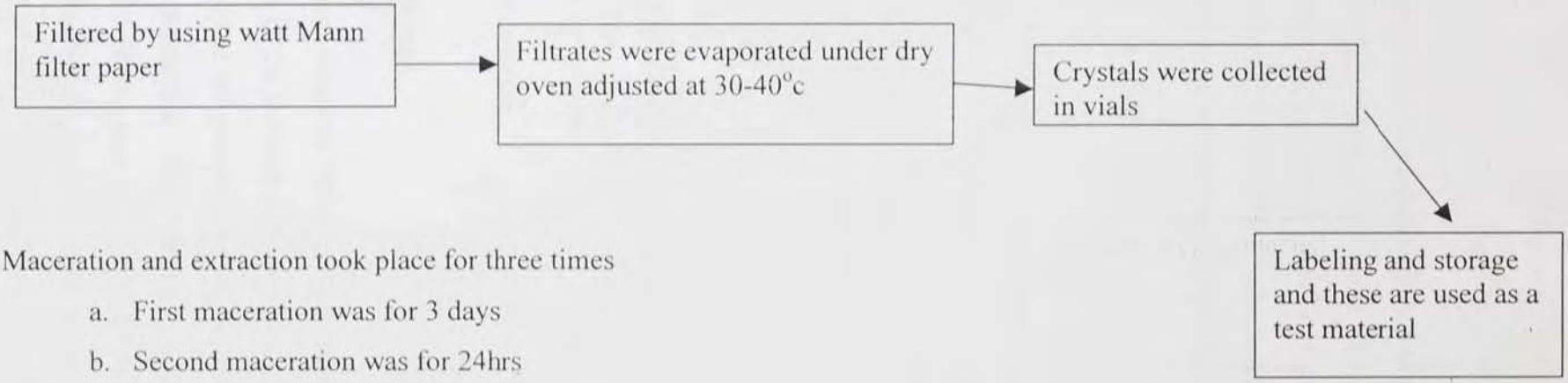
Step I: Herb preparations



Step II: Maceration



Step III: Extraction



Maceration and extraction took place for three times

a. First maceration was for 3 days
b. Second maceration was for 24hrs
c. Third maceration was for 24hrs

3.4.2. Herb collection

Plants/herbs were collected from their natural habitats and washed with tap water to remove unnecessary particles. They were allowed to dry under shade. They were chopped (by knife and axe) into pieces to facilitate drying and grounded to powder using a large mortar after drying. The material was sieved using ordinary sieve. The grounded powder was ready to be macerated in 80% methanol. The general information is summarized in table 6.

Table 6. General information

Plant/herb	Part used	Amount macerated in 80% Methanol (g)	Place of collection	Previous work done or reported activity on test bacteria
Burcea antidysenterica	Leaf and root	200	Around Bahir-Dar	Yes
Cyphostemma adenocaule	Root	300	Gaynt- South Gondar	Yes
Persicaria senigalense	Leaf	200	Debre-Zeit	Yes
Plantago lanceolata	Leaf	300	Around Bahir-Dar	Yes
Plantago lanceolata	Seed	100	Around Bahir-Dar	Yes
Zehneria scabra	Root	170	Around Bahir-Dar	Yes
Combertum molle	Leaf	50*	Gaynt- South Gondar	No

*- Was macerated in absolute methanol and aqueous solution

3.4.3.Preparations of crude extract for in *vitro* experiment

50-300 grams of each herb leave, root or both were weighed. The materials were macerated in 80% methanol (1 part test material and: 10 parts of the solvent) in large ground bottle and the contents mixed by a flask shaker (Gallenkamp) at maxmimum speed for 30 minutes and allowed to stand for 3 days at room temperature. Each extract was filtered by chromatographic filter paper No 3 size 113. The filtrate was transferred to evaporating dish and kept in an oven at 30^0C-40^0C to dry. The residue left after filtration was macerated again in methanol solution for 24 hours and filtered and added to evaporating dish. The procedure was repeated for the third time to have sufficient amount of extracts. The crystals were collected and were ready for use.

3.4.4. Preparation of antimicrobial discs from herb extracts for in *vitro* experiment

Six serial dilutions of herb extracts were made in test tubes. Point eight grams of plant extract was mixed with 2ml Dimethyl Sulfoxide (DMSO) in the first test tube to prepare 40% solution according to Shihata *et al.* (1983). The remaining test tubes were filled with 1ml of DMSO and 1ml of 40% solution from the first tubes was transferred to second test tube to prepare 20%. The procedure continued by transferring 1ml solution from 20% to third test tube to get 10% concentration, and this continue until 1.25% concentration is obtained. Discs of 12mm size were impregnated by adding 3 drops from each concentration and allowed to dry at 37^0C overnight. Dried discs were used to determine antibacterial effects. Each disc was gently pressed down to ensure complete contact with the agar and the plates were inverted and incubated at 37^0C for 24hrs. The diameter of zone of inhibition was measured in millimeter

In *vitro* antimicrobial test was carried out using the crude powder of each plant extract by plate diffusion method (Ieven *et al.*, 1979). Wells of 5mm diameter were made on the inoculated media using a sterile single gel cutter on the seeded plate equidistantly. The wells were filled with 0.02g, 0.01g, and 0.005g of each plant extract and zone of inhibitions were measured.

3.4.5. Antimicrobial sensitivity test

Antimicrobial sensitivity was conducted using agar disc diffusion method. The bacteria used for this study were major isolates (*Staphylococcus aureus* and *Streptococcus agalactiae*) from clinical mastitic quarters. Three to then well-isolated colonies of the same morphological type were selected from the nutrient agar and suspension was made in a sterile saline. Turbidity of the bacterial suspension was adjusted by comparing with 0.05 McFarland turbidity standard. The standard and the test suspension were placed in 10ml size test tubes and compared against a white background with contrasting black lines, until the turbidity of the test suspension equates to that of the turbidity standard. Adjustment of the turbidity was made by adding saline or colonies depending on the degree of turbidity. A sterile swab was dipped into the standardized suspension of the bacteria and excess fluid was expressed by pressing and rotating the swab firmly against the inside of the tube above the fluid level. The swab was streaked in three directions over the entire surface of the agar with the objective of obtaining a uniform inoculation and a final sweep with the swab was made against the agar around the rim of the petridish. The inoculated plates were allowed to stand for not more than 15 minutes and the discs were placed on the agar surface

using sterile forceps. Each disc was gently pressed with the point of a sterile forceps to ensure complete contact with the agar surface (Quinn *et al.*, 1999).

For this study oxytetracycline, penicillin G, streptomycin and neomycin (of Himedia laboratory product) were used to compare their efficacy with herbal preparations. The antimicrobials were selected based on the frequent use of the preparations for the treatment of clinical bovine mastitis and other disease problems in the two dairy farms. The activities of the conventional antimicrobials were compared against 20% concentrations of each test plant. The solvents (Methanol and DMSO) served as a control. As a test protocol each test herb preparation was put at the centre and conventional antimicrobial discs at the periphery of the plate.

Mueller- Hinton agar medium was used and was supplemented with 5% sheep blood for *Streptococcus agalactiae*. Ph= 7.2-7.4. Therefore, the agar was prepared by pouring 25ml in a 90mm diameter petridish. Barium sulphate solution used as a standard to determine the bacterial concentration was prepared as 1% solution in 1% H_2SO_4. The preparation was kept in the dark. For the preparation of bacterial suspension 3-10 colonies were picked from the culture under study and were placed in 4 ml of sterile physiological saline and the culture was standardized by comparing with McFarland solution.

Discs and test material were applied at a space of 24 mm apart from center to center and 15 mm away from the edge of the plate. This was made no latter than 15 minutes after the inoculum had been seeded. For this study the plant materials were put at the center of the plate and conventional discs at the periphery. Plates were incubated soon after the discs had been applied for 24 hours at 37^0C. Diameter of zone of inhibition was measured using a ruler in millimeter including the antibiotic discs and results were recorded as susceptible, intermediates or resistant by comparing with standard values for each antibiotic disc (Delaat, 1979; Carter, 1984; Quinn *et al.*, 1999).

McFarland turbidity standard was prepared by mixing 0.5ml of 1.175% aqueous solution of Barium chloride (0.048M $Bacl_2$ $2H_2O$) with 9.95ml of 1% H_2SO_4 (0.36N H_2SO_4 (Quinn *et al.*, 1999).

3.5. Data management and analysis

The data obtained were stored in Excel spreadsheet. Zone of inhibitions were summarized using Intercooled Stata 7. Agents isolated, severity of mastitis and diagnosis results were analyzed by descriptive statistics. Incidence of clinical bovine mastitis was expressed by 100 cows-month at risk. Soft Wares Package for Social Science (SPSS) was used for multiple comparisons and the results are indicated in appendix 8.6.

4. RESULTS

This study was conducted with the objective of assessing the incidence of clinical mastitis, identifying causal agents and evaluating the antimicrobial effects of selected herbs on *Staphlococcus aureus* and *Streptococcus agalactiae* isolates. Incidence of clinical mastitis was followed for six months (from September 2003- March 2004) in two dairy farms.

4.1. Investigation of clinical mastitis

4.1.1. Incidence of clinical bovine mastitis

Incidence of clinical mastitis during the study period was 52.8% and 33.96% in EARO and Fairfield dairy farms, respectively. The average incidence of clinical mastitis observed was 41.6%.

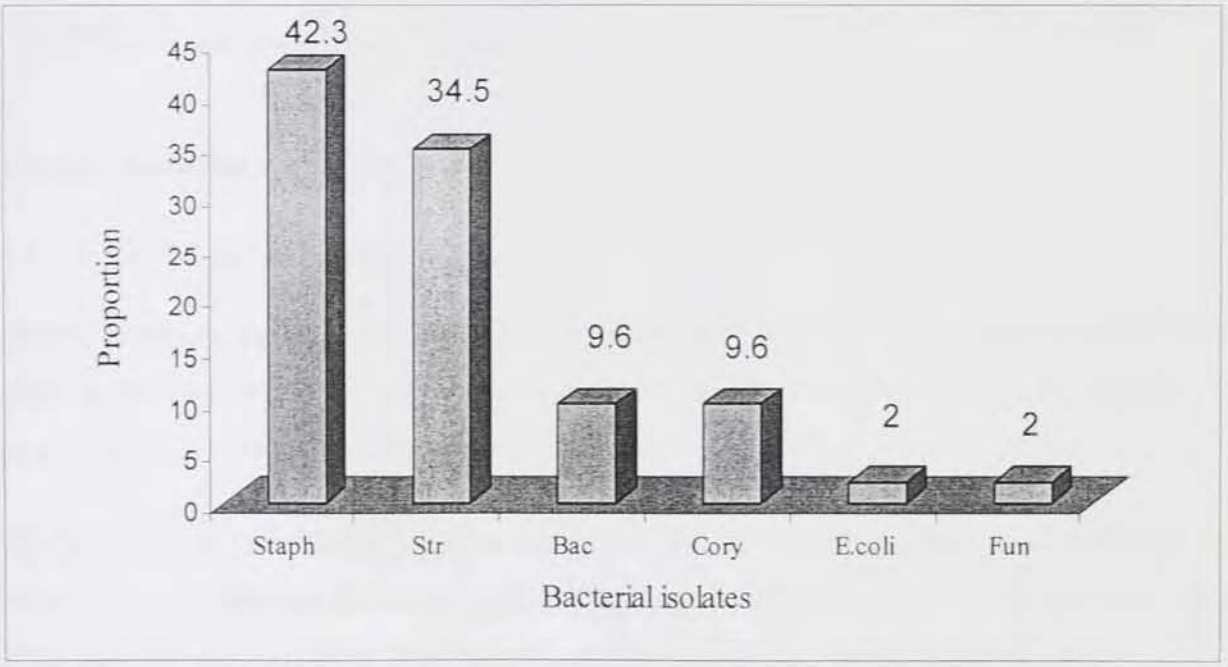
An incidence density of 12.4 new clinical mastitis cases/100cows-month (95% CI 8.0- 20.7) and 6.7 new clinical mastitis cases/100cows-month (95% CI 4.0- 10.6) at risk in EARO and Fair field dairy farms respectively was recorded. The overall incidence rate was 9 new clinical mastitis cases/100 cow-month (95% CI 6.4- 12.4) at risk for both farms.

4.1.2. Microbial agents isolated

Milk samples were collected from 32 mastitic clinical quarters out of 37 lactating cows that had clinical mastitic evidence in the two dairy farms. Of the isolates *Staphylococcus aureus* (32.7%), *Streptococcus agalactiae* (27.0%), showed highest frequencies. Other isolates included *Streptococcus uberis* (5.8%), *Staphylococcus intermidius* (3.8%), *Bacillus cereus* and *Bacillus* species (5.8%) each, *Corynebacterium bovis* (3.8%) and *Corynebacterium ulcerans* (5.8%). The bacterial species isolated are presented in Table 7. The distribution of isolates at genera level is given in Figure 1. The highest proportion of 42.3% was recorded for *Staphylococcus* species followed by *Streptococcus* species (34.5%) proceeded by *Bacillus* and *Corynebacterium* species (each contributing 9.6%).

