ADDIS ABABA UNIVERSITY

COLLEGE OF HEALTH SCIENCES

SCHOOL OF VETERINARY MEDICINE

A PREVALENCE STUDY ON BOVINE MASTITIS IN SELECTED AREAS OF ETHIOPIA AND EFFICACY TEST OF MEDICINAL PLANTS ON COMMONLY ISOLATED PATHOGENS OF BOVINE MASTITIS

BY

MANDEFRO DARGIE BEZABIH

JUNE, 2010

DEBRE ZEIT, EHIOPIA

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A thesis submitted to Addis Ababa University, College of Heath Science, School of Veterinary Medicine in partial fulfillment of requirements of Degree of Master in Tropical Veterinary Epidemiology

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Board of External Examiners:	**Signature**
Dr. Alemu Yami	_____
Prof. Tesfu Kassa	_____
Dr. Desalegn Lidetu	_____
Prof. Damen H/mariam	_____

Advisors:

Yasmin Jibril (DVM, MSc, Asst.Prof.)	_____
Fekadu Regassa (DVM, PhD, Asso. Prof.)	_____
Akafete Teklu (DVM, MSc, Asst.Prof.)	_____

ABSTRACT

The study was conducted with an objective of determining prevalence of mastitis in local zebu and cross breed lactating cows and assessing efficacy of seven phytopreparatons on commonly isolated pathogens of bovine mastitis. The study was based on 369 lactating cows with in small-scale extensive and semi-intensive husbandry practices in two selected highland and midland areas of Amhara region and in a large-scale intensive farm in Algae ATVET College. During sampling, each quarter was inspected for clinical case of mastitis and quarter milk from each cow was tested with CMT for sub-clinical mastitis. Plants were collected from their natural habitat, prepared, extracted and impregnated for efficacy test on (n=12) isolates of *Staphylococcus aureus* and *Streptococcus agalactiae*. With combined clinical inspection and CMT test, an overall prevalence of mastitis was 50.7% (16.0% clinical and 34.7% sub clinical) are cow base and 39.8% (6.3% clinical and 33.5% sub-clinical) at quarter level. A binary logistic regression analysis showed statistical associations of intensive farms (OR= 10.727, p= 0.000) with the disease than extensive husbandry practices. Cows at third and above lactation were statistically associated (OR= 5.418, p = 0.000) with mastitis than cows at first lactation. From seven plants tested for efficacy Bereza, *Commicarpus pediculosus*, *Cyphostemum adenocaule* and *Xanthium stramarium* showed visible zone of inhibitions. There was a significant difference observed within mean zone of inhibition of plant types. Mean zone of inhibition from 20% *Commicarpus pediculosus* (29.00mm) was found statistically higher than (P<0.01) mean from 20% Bereza (19.83mm). (P<0.01mm) mean from 20% *Cyphostemum* (26.17mm) mean zone of inhibition from 20% *Commicarpus pediculosus* was also found statistically higher (P<0.001) than mean from penicillin G resistant isolates (19.13mm) and (P<0.001) than mean from oxytetracycline resistant isolates (11.25mm), 66.7% and 33.3% of the isolates of *Staphylococcus aureus* were resistant to penicillin G and oxytetracycline, respectively. The higher prevalence of mastitis and drug resistance development of isolates results economic loss to increase in a farm with a subsequent effect at national level. To combat the loss an effective control program needs to b e implemented. Finding alternatives for drug resistance are of the actions taken for the control program. *In-vitro* experiment result of plant efficacy in this study will be used as an input to solve the problem.

Key words: Breed/ Cow/Drug resistance/Economic loss/Efficacy/Extraction/ Impregnation/ Plants/ Prevalence/ Mastitis/ Mean

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LIST OF ABBREVIATIONS

ANOVA	Analysis of Variance
ANRS	Amhara National Regional State
ATVTE	Agricultural Technical and Vocational Training Education
CI	Confidence Interval
Cm	Centimeter
CMT	California Mastitis Test
CSA	Central Statistics Agency
DMSO	Di Methyl Salfoxanide
DVM	Doctor of Veterinary Medicine
E	East
Gm	Gram
IIRR	International Institute of Rural Reconstruction
ITDG	Intermediate Technology Development Group
Km	Kilometer
M	Meter
M.a.s.l	meters above sea level
MHA	Muller – Hinton
ml	Mililiter
MSc	Master of science
OR	Odds Ratio