Table 7: Microbial agents isolated from clinical mastitis cases in two dairy herds from Debre Zeit, September 2003 to March 2004

No	Agents	EARO		Fair-field		Overall (%)	Contribution to clinical incidence (%)
		No of isolates	Relative isolation %	No of isolates	Relative isolation %		
1	*Staphylococcus aureus*	12	41.4	5	21.7	32.7(N=17)	42.3
2	*Staphylococcus intermidius*	2	6.9	0	0	3.8(n=2)	
3	*Staphylococcus hyicus*	1	3.4	0	0	1.9(n=1)	
4	*Staphylococcus epidermidis*	0	0	1	4.35	1.9(n=1)	
5	*Staphylococcus chromogens*	0	0	1	4.35	1.9(n=1)	
6	*Streptococcus agalactiae*	6	20.7	8	34.8	27.0(n=14)	34.5
7	*Streptococcus dysgalactiae*	1	3.45	0	0	1.9(n=4)	
8	*Streptococcus uberis*	2	6.9	1	4.35	5.8(n=3)	
9	*Bacillus cereus*	0	0	3	13.05	5.8(n=3)	9.6
10	*Bacillus species*	2	6.9	0	0	3.8(n=2)	
11	*Corynebacterium bovis*	2	6.9	0	0	3.8(n=2)	9.6
12	*Corynebacterium ulcerans*	1	3.45	2	8.7	5.8(n=3)	
13	*Esceririchia coli*	0	0	1	4.35	1.9(n=1)	2.0
14	Fungus	0	0	1	4.35	1.9(n=1)	2.0
	Total	**29**	**100.0**	**23**	**100.0**	**100.0(n=37)**	**100.0**



Staph = *Staphylococcus* species, Str = *Streptococcus* species, Bac= *Bacillus* species, Cory = *Corynebacterium* species, Fun = Fungus

Fig.1: Proportion of bacterial agents at genus level isolated from clinical mastitis

Isolates of clinical cases were single (one aetiological agent is involved), mixed (two etiological agents), or contaminated (more than two aetiological agents were involved). Out of 32 mastitic milk samples 46.9% (n = 15), 37.5% (n = 12) and 9.4% (n =3) were single, mixed and contaminated infections, respectively and 6.2% (n = 2) samples were found negative cultures.

4.1.3.Severity of clinical mastitis

The distribution of clinical mastitis graded as I or II is given in Table 8. Cows that showed mild changes in the milk were graded as I showed the highest frequency (83.8%, n = 31) compared to moderate changes in milk and the udder. A grade of III with involvement of systemic signs is not shown here because no animal was found showing the sign during the study period.

Table 8: Grade of clinical mastitis with their proportions

Grade	EARO		Fair-field		Overall	
	Cases	%	Cases	%	Cases	%
I	18	94.7	13	72.2	31	83.8
II	1	5.3	5	27.8	6	16.2
Total	**19**	**100.0**	**18**	**100.0**	**37**	**100.0**

4.2.Antimicrobial susceptibility testing

4.2.1. Effect of herbal preparations

Before commencing 20% concentrations comparison with conventional antimicrobial discs each plant extract was tested at different concentration levels (40%, 20%, 10%, 5%, 2.5% and 1.25%) to see their inhibitory effects against the isolates.

The mean zone of inhibition in mm for each plant extract at doubling concentrations ranging from 1.25 % to 40% are shown in Table 9. The zone of inhibition for *Combertum molle* increased from 11 to 22 mm and 10 to 20 mm for *S. aureus* and *Str. agalaciae*, respectively.

The efficacy of the test herbs/plants at 20% concentrations were evaluated against two major isolates, namely *Staphylococcus aureus* (n = 17) and *Streptococcus agalactiae* (n = 14). These mastitic causal agents were isolated from clinical mastitis cases during the study time. A higher

mean zone of inhibition on two bacterial isolates was found by *Combertum molle* and least mean zone of inhibition was obtained by *Brucea antidysentrica.*

Table 9: Efficacy of herb preparations at different concentrations

Herb	Agent		Concentrations (%)					
			40	20	10	5	2.5	1.25
Brucea antidysenterica	*S. aureus*		10	8	7	-	-	-
	S.agalactiae		11	10	8	-	-	-
Combertum molle	*S. aureus*	Mean	22	19	18	15	13	11
	S.agalactiae	Inhibition	20	18	16	13	11	10
Cyphostemma adenocaule	*S. aureus*	zone (mm)	14	12	10	9	-	-
	S.agalactiae		12	10	9	7	-	-
Persicaria senigalnsis	*S. aureus*		12	11	9	8	-	-
	S.agalactiae		11	10	8	7	-	-

Apart from 20% concentrations of plant preparations the herbal efficacies were observed at different quantities of the extract filled in a 5 mm diameter well. The amount added to each of the wells was equivalent to 0.02g, 0.01g and 0.005g powder of each plant extract. For this assessment 5 *Staphylococcus aureus* and 5 *Streptococcus agalactiae* isolates were tested and all quantities gave a measurable inhibitory zone (Table 10). But there was over flow of the powder at higher amounts and remaining at the bottom when using small quantities. Therefore, a disc impregnation and diffusion method was preferable for testing antimicrobial effect of the plants.

Table 10: Effect of plant extracts in grams

Agent	Herb		Effect of powders (g)		
			0.02	0.01	0.005
	Brucea antidysenterica		15	14	11
S. aureus	*Combertum molle*	Inhibition zone	25	23	20
	Cyphostemma adenocaule	(mm)	12	10	8
	Persicaria senigalnsis		11	10	8
	Brucea antidysenterica		13	12	8
S.agalactiae	*Combertum molle*		22	21	20
	Cyphostemma adenocaule		9	8	7
	Persicaria senigalnsis		11	11	9

4.3. Comparison of 20% four herbal preparations with four conventional antimicrobial discs.

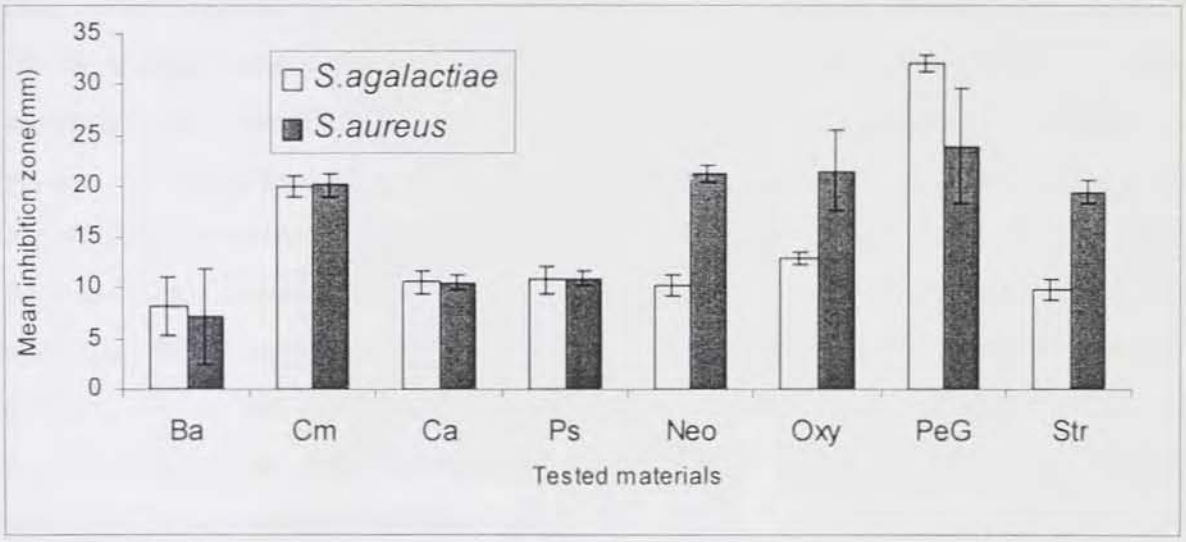


Fig. 2: Mean zone of inhibition (mm) of four 20% phytopreparations on *Streptococcus agalactiae* and *Staphylococcus aureus* in comparison with four conventional antimicrobial discs.

The comparative mean zone of inhibition in mm between herbal preparations and conventional antibiotics are shown in Fig 2. While the inhibition in conventional antibiotics shows differences

in the size of inhibition for both species of bacteria, those of herbal preparations were equal except *Brucea antidysentrica*.

Table 11: Mean inhibition zone in mm for the effect of 20% preparations of 4 herbs compared with 4 conventional antimicribial discs (AMD) on test organisms

Tested materials	*Staphylococcus aureus*			*Streptococcus agalactiae*		
	No of isol.	Mean	95% CI	No of isol.	Mean	95% CI
Brucea antydysentrica	17	7.23	2.60-11.92	14	8.21	5.33-11.10
Combertum molle	17	20.18	19.01-21.34	14	20.10	19.10-21.10
Cyphostemma adenecuale	17	10.53	9.73 –11.32	14	10.57	9.40-11.74
Persicaria senigalesis	17	10.94	10.16-11.72	14	10.79	9.50-12.11
Neomycin	17	21.30	20.34-22.20	14	10.21	9.22- 11.21
Oxytetracycline	17	21.59	17.57-25.61	14	12.86	12.22-13.50
Penicillin G	17	24.0	18.32-29.68	14	32.14	31.24-33.04
Streptomycin	17	19.53	18.34-20.72	14	9.86	8.90-10.81

4.4. Effect of 20% herbal preparations in comparison with AMD on resistant isolates

Streptococcus agalactiae was resistant 85.7% (n = 12), 100% (n = 14), 85.7%(n = 12) to neomycin, oxytetracycline and streptomycin, respectively while all isolates were sensitive to penicillin G. *Staphylococcus aureus* isolates were resistant 23.5% (n = 4) and 64.7% (n = 11) to oxytetracycline and penicillin G, respectively while it was sensitive to neomycin and streptomycin. Inhibition zone of $\leq$12mm and $\leq$ 11mm were considered as resistant for neomycin and streptomycin, respectively. Inhibition zone of $\leq$ 14mm and $\leq$ 18mm were taken as resistant for *Staphylococcus aureus* and *Streptococcus agalactiae*, respectively in the case of oxytetracycline. While, inhibition zone of $\leq$ 28mm and $\leq$ 19mm were considered as resistant for *Stapylococcus aureus* and *Streptococcus agalactiae,* respectively for penicillin G. The herbal efficacies in comparison with conventional antimicrobial agents to which test organisms developed resistant are summarized from fig 3-7.

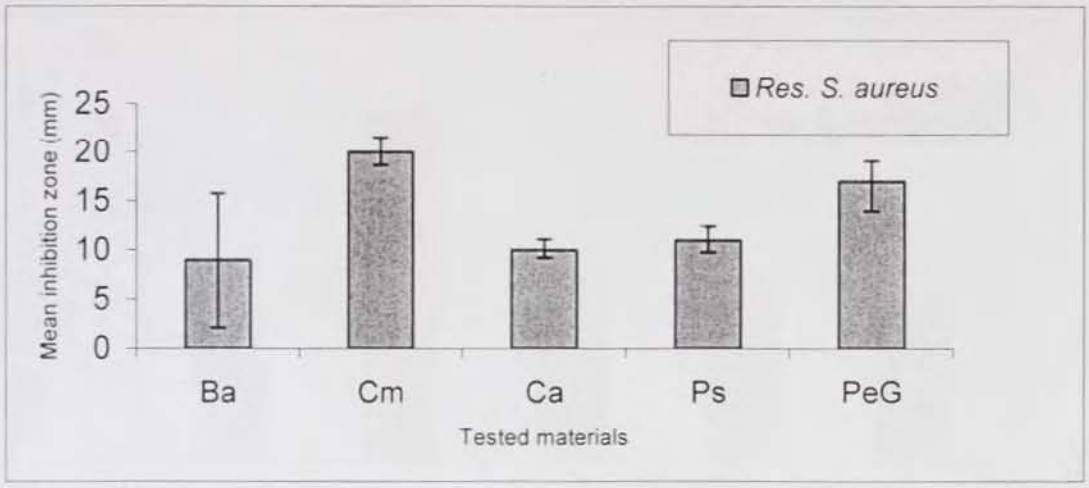


Fig.3: Mean zone of inhibition (mm) of four 20% Herb preparations in comparison with penicillin G - resistant isolates of *Staphylococcus aureus*.