P	P-value
PH	Power of hydrogen
Pp	Page
SCC	Somatic Cell Count
SPSS	Software Program for Social Science
TSB	Tryptic Say Broth
UK	United Kingdom
USA	United States of America

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Key words: Breed/ Cow/Drug resistance/Economic loss/Efficacy/Extraction/ Impregnation/ Plants/ Prevalence/ Mastitis/ Mean

1. INTRODUCTION

Ethiopia has high livestock resources potential, estimated at 47 million cattle, 26.12 million sheep, 21.7 million goats and 0.38 million camels (CSA, 2008). In spite of the potential, the expected benefit from livestock sub-sector seems minimal. The lower productivity are associated with both non-technical and technical constraints including inadequate year- round feed resources, losses from wide spread livestock diseases interacting with a lack of inadequate veterinary services, low input husbandry practices under which they are reared, low genetic potential for milk production of the indigenous livestock breeds and poor access to input and out put market services (Gary, 2001).

Global drivers of the livestock sector include economic growth and income, demographic and land use changes, dietary adjustments and technology change (Steinfeld *et al.*, 2006). Too much livestock product demand as compared to its supply having limited product demand as compared to its supply having limited land, favors increase productivity through intensification in urban and per urban areas. System with dairy components offers income- generating opportunities because demand exceeds supply; elasticity of live stock products is higher than cereals and global prices expected to increase (Ibrahim and Olaloku, 2000). It is with this truth that urban and per urban dairy production system has evolved in Ethiopian highlands. Dairy development program with little attention to veterinary services component could potentially be impaired due to the many diseases capable of emerging a result of intensification and improvement of breeds. Bovine mastitis could be clinical or sub- clinical mastitis is characterized by a wide range of findings including abnormal texture and milk, discolorations, flakes or clots in the milk, and increased temperature, swelling and pain of the gland. On the basis of clinical manifestations, mastitis could be mild, per acute, acute or chronic (Crist *et al.*, 1997). In the case of sub clinical 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 (Radostitis *et al.*, 1994).

Economic losses due to mastitis, one of the diseases of intensification, are recognized globally as a major problem in dairy industries. At the sub- clinical level, the loss is severe and unnoticed by framers; this is particularly true in setting like Ethiopia with impaired animal health service and no mastitis control program. Milk yield can drop as much as 20% per infected quarter. Loss due to clinical mastitis might be as much as 10% more on quarter level (Dijknizen and Renkema, 1978). Effective control measure for mastitis will reduce problem of production loss. Treatment is one way for controlling the disease.

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 with experimental and practical applications which are confirmed by *in-vitro* sensitivity test (Hussein *et al.*, 1997; Kerro, 1997; Nibret, 2007). Beside availability, drug cost is another problem, especially in rural areas where high proportion of live stock is reared. Finding an alternative effective therapy will complement modern animal health services giving efficient animal health delivery system throughout the country.

Traditional health care is the use of medicinal plants, surgical techniques and management practices to prevent and treat a range of disease and problems encountered by livestock holders. Researchers has shown that many of plants used to prepare indigenous medicine do in fact contain active ingredients, though much research remains to be done in this area (ITDG and IIRI, 1996).

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 medicine (Sheng, 1987) and good proportion of modern drugs have been developed from plants used in traditional medicine (Green and Baker, 1992).

Many African countries have now begun to recognize the use of herbal remedies as important partners to the conventional health care system. In Ethiopia, Madagascar, and Tanzania

practical steps are already being taken to ascertain efficacy and determine optimum dosages for several herbal medicine practices (MacCorkle and Mathias, 1992).

Due to absence of modern animal services particularly in rural areas of Ethiopia, livestock owners frequently visit traditional healers to get solutions for the ill health animals including mastitis problem (Sahle, 2002; Markos, 2003). From this point of view plants that are documented to have effects on the treatment of mastitis and other disease will have to be tested for their efficacy on disease causing organisms and be validated. 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). Activities in this area have to be encouraged to develop alternative phytomedicines to avail drug choices and efficacy and to solve problems of emergence of drug resistance. This will support the struggle against poverty, alleviation and initiates further study in the field of ethno-veterinary medicine leading to the exploitation of traditional knowledge.

In Ethiopia, ethno-veterinary practice has a long history and reach 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 when ever they get sick or health is compromised and prevent when there is any diseases problem circulating in and around their locality. Ethiopia still has a rich medicinal lore! It is amazing indeed to discover, while working in this field, that almost all plants of the Ethiopian flora are used some where some how medicinally (Getahun, 1976).

Traditional healers in Ethiopia use a number of plants for the treatment of bovine mastitis and the efficacy of some of these plants have been tested against causative agents of mastitis. *In-vitro* study conducted by Sahle (2002) indicates that *Periscaria sengalensis*, *Cyphostemum adenocaule* and *Cucumis ficifolius* have shown some degree of growth inhibitory effects. Markos (2003) and Araya (2004) screened some herbal preparations against mastitis causing pathogens and observed encouraging results.

Generally mastitis was an important disease in the dairy production. A study on the prevalence of the mastitis and actions taken on the control of the disease will increase productivity of the sector. The issue of drug resistance is a problem in treatment measures. Finding alternatives for the efficiency of treatment needs great attention.

Therefore, the objectives of this study were:

- To determine the prevalence of clinical and sub- clinical mastitis and assess associated risk factors in local zebu and cross breed dairy herds in the study area.
- To assess *invitro* antimicrobial effect of seven phytopreparations on *Staphylococcus aureus* and *Streptococcus agalactiae* isolated from bovine mastitis.

2. LITERATURE REVIEW

2.1. Bovine mastitis

2.1.1. Definition

Mastitis is an inflammation of the mammary gland caused by micro organisms, usually bacteria that invade the udder, multiply and produce toxins that are harmful to the gland. It is a complex disease occurring world wide. Mastitis results in milk compositional changes such as increase in leukocyte counts, leakage of plasma proteins in to the milk, cell damage resulting in leakage of intracellular constituents in to milk, changes in composition and decrease in milk production (Crist *et al.*, 1997).

2.1.2. Aetiology

More than 137 species of microorganisms have been isolated from mastitis. Causal agents of mastitis could also be classified in to two based on source of infections, namely contagious mastitis and environmental mastitis (Crist *et al.*, 1997). Agents of mastitis include contagious pathogens (*Staphylococcus aureus*, *Streptococcus agalactiae*, *Corynebacterium bovis* and *Mycoplasma bovis*); environmental pathogens (*Streptococcus dysgalac*tiae and *Escherichia coli*); minor pathogens (Coagulase negative *Staphylococcus*) and uncommon pathogens (*Actinomyces pyogenes*, *Myocardia* spp, *Pasteurella* spp, *Mycobacterium bovis*, *Bacillus cereus*, *Serratia mareescens*, anaerobic bacterial species, fungi and yeasts (Radostits *et al.*, 2000).

Agents of contagious mastitis

Staphylococcus aureus

It is found in the udders of chronically infected cows and also in cuts and chaps on the teat skin (Blowley, 1990). About 10% of the cows may have clinical mastitis but another 50% can have sub-clinical mastitis and act as a source of infection for further clinical cases (Quinn *et al.*, 1999). *Staphylococcus aureus* is not an obligate inhabitant of the mammary gland and it is the worst of the contagious bacterial organisms causing deep infection of the mammary gland causing fibrosis and abscess, which is extremely difficult to cure. It is very difficult to cure the

infection once it is established and chronic infections are resistance to antibiotics (Rebhun, 1995). Many cases are characterized by slowly developing indurations, atrophy with the occasional appearance of clots in the milk or wateriness of the first streams (Radostitis *et al.*, 1994). The type of mastitis ranges from sub-clinical to the per acute life treating form, one of which is gangrenous mastitis caused by the action of alpha toxin that damage the blood vessels resulting in ischemic coagalative necrosis of the adjacent tissues (Quinn *et al.*, 1999).

Streptococcus agalactiae

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

Corynebacteruim bovis

Corynebacteruim bovis is a normal inhabitant of the teat canal and quite commonly isolated from milk samples but is doubtful in causing clinical mastitis because of its low pathogenecity. It causes a persistent infection of the teat duct epithelium and a mild but significant rise in the leukocyte cell count (Quinn *et al.*, 1999)

Mycoplasma bovis

Although mycoplasmal mastitis can be clinically severe, there is rarely any systemic involvement. Cows of all ages and at all stages of lactation can be affected, with those that have recently calved showing the most severe sign. There can be long term persistence of the organisms in udders (up to 13 months) and some cows may become shedders of mycoplasmas with out sever clinical signs being seen. The secretion from an affected quarter appears fairly normal in the early stages of lactation, but if the milk is allowed to stand, a deposit of fine, flaky material settles out leaving turbid, whey- like supernatant fluid. Leukocyte counts in the milk are usually very high, often over 20 million cells\ml (Quinn *et al.*, 1999).