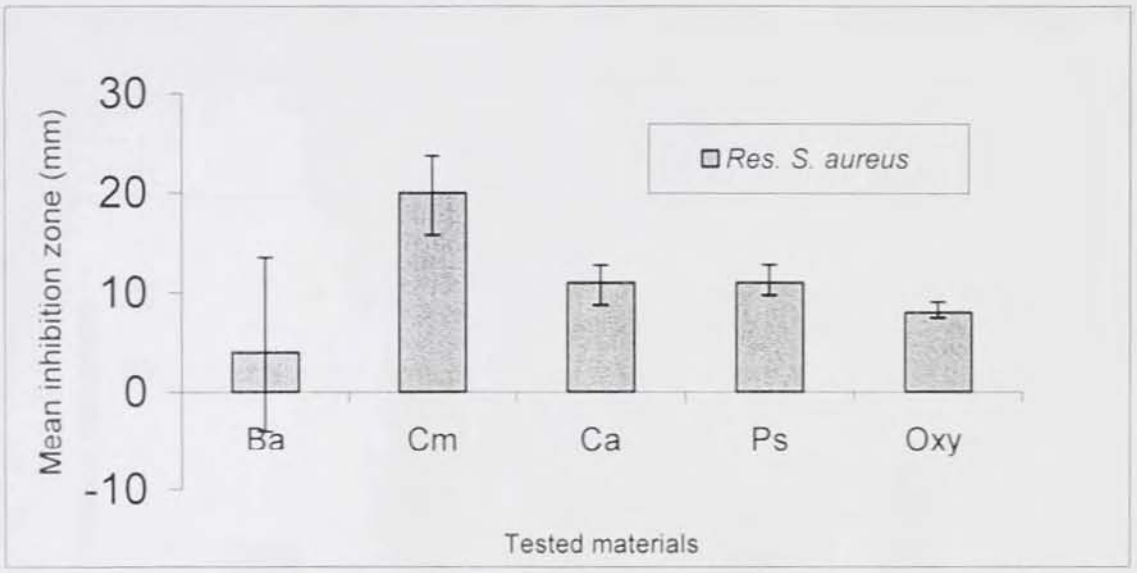


Fig.4: Mean zone of inhibition (mm) of four 20% Herb preparations in comparison with oxytetracycline - resistant isolates of *Staphylococcus aureus*.

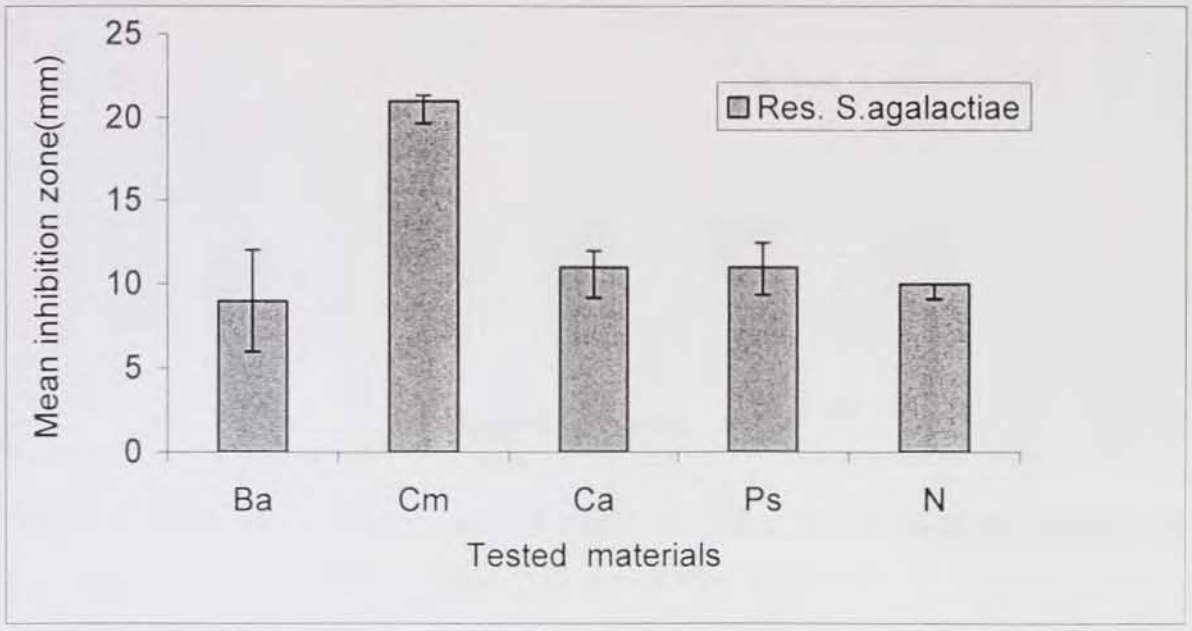


Fig.5: Mean zone of inhibition (mm) of four 20% Herb preparations in comparison with neomycin - resistant isolates of *Streptococcus agalactiae*.

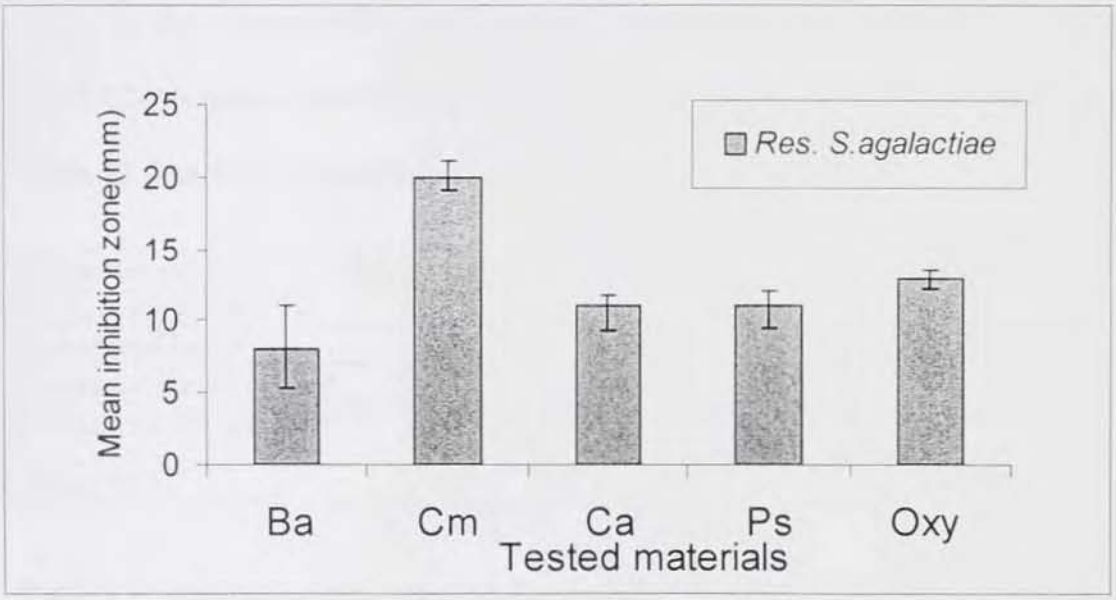


Fig.6: Mean zone of inhibition (mm) of four 20% Herb preparations in comparison with oxytetracycline - resistant isolates of *Streptococcus agalactiae*.

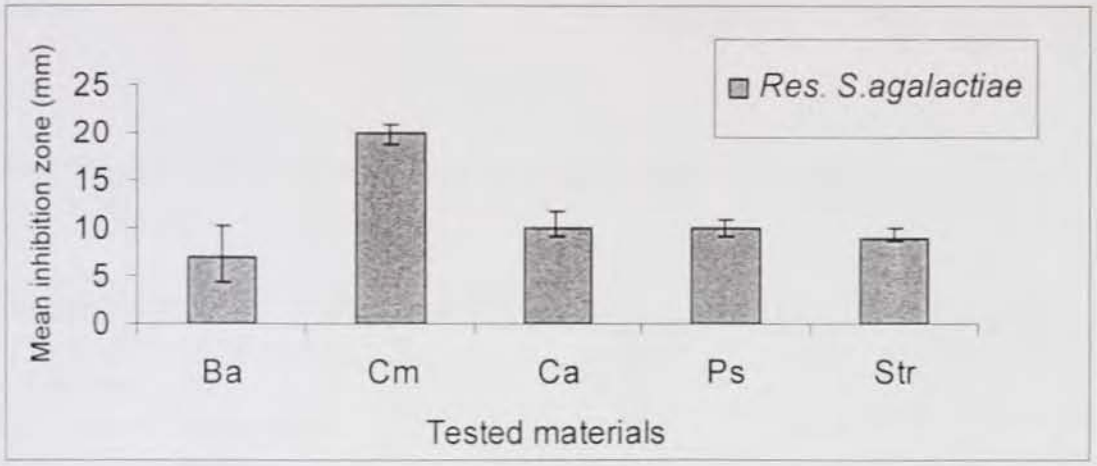


Fig.7: Mean zone of inhibition (mm) of four 20% Herb preparations in comparison with Streptomycin - resistant isolates of *Streptococcus agalactiae*.

Key

1. Neo(n)= Neomycin
2. Oxy(o) =Oxtetracycline
3. PeG(P)= Penicillin G
4. Str(s)= Streptomycin
5. Ba = *Brucea antidysentrica*
6. Cm = *Combertum molle*
7. Ca = *Cyphostemma adenecuale*
8. Ps = *Persicaria senegalensis*
9. Res. *S.aureus*= Resistant *S.aureus*
10. Res. S.agalactiae= Resistant *S.agalactiae*

Table 12- 16 summarizes the mean inhibition zone in mm obtained on resistant isolates.

Table 12: Penicillin G-resistant *Staphylococcus aureus* isolates

Test materials	No of isolates	Mean	[95% Conf. Interval]	
Burcea antidysenterica	11	8.91	2.10	15.72
Combertum molle	11	20.10	18.67	21.51
Cyphostemma adenocaule	11	10.18	9.24	11.12
Persicaria senegalensis	11	11.09	9.77	12.42
Penicillin G	11	16.51	13.97	19.12

Table 13: Oxytetracycline- resistant *Staphylococcus aureus* isolates

Test materials	No of isolates	Mean	[95% Conf. Interval]	
Burcea antidysenterica	4	4.75	-4.00	13.50
Combertum molle	4	19.75	15.77	23.73
Cyphostemma adenocaule	4	10.75	8.75	12.75
Persicaria senegalensis	4	11.25	9.73	12.77
Oxytetracycline	4	8.25	7.45	9.06

Table 14: Neomycin –resistant *Streptococcus agalactiae* isolates

Test materials	No of isolates	Mean	[95% Conf. Interval]	
Burcea antidysenterica	12	9.00	5.98	12.02
Combertum molle	12	20.50	19.66	21.34
Cyphostemma adenocaule	12	10.58	9.19	11.98
Persicaria senegalensis	12	10.92	9.37	12.46
Neomycin	12	9.58	9.16	10.01

Table 15: Oxytetracycline- resistant *Streptococcus agalactiae* isolates

Test materials	No of isolates	Mean	[95% Conf. Interval]	
Burcea antidysentrica	14	8.21	5.33	11.10
Combertum molle	14	20.07	19.10	21.10
Cyphostemma adenocaule	14	10.57	9.40	11.74
Persicaria senegalensis	14	10.79	9.46	12.11
Oxytetracycline	14	12.86	12.22	13.50

Table 16: Streptomycin- resistant *Streptococcus agalactiae* isolates

Test materials	No of isolates	Mean	[95% Conf. Interval]	
Burcea antidysentrica	12	7.25	4.30	10.24
Combertum molle	12	19.83	18.72	20.94
Cyphostemma adenocaule	12	10.42	9.05	11.78
Persicaria senegalensis	12	10.00	9.23	10.77
Streptomycin	12	9.33	8.71	9.96

In order to appreciate variability that exists among test plants against test pathogens the sensitivity test result is shown in Table 17 and 18.