Agents of environmental mastitis

Streptococcus dysgalactiae

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

Escherichia coli

Escherichia coli and other coliforms can multiply rapidly in quarters with a low cell count. This elicits an inflammatory reaction that destroys a large proportion of the bacterial population. When Gram-negative bacteria die and lyses, endotoxin is released from the cell wall. This massive and sudden liberation of endotoxin results in severe, life-threatening toxemia. A unique feature of coliform infections is that, in the cows that recover, the udder tissue gradually returns to normal with out fibrosis. In subsequent lactations the gland produces to its optimal capacity (Quinn *et al.*, 1999).

2.1.3. 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. It can be explained using three stages namely, invasion, infection and then inflammation (Radostitis *et al.*, 2000). If the interior environment is conducive for the growth and multiplication of invading microorganism, their metabolites irritate the delicate tissues causing an inflammatory response. The characteristic clinical signs developed are an expression of defense with the aim to destroy and eliminate the potential invaders 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).

2.1.4. Diagnosis

Clinical mastitis is recognized by the appearance of abnormal milk, gland swelling and or illness. Sub clinical mastitis is characterized by normal milk and hence requires indirect tests to

detect for example Somatic Cell Count (SCC), California Mastitis Test (CMT) and Culture (Dorgen- Mellna *et al.*, 1988).

Somatic Sell Count (SCC)

The SCC of milk is used to identify cows that are infected and to estimate the prevalence of mastitis in an entire herd. Milk from normal or uninfected cows will usually have a somatic cell count in the range of 50,000 to 200,000 (Hamann, 2003). The two most commonly used methods are the coulter milk counter, which counts particles as they flow through electric fields and of the fossomatic cell counter, which stains and then counts the number of fluorescing. cells with a fluorescent die (Schalm *et al.*, 1971). Somatic cell count is internationally recognized as a parameter for assessing milk quality and udder health (Hogan *et al.*, 1988). Dahoo *et al.* (2003) reported 250, 000 and 30,000 cells/ml threshold to quarter and cow, respectively. At present a threshold of 100,000 cells/ml can be assumed an internationally accepted definition of udder health (Hamann, 2003).

California Mastitis Test (CMT)

The CMT estimates the somatic cell content of milk. The scores are related broadly to the number of somatic cells in the milk. Somatic cell numbers in the milk tend to increase during milking and remain high for several hours afterwards, even in uninfected quarters. For reliable results, test should be conducted just before milking after stimulating the cow and discarding the fore milk (Auldist *et al.*, 1999). The CMT reagent reacts with genetic material of somatic cells present in milk to form gel.

Culture

Culturing is used to know the types of microorganism causing infections in a mastitis problem herd. This is performed by microbiologic culture of milk samples from individual quarter or of composite samples from all four quarter of individual cows. The culture results are important for adequate recommendations for therapy and for making culling decisions on individual cows (Radostitis *et al.*, 2000).

2.1.5. Control

Different mastitis organisms require different treatment regimes and control strategies such as mastitis therapy, control of contagious and environmental mastitis. Udder health management during the dry period is an integral period of elimination of existing and prevention of new intra-mammary infection (Winkler, 1982).

Mastitis therapy

Successful treatment of clinical mastitis requires a history of the herd, identification and susceptibility pattern of the bacteria involved as well as information on the milking management. Intra-mammary drug formulation is a suitable route for administrations of long acting antimicrobial preparations at "dry off" as part of mastitis control program (Quinn *et al.*, 1999).

Erskine (2001) has suggested that the prevalence of *Streptococcus agalactiae* intra-mammary infection can be reduced rapidly by "blitz" treatment. With this methods an entire herd, or more economically, all the culture positive cows in a herd, are treated with antimicrobials. The most efficacious and cost effective regime is to use intra-mammary β- lactam therapy. Studies of treatment efficacy of *Staphylococcus aureus* mastitis have found in experimental infections that were evaluated from 21 to 60 days after infection (Owens *et al.*, 1997). However, natural infections are usually of longer duration before therapeutic intervention is used and thus more retractile to therapy.

Hussein *et al.*(1997) reported in Ethiopia that out of five isolated of *Staphylococcus aureus* four were sensitive to penicillin and streptomycin, all to erythromycin, three to chloramphenicol and ampicillen but four isolates were less sensitive to tetracycline. Nibret (2007), Gebreyohannes (2008) and Rediet (2009) were reported the level of drug resistance by pathogens from bovine mastitis.

Therefore, therapy of clinical intra-mammary infection may have the best probability of success by including parental in addition to intra-mammary therapy, preferentially it should be administered for periods long enough to ensure drug levels above minimal inhibitory concentration to allow effective killing of the pathogens (Eriskine, 2001).

Controlling contagious mastitis

Proper milking procedures are important for the prevention of mastitis and for ensuring complete milk removal from the udder. Milking may begin with a check of all quarters for mastitis, strip milk on to the floor in a milking parlor or flat barn. The procedure for pre dipping involves washing teats with water, then dried with an individual towel and dipped or sprayed with an effective germicidal (Owen *et al.*, 1997). Post milking teat disinfection, treating all quarters of all cows at drying off with antibiotic products specifically designed for dry cow therapy and culling chronically infected cows are parts of the control program (Smith *et al.*, 1985).

Controlling environmental mastitis

Cow environment should be as clean and dry as possible and should not have access to manure, mud or pools of stagnant water. Calving areas must be clean. Adequate accommodation for cows, good udder preparation and pre milking disinfection are recommended (Sori *et al.*, 1997).

2.1.6. Epidemiology in Ethiopia

In Ethiopia, there have been a number of epidemiological studies of bovine mastitis. Hussein *et al.* (1997) reported prevalence of clinical and sub-clinical mastitis to be 1.9% and 5.3% on cow basis, and 1.9% and 7.4% on quarter basis, respectively in the Central regions of Ethiopia. Kerro and Tareke (2003) in selected areas of Southern Ethiopia reported a prevalence of mastitis to be 40.39% of which 41.93% clinical and 58.06% sub-clinical infection. Bishi (1998) reported the overall prevalence to be 30.2% and 5.5% for sub clinical and clinical mastitis, respectively, in a study conducted in urban and per urban dairy production system in and around Addis Ababa. Mungube (2001) reported an overall prevalence of sub clinical mastitis in urban and semi-urban areas of Addis Ababa at cow level 46.6% and at quarter level 27.8%. According to the production system the prevalence was 60% and 46.9% at cow level in urban and peri-urban, respectively. A sub-clinical mastitis prevalent rate of 36.7%, 45.5% and 38.7% in Repi, Debre Zeit and Mojo, respectively, was reported by Dagne and Abdicho (2001). Girum (2005) reported prevalence of 42.1%, 3.8% and 2.1% of sub-clinical, clinical and blocked teat, respectively in dairy farms of Eastern part of Amhara Region. A sub-clinical prevalence of

34.72% and 17.54% at cow level, and 4.99% and 4.15% at quarter level in cross-breeds and local zone, respectively, was reported by Nibret (2007).

3. MATERIALS AND METHODS

3. 1. Study area

The prevalence study was conducted in three selected areas. Two districts with in the Amhara National Regional State (ANRS) North western Ethiopia namely, Bassona Worana district in North Shoa zone and Dembia district in North Gondar zone, and the third area is in Alage Agricultural Technical Vocational Educational Training College. The two districts are characterized by having typical highland and midland climate of Ethiopia. The production systems lay with in one of the following: Crop-livestock extensive system, small scale semi-intensive and large-scale intensive system.

Basona Worana district is found in North Shoa zone of Amhara National Regional State (ANRS). North Shoa is located 130 Km North East of Addis Ababa at 9^0 36′ N Latitude and 39^0 36′ E Longitude. The area is plateau found in Central Ethiopia at an altitude of 2780 m.a.s.l. with a bimodal rainfall pattern consisting of a long rainy season from June to September and short rainy season from February to March. The mean annual temperature is 12.6^0 C (6.3^0 C-18.8^0 C). The average annual rainfall is 956 mm and a relative humidity of 59.6 %.