Table17: Raw data showing inhibition zone in mm obtained by 20% herbal preparations in comparison with conventional antimicrobial discs for Staphylococcus *aerues*

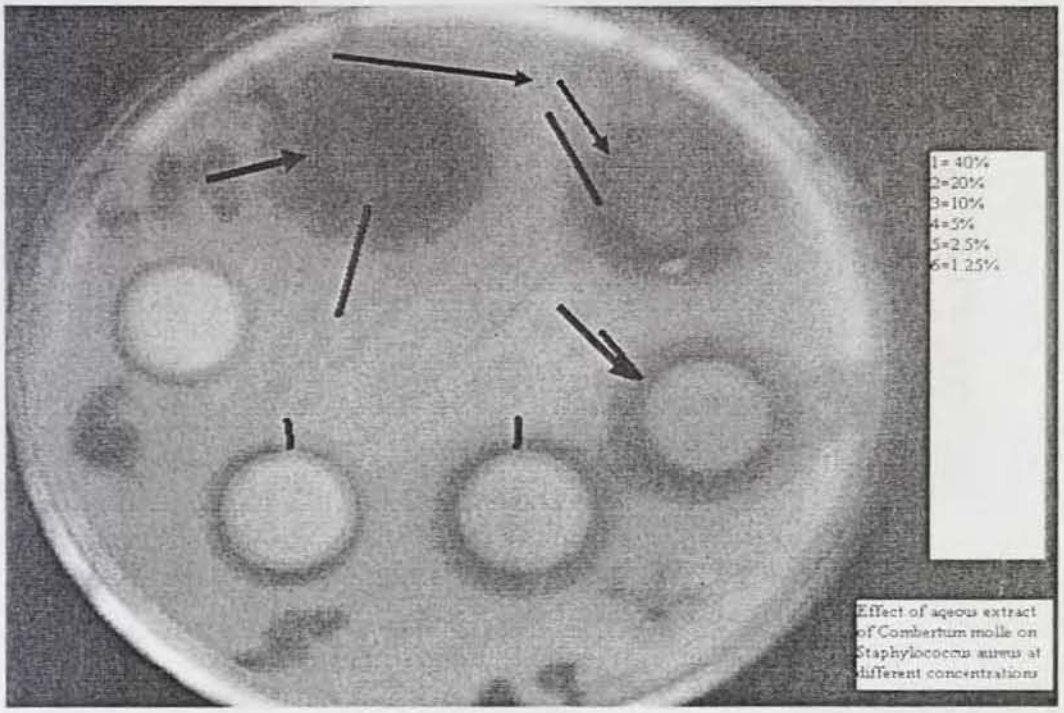
	Staphylococcus aerues							
Isolate	Ba	Cm	Ca	Ps	Neo	OXT	PeG	Str
1	0	18	14	11	20	28	44	18
2	0	19	12	10	20	25	38	14
3	10	23	9	11	20	8	13	19
4	22	24	11	15	23	28	23	14
5	25	20	12	12	23	29	20	19
6	9	17	12	12	21	9	16	21
7	0	22	11	11	20	25	35	20
8	0	18	10	11	20	22	37	18
9	9	20	10	12	22	25	20	20
10	0	21	10	9	25	27	19	25
11	0	18	8	9	20	24	12	19
12	11	20	9	10	20	25	33	21
13	0	19	11	12	21	8	15	20
14	0	18	8	9	20	24	12	19
15	14	25	11	10	25	27	39	23
16	23	21	10	12	22	25	19	18
17	0	20	11	10	20	8	13	19

Table18: Raw data showing inhibition zone in mm obtained by 20% herbal preparations in comparison with conventional antimicrobial discs for *Streptococcus agalactiae*

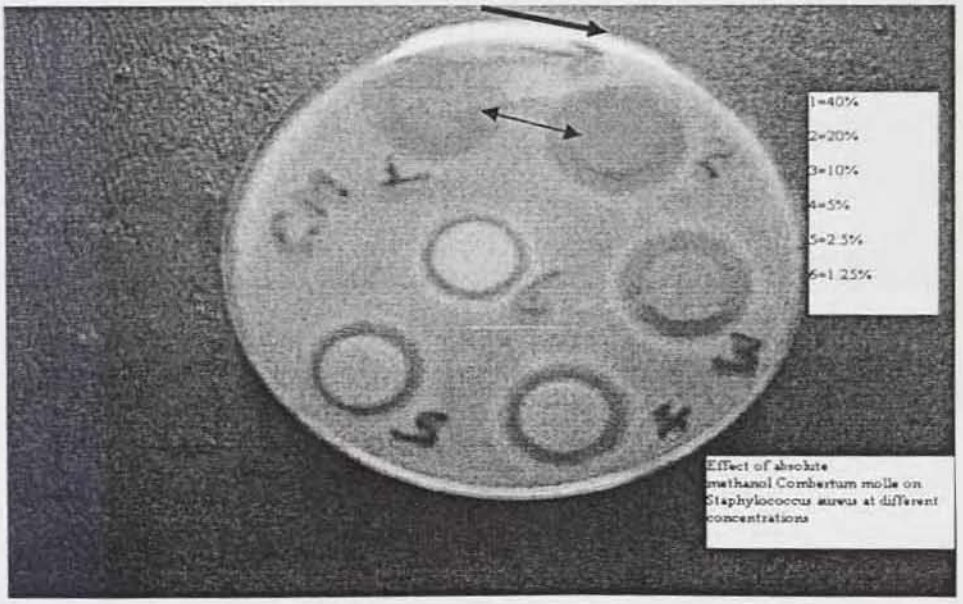
	Streptococcus agalactiae							
Isolate	Ba	Cm	Ca	Ps	Neo	OXT	PeG	Str
1	0	16	11	9	14	13	31	9
2	7	19	10	11	14	14	31	10
3	12	21	9	9	8	14	33	10
4	15	21	11	16	10	13	32	12
5	13	22	12	15	10	12	32	14
6	12	20	10	11	10	11	34	9
7	8	18	11	8	9	14	33	8
8	9	21	12	11	10	13	30	9
9	0	22	8	9	10	11	31	8
10	8	19	9	12	10	14	34	10
11	0	20	11	10	9	14	35	8
12	8	22	16	11	10	12	31	10
13	11	19	8	9	10	12	33	10
14	12	21	10	10	9	13	30	11

Inhibition zones

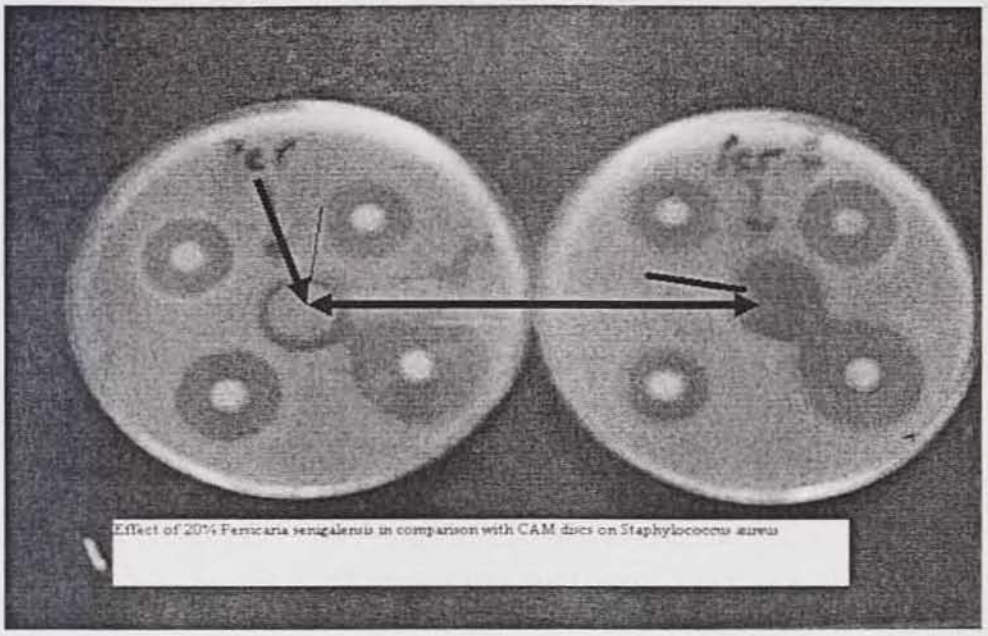
A. Inhibition zone of aqueous extract of *Combertum molle* at different concentrations on *Staphylococcus aureus* isolate (from higher to lower concentration, clock wise)



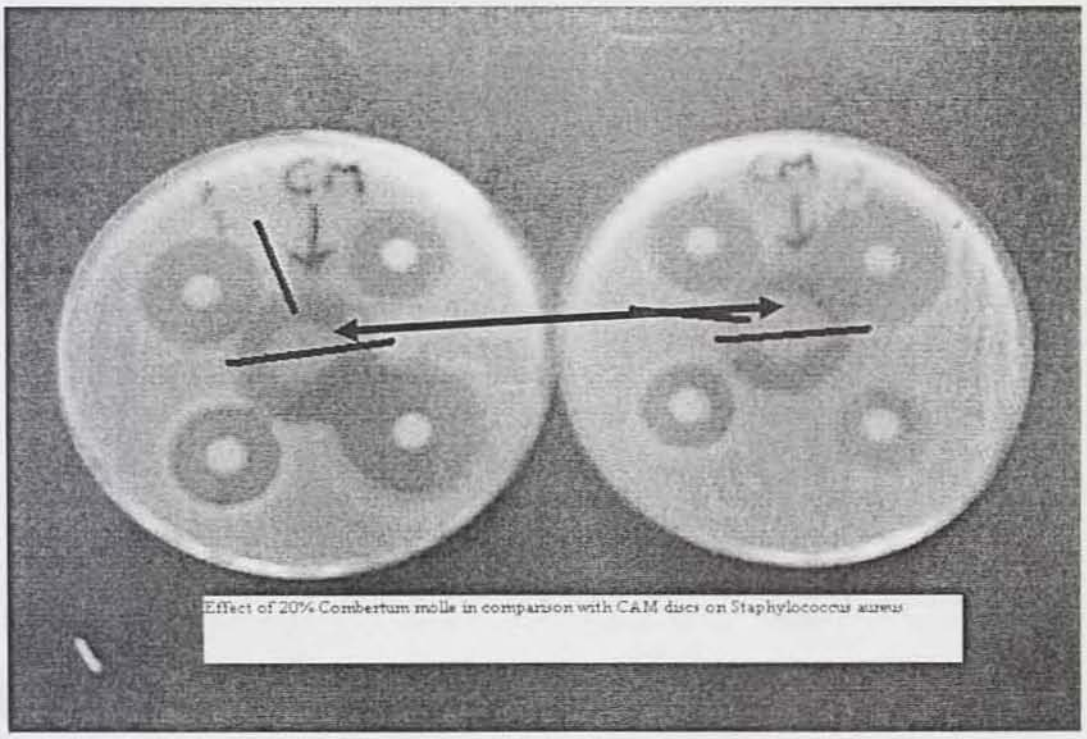
B. Inhibition zone of absolute methanol extract of *Combertum molle* at different concentrations on *Staphylococcus aureus* isolate (from higher to lower concentration, clock wise)



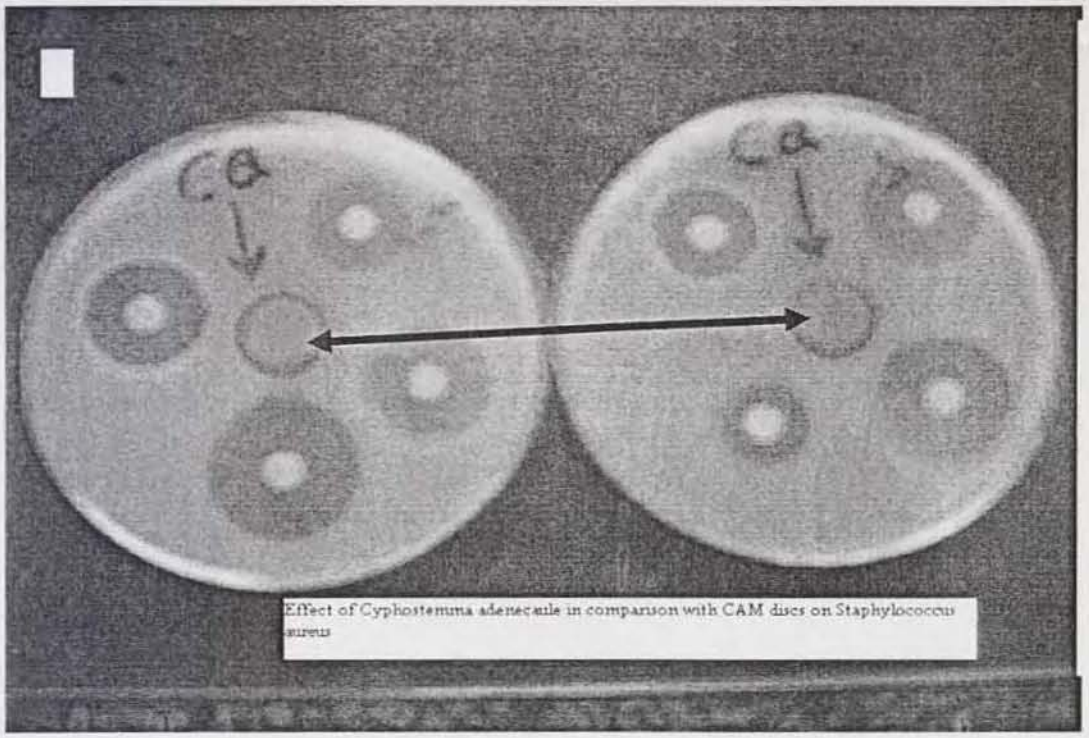
C. Inhibition zone of aqueous methanol extract of *Persicaria senegalensis* at 20% on *Staphylococcus aureus* isolate in comparison with AMD



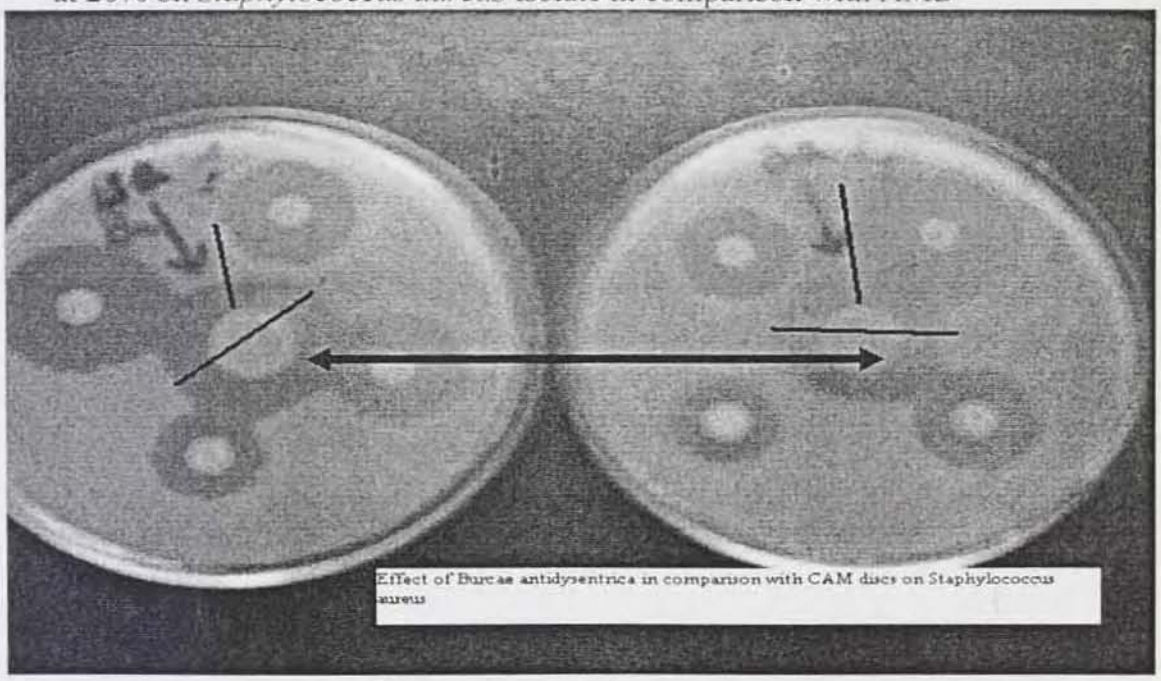
D. Inhibition zone of aqueous methanol extract of *Combertum molle* at 20% on *Staphylococcus aureus* isolate in comparison with AMD



E. Inhibition zone of aqueous methanol extract of *Cyphostemma adenocaule* at 20% on *Staphylococcus aureus* isolate in comparison with AMD



F. Inhibition zone of aqueous methanol extract of *Brucea antidysenterica* at 20% on *Staphylococcus aureus* isolate in comparison with AMD



* Arrows indicate the test herb and inhibition zone.

The overall incidence was 9 new clinical mastitis cases/100 cows-month (at 95% CI - 6.4-12.4) at risk and *Staphylococcus* and *Streptococcus* species were the main isolates and *Staphylococcus aureus* and *Streptococcus agalactiae* served as test organisms. *Staphylococcus aureus* showed resistant to penicillin G and oxytetracycline while *Streptococcus agalactiae* showed resistant to neomycin, streptomycin and oxytetracycline. Four herbs at 20% preparations were showing inhibitory effect against the test organisms. Among the herbs *Combertum molle* were having a better effect.

5. DISCUSSION

The study of incidence of bovine clinical mastitis was carried out for six months from September 2003 to March 2004 in two selected dairy farms in Debre-zeit town. There was no study conducted in the two farms in relation to incidence of clinical mastitis in these farms before. The main aim of studying incidence in these two farms was to determine the problem of clinical mastitis, to obtain and determine the prevalence of antibiotic resistant mastitis pathogens and then to evaluate the antibiogram profiles using conventional methods and herbal extracts.

Mastitis is the most common disease in dairy cows (Sonhua, 2000) and it occurred at any times of the year with varying degree of severity and incidence rates. Mastitis is responsible for decreasing milk production by 3-50% of the potential and also compromises the quality of milk, which represents a risk to public health (Costa, 1998; Benites *et al.*, 2003). In this study an incidence density that includes repeated cases of episodes in the same cows was 12.4 new clinical mastitis cases/100cows-month at risk and 6.7 new clinical mastitis cases/100 cows-month at risk were recorded in EARO and Fair-field dairy farms, respectively. The overall incidence density was 9 new clinical mastitis cases/100 cows-month (at 95% CI - 6.4-12.4) at risk. This finding was higher than the reports of Miltenburg *et al.* (1996) 17.9, Peeler *et al.* (2000) 22.8, Bartlett *et al.* (2000) 48 and Milne *et al.* (2002) 73 new clinical mastitis cases/100cows-year at risk. This might be related to the presence of unhygienic milking and poor management practices in the two farms in general. Miltenburg *et al.*, (1996) reported that differences in the incidence rate of clinical mastitis in dairy cows are associated with factors such as climate, breed, level of production and management. A higher incidence rate of bovine clinical mastitis in EARO was recorded than Fair-field dairy farm. In the private dairy farm, (Fair field), they do have a good milking practice. They wash and dry the udder and the teats before milking and apply post milking dipping, allowed the cows to stand for a certain period of time after milking, change beddings every day and above all in most of the cases the same person is milked the same animal and responsibility to report any change in the milk and the udder for immediate action. The aforementioned observations were stated as a control measures for clinical mastitis by (Fox and Gay, 1993; Crist *et al.*, 1997). Mastitic cows were also milked at last in this farm. Removal of dirt or slurry from the cow's immediate environment was suggested as a fundamental controlling strategy for bovine toxic mastitis and they recommend strategies to encourage the cows to remain standing after milking (Menzies and Mackie, 2001).

Clinical cases observed were categorized into 3 grades; viz, grade I, grade II and grade III depending on the severity or degree of clinical signs manifested and disease condition. Out of 37 clinical cases registered in the two dairy farms 83.8% of the cases were grade I and 16.2% were grade II. There was no grade III case with involvement of systemic signs during the study time. Bradley and Green (2001) in the UK reported that 67.4% of the cases were reported as being grade I and 26.4% as grade II and the current finding is in agreement with these researchers. Both grades resulted from either single, mixed, contaminated or with no identified aetiological agents. The reason why the majority of cases were Grade I might be attributed to the early disease detection, the nature of the aetiological agents, the immunological status of the udder and the general health condition of the animal. Bradley and Green (2001) stated that mastitis was significantly more severe (grade II and III) in the herd with the lowest bulk milk somatic cell count. This finding suggests that mild clinical cases are more important than others and is more common in the farms that could cause a considerable milk loss and compromises the cow's health and might serve as a source of infection to healthy animals if passed unnoticed. Hence, it is alarming to the farms to seriously attend their cows at milking times in order to reduce the risk of spread of infectious agents and farm contaminations.

The diagnoses of clinical mastitis were made according to the number of isolates obtained or involved during the disease process after cultural, morphological and biochemical identification of the causal agents. Out of 32 mastitic milk samples taken from the two farms 46.9% (n=15) and 37.5% (n=12) and 9.4% (n=3) were single, mixed and contaminated, respectively and 6.2%(n=2) samples were found negative cultures. Bhattacharya (2002) indicated that in a district of India 88.8% and 11.11% of samples revealed single and mixed infections/isolates respectively. In another place of India from 48 clinical mastitic milk samples 56.25% and 43.75% were single infection and multiple infections, respectively (Barbuddhe *et al.*, 2001). Haile (1995) from 151 milk samples he fond 22.6% mixed bacterial isolates. Philip *et al.* (1991) reported multiple isolates were more significantly common from premilking samples. The current finding indicates that emphasis should be given as to the aetiological agents during any mastitis cases occurrence. Most professionals and paraprofessionals suspect the involvement of single agents in any intramammary infection in most of the cases and treat cases with a medicament what they have at their disposal. Hence, since the finding of mixed and contaminated infections couldn't be undermined, care must be taken during drug selection and if possible, it is advisable to carry out bacteriological isolations before commencing any treatment.

From 32 mastitic milk samples cultured *Staphylococcus aureus* (32.7%) were predominant isolates followed by *Streptococcus agalactiae* (27.0%). Both agents are major and contagious pathogens. These agents have been reported in Tanzania (Shem *et al.*, 2001), in Jamaica (Zingser *et al.*, 1991) and in Ethiopia (Kero and Tareke, 2003). Zingser *et al.* (1991) in a survey conducted in Jamaica, the most common bacteria isolated were *Staphylococcus aureus* (27%). In India, Barbuddhe *et al.* (2001) reported 23.25% and 11.6% *Staphylococcus aureus* and *Streptococcus* species, respecively. Haile, (1995) found 38.8% of *Staphylococcus aureus* isolate from milk samples as a dominant isolate and 6.8% of *Streptococcus* species. Most of these findings agree with the current study. Among the *Staphylococcus* species Coagulase Positive *Staphylococcus* (CPS) species contribute 90.9% and this indicates their important role in the disease occurrence while their counter part Coagulase Negative *Staphylococcus* (CNS) species contribute 9.1% and agrees with the finding made by Milne *et al.* (2002), which is 10%. Though CNS species are considered as less pathogenic the current finding points out that these organisms are now becoming as an important causal agents to bovine clinical mastitis. Bhattacharya (2002) in his study indicated a high incidence of *Staphylococcus* (44.4%) mastitis, which is almost equivalent to the current finding of 42.3%. The isolation of relatively higher number *Staphylococcus aureus* and *Streptococcus agalactiae*, since they contribute 60% to the total isolates, in the two farms indicates that contagious pathogens are the most important causal agents of clinical mastitis in the herds investigated and assures the presence of poor hygienic practice and this agrees with Benits *et al.* (2003), who reported 56.3% of contagious mastitis. In general *Staphylococcus* species were the predominant isolates and this agrees with the findings of Buragohain and Dutta (2000) and Bhattacharya (2002), followed by *Streptococcus* species (Zingeser *et al.*, 1991; Shem *et al.*, 2001; Barbuddhe *et al.*, 2001).

Streptococcus uberis was isolated from 5.8% of clinical cases. Studies conducted by Parkinson *et al.* (2000) indicated that *Streptococcus uberis* was the most commonly isolated pathogen from cows that developed clinical mastitis within 10 days of calving and in another study carried out by Milne *et al.* (2002) 37% was *Streptococcus uberis* from the total isolates. Miltenburg *et al.* (1996) also reported 12.1% *Streptococcus uberis* isolates. Though the current finding indicates that *Streptococcus uberis* is a potential pathogen to the dairy farms.

Bacillus species and *Coryenbacterium bovis* and *Coryenbacterium ulcerans* were the third dominant isolates in the two farms. Isolation of *Bacillus* species from mastitic cases has been reported (Radostits *et al.* 1994). Haile (1995) reported 11.56% of *Bacillus* species, which is greater than the current finding. The finding of *Bacillus* species lies between the findings obtained by Bhattacharya (2002), 15.27% and Barbuddhe *et al.* (2001), 8.14%. In a similar study 1.4% of *Coryenbacterium* species was reported (Haile, 1995) and Tolla (1996) reported 5.3%. However, in the current finding this species accounted 9.6% from the total isolates. *Escherichia coli*, and *Streptococcus dysgalactiae* were isolated from contaminated and mixed clinical cases, respectively. Though environmental pathogens are found in cow's surroundings such as bedding, manure, soil, etc., (Jones and Baily, 1998) this finding was lower compared to the reports made by (Haile, 1995; Tolla, 1996; Bradly and Green, 2001; Markos, 2003).

Mastitis is one of the most frequent diseases affecting dairy cattle (Danusre *et al.*, 1987). Its economical relevance in the dairy industry is a worldwide accepted fact, hence a wide variety of drugs have been used to treat the various clinical forms of bovine mastitis. It always happens as a multi-etiological, multifactorial disease with treatments changing with time Sumano and Ocampo (1992). Antibiotic resistant bacterial strains are increasingly emerging worldwide as a result of abuse or indiscriminate use of antimicrobial drugs that resulted in significant public health problems (Sheers, 1993; Hart and Kariuri, 1998). In this study antimicrobial susceptibility test was conducted on major isolates of bovine clinical mastitis; namely, *Staphylococcus aureus* and *Streptococcus agalactiae*. Commercially available antimicrobial discs; viz, Neomycin (30mg), Oxytetracycline (30mg), Penicillin G (10 IU) and Streptomycin (10mg) of Himedia products and 20% herbal preparations were used for sensitivity test. The results indicated that *Staphylococcus aureus* isolates were resistant to Penicillin G (64.7%) and Oxytetracycline (23.5%) and *Streptococcus agalactiae* isolates were resistant to Oxytetracycline (100%), Neomycin (85.7%) and Streptomycin (85.7%). In Italy, high incidence of resistance to penicillin was observed among *Staphylococcus aureus* strains (Barberio *et al.*, 2001). In India, in one farm many isolates of *Staphylococci* species resistant to penicillin were reported by Buragohain *et al.*, (2000). Zingeser *et al.* (1991) reported 38% resistant *Staphylococcus aureus* isolates to penicillin in Jamaica dairy farms. Haile (1995) indicated 40% resistant *Staphylococcus aureus* to Tetracycline and Tolla (1996) reported 37.5% and 87.5% resistant *Staphylococcus aureus* isolates to Oxytetracycline and Penicillin G, respectively. Haile (1995) reported 66.67% and 33.33% resistant *Streptococcus agalactiae* isolates to Streptomycin and Tetracycline, respectively. Tolla

(1996) reported 60% and 40% resistant *Streptococcus agalactiae* isolates to Streptomycin and Oxytetracycline, respectively. Though, the in *vitro* antibiotic susceptibility test does not exactly reflect the in-*vivo* therapeutic value of the antibiotic (Radositis *et al.*, 1994) the current finding indicates the existence of resistant *Streptococcus agalactiae* and *Staphylococcus aureus* isolates in the two farms and this is in agreement with above authors.

Six medicinal herbs/plants were selected and tested for their effect on the major isolates both resistant and susceptible to conventional antibiotics. The efficacy of each plant was first assessed alone at different concentrations of 40%, 20%, 10%, 5%, 2.5% and 1.25% and at different quantities in powder forms of 0.02g, 0.01g and 0.005g. In the mean time the vehicles used as a solvent to make different concentrations were used as a control and none of them were showed inhibitory effect and the effects obtained from this study purely related to the efficacy of the phytopreparations.

Brucea antidysenterica was effective at a concentration of 40%-10% only on both *Streptococcus agalactiae* and *Staphylococcus aureus* isolates. As to its effect on test organisms this finding is consistent with the finding obtained by Belay (2003), in which he noted inhibition of both organisms at 40%, 20%, 10%, 5% and 2.5% concentration levels. The plate diffusion using powder alone also gave better inhibitory zone at all amounts on both test organisms and this method can be used to evaluate the activity of herbs where standard plain antibiotic discs are not available.

The absolute methanol extract of *Combertum molle* showed a good inhibitory effect on test organisms. There was no work done on this plant previously, especially as antimicrobial on the causal agents of mastitis. It inhibited the growth of *Streptococcus agalactiae* and *Staphylococcus aureus* isolates at all concentrations. It is the only plant observed inhibiting at all concentrations when compared to its partners and showed a wider zone of inhibition than the others. Its mean inhibition zone value is greater to the inhibition zone obtained by Penicillin G to resistant strains of *Staphylococcus aureus* isolates and it is almost twice greater than the mean inhibition zone obtained by Neomycin, Oxytetracycline and Streptomycin against *Streptococcus agalactiae* and *Staphylococcus aureus*. The plate diffusion using powder alone also gave relatively large inhibition zone at all amounts on both test organisms and the inhibition zone was wider than the rest of the plants. The aqueous extract of the plant was not effective as to that of absolute methanol extract. It inhibited *Staphylococcus aureus* at all concentrations (40%-1.25%) and

Streptococcus agalactiae was inhibited only at 40% and 20%. From this simple observation it is possible to hypothesize that different ingredients of the plant/herb have different solubility depending on the solvent used. Hence, the type of solvents used matter for the efficacy of the plant/herb.

Cyphostemma adenocaule inhibited the growth of *Streptococcus agalactiae* and *Staphylococcus aureus* isolates at 40%-5% concentration levels. Sahle (2002) reported the efficacy of this herb at all concentrations with respective mean inhibition zones. The difference encountered might be related to many factors such as collection site, seasonality, age of the plant and procedures followed. Anyhow its repeatability is checked, though there was efficacy variation.

Among the plants tested for their efficacy it is *Persicaria senegalensis* herb that had a better history and trail by other researchers. In this study an inhibition zone up to 5% were recorded on *Streptococcus agalactiae* and *Staphylococcus aureus* isolates. However, a study conducted by Sahle (2001) indicated observable inhibition zones down to 1.25% concentrations. The current finding is not in agreement with his finding concerning the concentration levels. Otherwise, as to its effect on these isolates is consistent. Dagne and Abdicho (2001) found an inhibition zone of about 18mm on *Staphylococcus aureus* using 820micrograms of the herb extract. Similarly, although the quantity used was not equal the herb gave a good inhibitory effect on *Streptococcus agalactiae* and *Staphylococcus aureus* isolates in this study.

Plantago lanceolata was another test material and it didn't show visible inhibitory zone on *Streptococcus agalactiae* and *Staphylococcus aureus* isolates. Desta (1995) indicated its antimicrobial effect and Belay (2003) reported the effect of the leaf at 40%, 20% and 5% and from 40%-1.25% concentrations against *Staphylococcus aureus* and *Streptococcus agalactiae* isolates, respectively.

A popular herb by many traditional healers and communities in various parts of the country called *Zehneria scabra* was tested for its effect as antimicrobial against *Streptococcus agalactiae* and *Staphylococcus aureus* isolates. Like to *Plantago lanceolata* visible zone of inhibition was not recorded. The current finding is contrary to the findings obtained by Belay (2003). He noted inhibition zone at all concentrations against *Streptococcus agalactiae* and *Staphylococcus aureus* isolates with a wider mean zone of inhibition.

The variation of efficacy among the above-mentioned phytopreparations could be attributed to the way of plant preparation, season of collection, stage of the plant, place of collection, way of extract drying, means of extraction, solvent used, preservation or storage of the extract till evaporation and other unnoticed factors.

Finally, 20% phytopreparations from the different plants were compared with conventional antimicrobial discs and the efficacy of these preparations at the mentioned concentration was satisfactory. The mean inhibition zone (to the nearest value) obtained by *Brucea antidysenterica* was (7mm and 8mm), *Combertum molle* (21mm and 19mm), *Cyphostemma adenocaule* (11mm and 11mm) and *Persicaria senegalensis* (11mm and 11mm) against *Staphylococcus aureus* and *Streptococcus agalactiae*, respectively. At this concentration a better inhibitory effect was observed by *Combertum molle* against the test organisms. The effect of *Cyphostemma adenocaule* and *Persicaria senegalensis* against the test organisms was the same and it approximately half to the effect obtained by *Combertum molle* against *Staphylococcus aureus*. The least mean inhibitory zone was recorded by *Brucea antidysenterica* against test organisms. The effect of this plant showed variation in its effect and as it is mentioned earlier the capacity of the plant extract might be incapable of penetrating through the media. The result ranged from 0-25mm and that is why the mean inhibition zone is smaller.

Comparison of herbal preparations with conventional antimicrobial discs was made only by the size of mean zone of inhibition obtained by each test materials against test organisms. The mean inhibition zone obtained by Oxytetracycline was almost comparable to that obtained by *Combertum molle* and the distance obtained by *Cyphostemma adenocaule* and *Persicaria senegalensis* were half of the drug while *Brucea antidysenterica* showed one-third inhibitory zone of that of Oxytetracycline, which is a big difference.

The mean inhibition zone obtained by Penicillin G on *Streptococcus agalactiae* was so large and not comparable to none of the phytopreparations. But the mean inhibition zone obtained on *Staphylococcus aureus* was comparable to *Combertum molle* and *Cyphostemma adenocaule* and *Persicaria senegalensis* gave a half mean inhibition zone to Penicillin G and as usual *Brucea antidysenterica* was the least herb having lowest mean zone of inhibition.

The mean inhibition zone obtained by Streptomycin against *Staphylococcus aureus* was almost comparable to that of *Combertum molle* but the mean inhibitory zone obtained on *Streptococcus*

agalactiae were smaller and almost half to the result obtained by *Combertum molle*. The mean inhibitory zone obtained by the drug against *Streptococcus agalactiae* was comparable to that obtained by *Cyphostemma adenocaule* and *Persicaria senegalensis* and is a bit greater from that of *Brucea antidysentrica*. On the other hand the inhibition zone obtained by the drug against *Staphylococcus aureus* was greater than the inhibition zone obtained by *Cyphostemma adenocaule*, *Persicaria senegalensis* and then to *Brucea antidysentrica*. The mean inhibition zone obtained by Neomycin follows a similar pattern to that of Streptomycin.

The comparison among these test materials suggests that the herbal preparations do have a capacity to inhibit the growth of test organisms with a similar or a different manner to that of conventional antimicrobial agents, though there is no established standard formulae to judge the level of zone of inhibition to say resistant, intermediate and susceptible for phytopreparations.

As antibiotic use increases in veterinary medicine, the issue of bacterial resistance to antimicrobial therapy becomes more worrisome. The emergency of increasing numbers of antibiotic resistant pathogens has implications for not only veterinary patients, but also humans (Hoffman, 2001). The mean inhibition zone obtained by Penicillin G against resistant isolates of *Staphylococcus aureus* was lower to that obtained by *Combertum molle*. The mean inhibition zone obtained by Oxytetracycline against *Staphylococcus aureus* was smaller than that obtained by *Combertum molle* and still it was less than the result obtained by *Cyphostemma adenocaule* and *Persicaria senegalensis*.

Combertum molle showed a greater inhibitory zone to Neomycin, Oxytetracycline and Streptomycin resistant isolates of *Streptococcus agalactiae*. Almost nearly equivalent mean zone of inhibition were noted by *Brucea antidysentrica*, *Cyphostemma adenocaule* and *Persicaria senegalensis* to that of Oxytetracycline, Neomycin and Streptomycin against the organism, though the mean values for Neomycin and Streptomycin were a bit lower than *Cyphostemma adenocaule* and *Persicaria senegalensis* and a bit greater than that of *Brucea antidysentrica*.

From this result its possible to suggest that phytopreparations could play an important role in the treatment of resistant isolates of mastitis causal agents and it could be the alternative solution to prevent the problem of an ever emerging resistant isolates in any disease situation.

6. CONCLUSION

There was an incidence rate of 9 new clinical cases of bovine mastitis/100cows-month at risk during the six month of study period and this indicated that mastitis is a great problem in the two dairy farms. This may hamper the expansion of dairy development in the country. Adoption of good milking and management practices could alter the situation.

In both farms mild (Grade I) mastitis cases were most common and this needs serious attention and follow-ups especially during milking times, because they might be passed un noticed and in turn affects the milk quality and quantity and above all might serve as a contaminant to the environment and spread of infection to healthy quarters and cows.

Cultural identifications revealed the involvement of single, mixed and multiple causal agents in the disease process and single infection was the predominant one although the contribution of mixed infections could not be undermined. The finding of mixed or multiple isolates indicated that before any attempt to treat clinical cases, aetiological agent identification seems important giving a chance to select drug/s having a satisfactory result against them.

Staphylococcus aureus and *Streptococcus agalactiae* were predominant contributed for 60% of the isolates. This indicates that contagious mastitis is important in the two dairy farms and in general this shows the poor hygienic status in the two dairy farms.

Resistant isolates of *Staphylococcus aureus* to Penicillin G and Oxytetracycline and *Streptococcus agalactiae* to Neomycin, Streptomycin and Oxytetracycline were found in the two dairy farms. Resistance development to the drugs might be due to continuous use of these drugs for any clinical cases and they should ready themselves to use drugs alternatively before things became more worthy.

Out of six herbs tested in *vitro* four inhibited the growth of both susceptible and resistant isolates of *Staphylococcus aureus* and *Streptococcus agalactiae* at different concentrations and this result indicated their future potential use in the synthesis of new medicaments. The current result, in fact needs to be assessed for its repeatability and further detailed study, like phytochemistry, toxicity, cytotoxicity, and etc., is highly mandatory. One way to control drug resistant problems

is through the development of alternative antimicrobials by screening and testing medicinal plants for their possible antimicrobial effects. Wide spread use of antibiotic for the treatment of bovine mastitis has a potential to cause contamination of milk, which has become a subject of public concern, therefore medicinal herbs/plants are natural and safe approaches to alleviate the problems.

Among the herbs/plants the absolute methanol extract of *Combertum molle* performed well against test organisms. The efficacy of the plant was assessed for the first time and many works are expected to be done on this plant. On the contrary *Brucea antidysenterica* showed a variable effect against test organisms.

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8. APPENDICES

8.1: General farm characteristics

No	Description	Farm		Comments
		EARO	Fair-Field	
1	Replacement	From farm	From farm	
2	Ownership	Government	Private	
3	Purpose	Research	Business	
4	Milking paroul	Available	Not available	
5	Pre-milking wash	Yes	Yes	
6	Drying after washing	No	No	
7	Post-milking dipping	No	Yes	
8	Management	Intensive	Intensive	
9	Milking practice	Hand	Hand	
10	Milking time (frequency)	Two times	Two times	Morning & evening
11	Tick infestation	Minimal	Minimal	Sprayed
12	Wound on the udder	Two cases	No case	
13	Dry cow follow-ups	Yes	Yes	
14	Dry cow treatment	No	No	
15	Feeding	Stall & occasional grazing	Stall & grazing	
16	Watering	Using trough	Using trough	
17	Culling of cows with repeated attack of mastitis in a single lactation period	No	No	
18	Medicaments used to treat mastitic cases	*Mastitis injector, penstrep and oxytetracycline	*Mastitis injector, penstrep and oxytetracycline	* Principally containing penicillin G, streptomycin and neomycin
19	Barren sanitation	Yes	Yes	
20	Animal wash	No, during calving only	Yes	
21	Visual checking of mastitis	Yes	Yes	During milking
22	California Mastitis Test screening	No	No	
23	Breed	Holstein, Barka, Boran cross	Holstein with Boran cross many years back	
	Calf feeding	Bucket	Bucket	At early stage calves are allowed to suckle
29	Fate of male calves	Sold	Sold	

EARO: Ethiopian Agricultural Research Organization

8.2. Biochemical tests (Carter, 1984, Quinn et al., 1999)

Catalase test: The test detects the enzyme catalase that converts hydrogen peroxide to water and gaseous oxygen. Hydrogen peroxide was kept at 4^{0}C in a dark bottle. Bacteria grown on blood agar were not used for the test since false positive reactions is a possible result.

a. A loop of a colony was taken from the nutrient medium
b. Placed on a clean slide
c. Three percent hydrogen peroxide was dropped
d. Effervescence of oxygen gas within few seconds indicate a positive reaction

Oxidase test: The test depends on the presence of cytochrome oxidase in bacterial cell. Anaerobes are oxidase negative. Reagents were stored at 4^{0}C, in a dark bottle.

a. A piece of filter paper was moistened in a petri dish with 1% aqueous solution of tetramethyl-p-phenylenediamine dihydrochloride.
b. The test bacterium was streaked across the filter paper with a glass rod
c. Dark purple colour along the streak line within 10 seconds indicated a positive reaction.

Motility test:

a. A hanging drop preparation was made by placing a drop of the broth culture on the centre of the clean cover slip
b. It was inverted over a transparent plastic or glass ring (about 5mm deep) fixed to a microscope slide
c. Observation was made under low power magnification and then with the high power dry objective

KOH test:

1. A loopful of the culture from a non-selective medium (blood agar) was put on a clean slide
2. An equal amount of 3% potassium hydroxide (KOH) was added
3. It was mixed thoroughly
4. Observation of the formation of a gel was made by lifting the loop at intervals
5. A vicious gel formation observation within 60 seconds was an indication of Gram-negative bacteria.

CAMP test:

1. A beta haemolytic *Staphylococci aureus* was streaked with a loop down the middle of a blood agar
2. The *streptococci* under study was streaked at 90^0 to the *Staphylococci*
3. A known *Streptococcus agalactieae* was also streaked in a similar way to serve as a positive control
4. Plates was incubated at 37^0C overnight
5. Plates were examined for the presence of characteristic "arrow head" of complete clearing of the beta haemolytic zone. *Streptococcus agalactieae* is positive for CAMP test.

Coagulase test:

A. Slide coagulase test

1. A loop of the *Staphylococcal* culture was emulsified in a drop of distilled water on a slide
2. A loopful of rabbit plasma was added and mixed well with the bacterial suspension
3. The slide was gently rocked
4. Observation of clumping was made within 1-2 minutes

B. Tube coagulase test

1. 0.5 ml of rabbit plasma was placed in a small (7mm) test tube
2. Two drops of an over night broth culture of the *Staphylococcus* or a heavy suspension made from the culture on an agar plate in sterile water was added
3. The tube was rotated gently to mix the contents
4. The tube then was incubated at 37^0C
5. Clotting of plasma was observed within 2-4hrs and if not, overnight incubation was performed.

IMVIC Test

1. Methyl Red test

   To 5ml of culture grown on MR-VP broth incubated for 48hrs at 37^0C 5 drops of Methyl Red solution was added and a positive reaction indicated by a distinct red colour indicating acidity (Ph= 4.4-6.0).

2. Vogous-Proskauer test

   From one milliliter of a 48hrs culture incubated at 37^0C in MR-VP broth 0.6ml of 5% alpha-naphtol solution was added, then 0.2ml of 40% KOH containing 0.3% creatine was added. The solution was shaked well and left for 5-10 minutes. Development of bright orange or a cherry red color developed that gradually extends through out the broth was considered as positive for the test.

3. Indole test

   One millilitre of ether was added to a 5ml portion of a 48hrs culture grown at 37^0C in a peptone water and shaked well and allowed to stand until the ether rises to the top. Gently, Kovac's reagent was added down the side of the test tube and the formation of brilliant red ring between the medium and ether was indicative of an indole production.

4. Citrate utilizations

Simmon citrate agar (Difco, USA) slope surface was streaked with the suspected bacterial colonies and incubated at 37^0C for 24hours.Typical reaction for citrate utilization and positivity was declared by the change of the medium from green to blue color (Quinn *et al.*, 1999).

8.3: Media used

1. Blood agar base (Merck, Germany)

Composition (g/l): Nutrient substrate (heart extract and peptones) 20.0; sodium chloride 5.0; agar-agar 15.0.

Preparations

Forty grams was suspended in 1litre of deminiralized water by heating in a boiling water bath and autoclaved at 121^{0}c for 15 minutes. Cooled to 45-50^{0}C and 5-8% sterile defibrinated blood was added and mixed taking care to avoid bubble formation. Poured to plates. Ph 6.8 $\pm$ 0.2 at 25^{0}C.

2. Simon's citrate agar

Composition (g/l) approximate formula per liter purified water

Ammonium dihydrogen phosphate	1.0
Dipotassium phosphate	1.0
Sodium chloride	5.0
Sodium citrate	2.0
Magnesium sulphate	0.2
Agar	15.0
Bromothymol blue	0.08
Ph	6.9$\pm$0.2

Preparation: Twenty four point two (24.2) grams of Simmons citrate agar was suspended in one liter distilled water, boiled to dissolve completely, sterilized at 121^{O}C for 15 minutes and dispensed into test tubes and allowed the medium to solidify to give slant agar tubes.

3. Edwards medium (modified) 500g, Oxoid, England.

Composition (g/l): 'Lab-Lemco' powder 10.0; peptone 10.0; asculin 1.0; sodium chloride 5.0; crystal violet 0.0013; thallous sulphate 0.3; agar 15.0.

Preparation

Forty-one grams of the media was suspended in 1liter of distilled water. Brought to the boil to dissolve completely. Sterilized by autoclaving at 115^{0}C for 20 minutes. Cooled to 50^{0}Cand 5-7% of sterile sheep blood was added and mixed well and poured to plates. Ph 7.4$\pm$ 0.2.

4. Nutrient agar (500g), Oxoid, England.

Composition (g/l): 'Lab-Lemco' powder 1.0;m yeast extract 2.0; peptone 5.0; sodium chloride 5.0; agar 15.0.

Preparation

Twenty grams of the media was suspended in 1litre of distilled water. Brought to the boil to dissolve completely. Sterilized by autoclaving at 121^{0}C for 15 minutes. Ph 7.4$\pm$ 0.2.

5. OF- basal medium (oxidation- fermentation) (500g) Merck, Germany.

Composition (g/l): Peptone from casein 2.0; yeast extract 1.0; sodium chloride 5.0; dipotasssium hydrogen phosphate 0.2; bromothymol blue 0.08; Agar-agar 2.5.

Preparation

Eleven grams of the media was suspended in 1litre of demineralised water by heating in a boiling water bath and autoclaved for 15 minutes at 121^{0}C; at approximately 50^{0}C 100ml/lt of a filter sterilized 10% solution of D (+) glucose. Dispensed into tubes to give depth of approximately 5cm, in half of the tubes immediately overlaid the medium with a 1cm layer of sterile paraffin viscous. Ph 7.1$\pm$ 0.2.

6. MR-VP broth (Methyl Red- Vogos-prouskauer) (500g), Merck, Germany

Composition (g/l): Peptone from meat 7.0; D(+) glucose 5.0; tampon phosphate 5.0.

Seventeen grams of the media was suspended in 1litre of demineralised water; dispensed into 5ml portions into tubes and was autoclaved for 15 minutes at 121^{0}C. Ph 6.9$\pm$ 0.2.

7. Phenol red broth base (500g), Merck, Germany.

Composition (g/l): Phosphate from casein 5.0; peptone from meat 0.5; sodium chloride 5.0; phenol red 0.018.

Preparation

Fifteen grams of the media was suspended in 1litre of demineralised water and dispensed into tubes, and then was autoclaved for 15 minutes at 121^0C; at less than 60^0C the reactants were added (final concentrations 5-10g/l) as sterile solutions. Ph 7.4$\pm$ 0.2.

8. Mac Conkey (500g), Merck, Germany.

Composition (g/l): Peptone from casein 17.0; peptone from meat 3.0; sodium chloride 5.0; lactose 10.0; bile salt mixture 1.5; neutral red 0.031; crystal violet 0.001; agar-agar 13.5.

Fifty grams was Suspended in 1litre of demineralised water by heating in boiling water bath and autoclaved for 15 minutes at 121^0C. Ph 7.1$\pm$ 0.2.

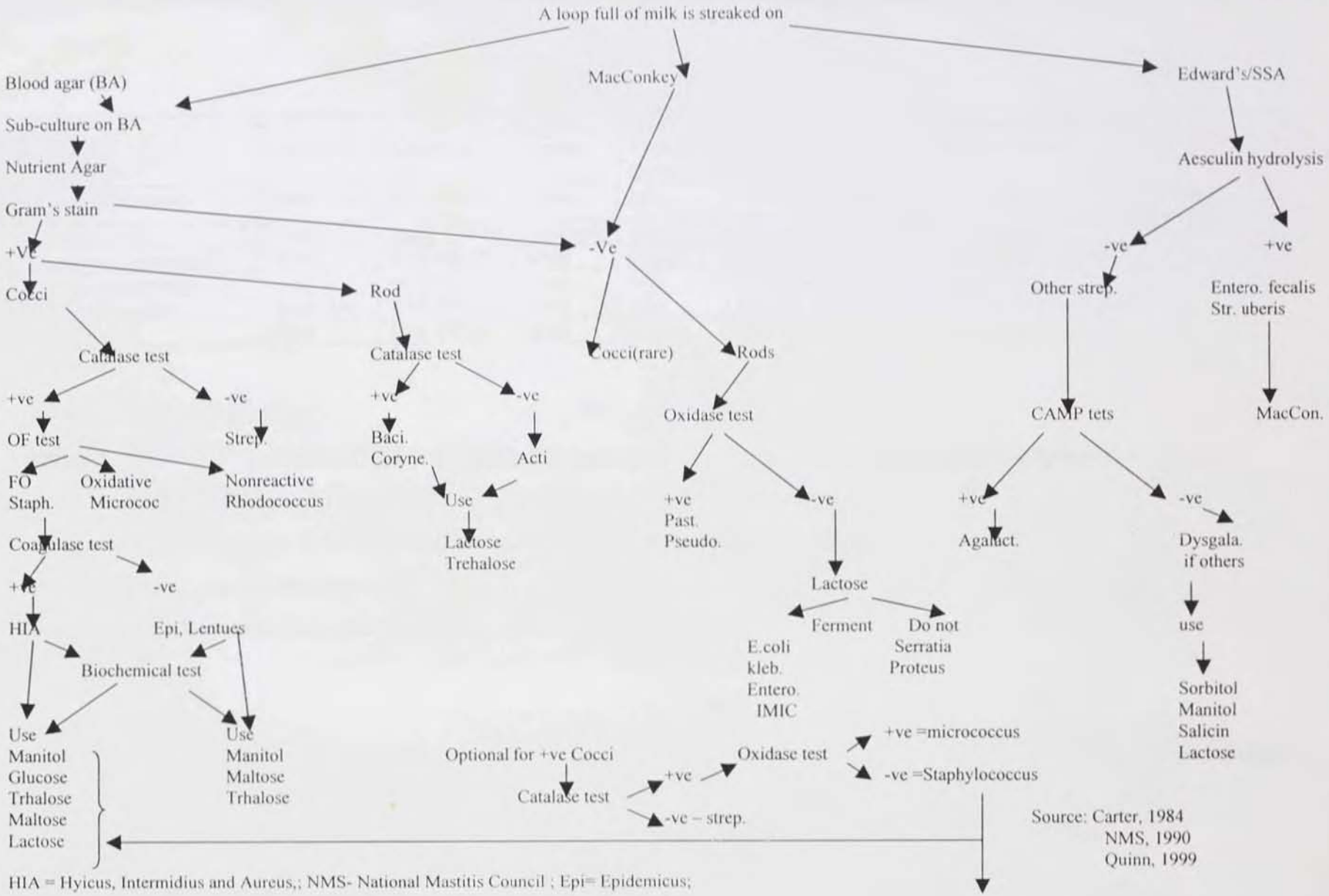
9. Buffered peptone water (BPW) (Sifin, Germany)

Typical composition (g/liter): Peptone from casein10.0; Sodium chloride 5.0; Di-sodium hydrogen phosphate 3.5; Potassium dihydrogen phosphate1.5
Preparation: Twenty grams of this media was dissolved in one liter of distilled water and sterilized by autoclaving at 121^OC for 15 minutes.

10. Tryptic soy agar (DIFCO TM, 500g)

Composition	
Pancreatic digest of casein	15.0g
Enzymatic digest of soybean meal	5.0g
Sodium chloride	5.0g
Agar	15.0g

Forty grams of the powder was suspended in 1lt of purified water and mixed thoroughly. It was heated with frequent agitation and boiled for 1 minute to completely dissolve the powder and autoclaved at 121^0C for 15 minutes and then dispended to sterile petridishs after reaching at 50^0C.


A loop full of milk is streaked on
Blood agar (BA)
MacConkey
Edward's/SSA
Sub-culture on BA
Nutrient Agar
Gram's stain
Aesculin hydrolysis
+Ve
-Ve
-ve
+ve
Cocci
Rod
Other strep.
Entero. fecalis
Str. uberis
Catalase test
Catalase test
Cocci(rare)
Rods
+ve
-ve
+ve
-ve
Oxidase test
CAMP tets
MacCon.
OF test
Strep.
Baci.
Coryne.
Acti
FO
Staph.
Oxidative
Micrococ
Nonreactive
Rhodococcus
Use
Lactose
Trehalose
+ve
Past.
Pseudo.
-ve
+ve
Agalact.
-ve
Dysgala.
if others
Coagulase test
Lactose
+ve
-ve
HIA
Epi, Lentues
Ferment
Do not
E.coli
kleb.
Entero.
IMIC
Serratia
Proteus
use
Biochemical test
Sorbitol
Manitol
Salicin
Lactose
Use
Manitol
Glucose
Trhalose
Maltose
Lactose
Use
Manitol
Maltose
Trhalose
Optional for +ve Cocci
Oxidase test
+ve =micrococcus
Catalase test
+ve
-ve =Staphylococcus
-ve – strep.
Source: Carter, 1984
NMS, 1990
Quinn, 1999
HIA = Hyicus, Intermidius and Aureus,; NMS- National Mastitis Council ; Epi= Epidemicus;

8. 5: Means of extractions and their effects on major bacterial isolates and problems encountered

Plant/herb	Part used	Extractions	Drying	Characters during drying	20% amount used	Observed efficacy	Problems during mixing with the solvent
Brucea antidysenterica	Root + leaf	80% MEOH	Oven	Jelly	3 drops	Variable	No problem
Combertum molle	Leaf	100% MEOH	Incubator	Crystal	3 drops	Excellent	No problem
Cyphostemma adenecuale	Root	80% MEOH	Oven	Crystal	3 drops	Good	Very difficult to mix
Persicaria senegalensis	Leaf	80% MEOH	Oven	Jelly	3 drops	Good	No problem
Plantiago lanceolata	Seed, leaf	80% MEOH	Oven	Jelly	3 drops^{x}	No	Difficult to mix the seed
Zehneria scabra	Root	80% MEOH	Oven	Crystal	3 drops^{x}	No	Not simple

MEOH= Aqueous Methanol

Oven = at 30-40^{0}C, the oven was fluctuating during 24 hours time depending on the environmental temperatures variation

Incubator = at 37^{0}C over night by using petridishes with an amount of about 20 ml

Jelly substances took more time to dry and became more jelly upon prolonged drying

3 drops^{x} = Even more amount were used

Both herbs were not showing visible inhibitory effect on *Escherichia coli*.

8.6: Multiple comparison results

Multiple Comparisons for *staphylococcus aurues*

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	4694.875	7	670.696	23.715	.000
Within Groups	3620.000	128	28.281		
Total	8314.875	135			

Dependent Variable: Zone of inhibition
Bonferroni

(I) TEST	(J) TEST	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
1	2	-12.94(*)	1.824	.000	-18.76	-7.12
	3	-3.29	1.824	1.000	-9.11	2.53
	4	-3.71	1.824	1.000	-9.53	2.11
	5	-14.06(*)	1.824	.000	-19.88	-8.24
	6	-14.06(*)	1.824	.000	-19.88	-8.24
	7	-16.76(*)	1.824	.000	-22.58	-10.94
	8	-12.29(*)	1.824	.000	-18.11	-6.47
2	1	12.94(*)	1.824	.000	7.12	18.76
	3	9.65(*)	1.824	.000	3.83	15.47
	4	9.24(*)	1.824	.000	3.42	15.06
	5	-1.12	1.824	1.000	-6.94	4.70
	6	-1.12	1.824	1.000	-6.94	4.70
	7	-3.82	1.824	1.000	-9.64	2.00
	8	.65	1.824	1.000	-5.17	6.47
3	1	3.29	1.824	1.000	-2.53	9.11
	2	-9.65(*)	1.824	.000	-15.47	-3.83
	4	-.41	1.824	1.000	-6.23	5.41
	5	-10.76(*)	1.824	.000	-16.58	-4.94
	6	-10.76(*)	1.824	.000	-16.58	-4.94
	7	-13.47(*)	1.824	.000	-19.29	-7.65
	8	-9.00(*)	1.824	.000	-14.82	-3.18
4	1	3.71	1.824	1.000	-2.11	9.53
	2	-9.24(*)	1.824	.000	-15.06	-3.42
	3	.41	1.824	1.000	-5.41	6.23
	5	-10.35(*)	1.824	.000	-16.17	-4.53
	6	-10.35(*)	1.824	.000	-16.17	-4.53
	7	-13.06(*)	1.824	.000	-18.88	-7.24
	8	-8.59(*)	1.824	.000	-14.41	-2.77
	7	-2.71	1.824	1.000	-8.53	3.11
	8	1.76	1.824	1.000	-4.06	7.58

(I) TEST	(J) TEST	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
5	1	14.06(*)	1.824	.000	8.24	19.88
	2	1.12	1.824	1.000	-4.70	6.94
	3	10.76(*)	1.824	.000	4.94	16.58
	4	10.35(*)	1.824	.000	4.53	16.17
	6	.00	1.824	1.000	-5.82	5.82
	7	-2.71	1.824	1.000	-8.53	3.11
	8	1.76	1.824	1.000	-4.06	7.58
6	1	14.06(*)	1.824	.000	8.24	19.88
	2	1.12	1.824	1.000	-4.70	6.94
	3	10.76(*)	1.824	.000	4.94	16.58
	4	10.35(*)	1.824	.000	4.53	16.17
	5	.00	1.824	1.000	-5.82	5.82
	7	-2.71	1.824	1.000	-8.53	3.11
	8	1.76	1.824	1.000	-4.06	7.58
7	1	16.76(*)	1.824	.000	10.94	22.58
	2	3.82	1.824	1.000	-2.00	9.64
	3	13.47(*)	1.824	.000	7.65	19.29
	4	13.06(*)	1.824	.000	7.24	18.88
	5	2.71	1.824	1.000	-3.11	8.53
	6	2.71	1.824	1.000	-3.11	8.53
	8	4.47	1.824	.437	-1.35	10.29
8	1	12.29(*)	1.824	.000	6.47	18.11
	2	-.65	1.824	1.000	-6.47	5.17
	3	9.00(*)	1.824	.000	3.18	14.82
	4	8.59(*)	1.824	.000	2.77	14.41
	5	-1.76	1.824	1.000	-7.58	4.06
	6	-1.76	1.824	1.000	-7.58	4.06
	7	-4.47	1.824	.437	-10.29	1.35

* The mean difference is significant at the .05 level.

Multiple Comparisons for *Streptococcus agalactiae*

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	6348.536	7	906.934	155.499	.000
Within Groups	606.571	104	5.832		
Total	6955.107	111			

Dependent Variable: Zone of inhibition
Bonferroni

(I) TEST	(J) TEST	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
1	2	-11.86(*)	.913	.000	-14.78	-8.93
	3	-2.36	.913	.314	-5.28	.57
	4	-2.57	.913	.162	-5.50	.36
	5	-2.00	.913	.859	-4.93	.93
	6	-4.64(*)	.913	.000	-7.57	-1.72
	7	-23.93(*)	.913	.000	-26.86	-21.00
	8	-1.64	.913	1.000	-4.57	1.28
2	1	11.86(*)	.913	.000	8.93	14.78
	3	9.50(*)	.913	.000	6.57	12.43
	4	9.29(*)	.913	.000	6.36	12.21
	5	9.86(*)	.913	.000	6.93	12.78
	6	7.21(*)	.913	.000	4.29	10.14
	7	-12.07(*)	.913	.000	-15.00	-9.14
	8	10.21(*)	.913	.000	7.29	13.14
3	1	2.36	.913	.314	-.57	5.28
	2	-9.50(*)	.913	.000	-12.43	-6.57
	4	-.21	.913	1.000	-3.14	2.71
	5	.36	.913	1.000	-2.57	3.28
	6	-2.29	.913	.387	-5.21	.64
	7	-21.57(*)	.913	.000	-24.50	-18.64
	8	.71	.913	1.000	-2.21	3.64
4	1	2.57	.913	.162	-.36	5.50
	2	-9.29(*)	.913	.000	-12.21	-6.36
	3	.21	.913	1.000	-2.71	3.14
	5	.57	.913	1.000	-2.36	3.50
	6	-2.07	.913	.709	-5.00	.86
	7	-21.36(*)	.913	.000	-24.28	-18.43
	8	.93	.913	1.000	-2.00	3.86

(I) TEST	(J) TEST	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
5	1	2.00	.913	.859	-.93	4.93
	2	-9.86(*)	.913	.000	-12.78	-6.93
	3	-.36	.913	1.000	-3.28	2.57
	4	-.57	.913	1.000	-3.50	2.36
	6	-2.64	.913	.129	-5.57	.28
	7	-21.93(*)	.913	.000	-24.86	-19.00
	8	.36	.913	1.000	-2.57	3.28
6	1	4.64(*)	.913	.000	1.72	7.57
	2	-7.21(*)	.913	.000	-10.14	-4.29
	3	2.29	.913	.387	-.64	5.21
	4	2.07	.913	.709	-.86	5.00
	5	2.64	.913	.129	-.28	5.57
	7	-19.29(*)	.913	.000	-22.21	-16.36
	8	3.00(*)	.913	.039	.07	5.93
7	1	23.93(*)	.913	.000	21.00	26.86
	2	12.07(*)	.913	.000	9.14	15.00
	3	21.57(*)	.913	.000	18.64	24.50
	4	21.36(*)	.913	.000	18.43	24.28
	5	21.93(*)	.913	.000	19.00	24.86
	6	19.29(*)	.913	.000	16.36	22.21
	8	22.29(*)	.913	.000	19.36	25.21
8	1	1.64	.913	1.000	-1.28	4.57
	2	-10.21(*)	.913	.000	-13.14	-7.29
	3	-.71	.913	1.000	-3.64	2.21
	4	-.93	.913	1.000	-3.86	2.00
	5	-.36	.913	1.000	-3.28	2.57
	6	-3.00(*)	.913	.039	-5.93	-.07
	7	-22.29(*)	.913	.000	-25.21	-19.36

* The mean difference is significant at the .05 level.

1= *Brucea antidysenterica*
2= *Combertum molle*
3= *Cyphostemma adenocaule*
4= *Persicaria senegalensis*
5= Neomycin
6= Oxytetracycline
7= Penicillin G
8= Streptomycin

9. CURRICULUM VITAE

1. Personal data

   Name : Araya Mengistu Kassa

   Date of birth : July 27 1967

   Birth place: Debretabor (South Gondar, Amhara National Regional State)

   Sex: Male

   Occupation: Civil servant

   Nationality: Ethiopian

2. Academic background

   Primary education :1975-1979, Shime Mariam Elementary School- Dera District, South Gondar- Arbgebiya.

   Junior Secondary High School : 1980-1981, Fasilo Junior Secondary high School - Bahir- Dar

   Secondary High School : 1982-1985, Lake Tana Secondary High School- Bahir-Dar

   University : 1986-1991, Addis Ababa University, Faculty of Veterinary Medicine.

3. Work experience

   Served as district veterinarian in North Gonadr zone: 1992- 1998

   Worked as junior research officer in Kombolcha regional veterinary: 1999-2002

   Postgraduate program and research work, Addis Ababa University, Faculty of Veterinary Medicine- 2003-2004.

4. Language proficiency: Amharic and English – Speaking and writing.

5. Research work: 1. Anticoagulant effect of *Allium sativum* in comparison with EDTA, DVM thesis.

   2. Ethnoveterinary knowledge and practices in South Wollo Zone.

6. Training: Computer science (Microsoft Word, Excel and access)

7. Publications: Fente, T., Mengistu , A. and Abeto, G . (2002): Ethnoveterinary knowledge and practices in South Wollo Zone. Proceeding of the Ethiopian Veterinary Association. 15th Annual Conference. Addis Ababa.

10. SIGNED DECLARATION SHEET

I under sign, declare that the thesis is my original work and has not been presented for a degree in any university.

Name Araya Mengistu

Signature_____

Date of Submission June 15 2004

This thesis has been submitted for examination with our approval as university advisors.

Dr Fekadu Regassa _____

Dr Ademe Zerihun _____

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This thesis has been submitted for examination with our approval as university advisors.

Dr Fekadu Regassa _____ 15.06.2004

Dr Ademe Zerihun _____ 15-06-2004

The Effect of Herbal preparations on st appylococcus Aureus & Streptococcus Agalactiae isolated from clinical bovine Mastitis

Araya Mengistu