Dembia district is found in North Gondar Administrative zone of Amhara National Regional States (ANRS). Gondar is located in the North- Western part of Ethiopia, 710 km NorthWest of Addis Ababa. The Study area is found at 12^0 40'N Latitude and 37^0 45'E Longitude with an altitude range of 1802-2200 m.a.s.l. The soil type falls in to three categories: Heavy black clay soil, loam brown and red soil and sandy loam soil. The ranges of maximum and minimum temperature vary between 22^0C -30.7^0c and 12.3^0C -17^0C, respectively. It receives a bimodal rainfall; with short rains between March and May and the long rains extend from June to September, with an average annual precipitation rate of 1000mm.

The Alage Agricultural Technical Vocational and Educational Training (ATVET) College situated at 217 kilometers SouthEast of Addis Ababa in the vicinity of Abyata and Shalla lakes. It lies with a longitude of $38^0$30'East and latitude of $7^0$30' North location .It rests on 4200 hectares of land with an altitude of 1600 m.a.s.l. The area is characterized by mild subtropical weather with minimum and maximum temperature ranging from 11^0C- to 29^0C. The area experiences bimodal rainfall distribution with annual precipitation average of 700 to 900 mm.

The three defined seasons are short rainy (March to April), long rainy (June to September), and long dry seasons (October to January). The dominate soil type is black clay soil with a PH of 7.9 (CSA, 2004).

3.2. Study period and subject

The study was conducted between October 2009 and April 2010. Local zebu and cross breeds of Hollistein Friesian were used as a study population for the prevalence study. Isolates from bovine mastitis causing organisms and plants were also used as study subjects for efficacy test of plants.

Plants used for efficacy test are among those commonly reported from surveys done on traditional plants for treatment of mastitis in different parts of Ethiopia. Seven plants are selected and used for this study. These plants are described by their growth, distribution and morphological characteristics (Jansell, 1981; Tadesse, 2004).

Adhatoda schimpserinia

It, "Sensel" in Amharic, "Dumuga" in Oromiffa, is an erect, much branched shrub growing up to 5m high with woody stem and large elliptic leaves. The plant is growing in scrap mountain forests, at the edges of evergreen forests and bush land and is a very common hedge- forming plant at altitudes between 1400-2600 m.a.s.l. The leaves are used for traditional treatment of mastitis.

Ajuga integrifola

It is herb laying in the ground and rooting at the nodes, covered with soft hairs, stems growing up to 40cm high. Leaves are oblanceolate and coarsely toothed, and are a common herb of wetter areas in disturbed grassland, along road sides and in ditches. It is found at an altitude between 1500-3200 m.a.s.l.

Bereza (Local name)

It is a small seasonal plant growing in lowland areas having triangular and branched leaves.

Commicarpus pediculosus

It, "Tihuan Tila" in Amharic, is a trailing or scan dent annual herb with stems growing to 3m long from a woody root stock. Leaves ovate, entire or with heavy margins. Flowers white (very rarely pale pink) in umbels, usually aggregated into irregular panicles. It is found in drier regions, growing among hedge plants and in scrub, in wood lands, often with *Acacia*, mostly near water ways and often in disturbed habitats at altitudes between 500 and 2000 m.a.s.l.

Cyphostemmum adenocaule

It is herbaceous climber or scrambler with stems growing to 6m long. The plant grows at an attitude between 850-2650 m.a.s.l. and is found on the wooden grass land, riverine forest and clearing of forest. The root of the herb is used as treatment of mastitis.

Plantago lanceolata

It, "Gorteb"in Amharic, "Korrisa" in Oromiffa, is a cosmopolitan weed and herb with flowering stems growing up to 40cm high. Leaves lanceolate, mostly erect, and sometimes also flat on the ground. It grows along paths and road sides in pastures and cultivated field at an altitude between 1200-3200 m.a.s.l. The seed and leaves are used for treatment of mastitis.

Xanthium stramarium

It, "Deha nikel" in Amharic, "Bandoo" in Oromiffa, is an erect, sturdy annual or short- lived perennial plant growing from 3 to 75 cm high and up to 3m high along river banks with unarmed stems. Leaves long petiolate, blade cunneatevoate to triangular. Obscurely 3- or 5-lobed, and 3-5 veined from base; margins irregularly crenate. Fruiting capitula scabrous to tomatoes, green to brown, armed with straight or hooked spines, beaks divergent or parallel. It grows at an attitude between 600-2400 m.a.s.l.

3.3. Study design

3.3.1 Sample size determination

Thrusfield's (1995) sample size determination formula was used to determine sample size of the prevalence study.

$$n = \frac{1.96^2 \times Pex\ (1\text{-}Pexp)}{d2}$$

Where, n= required sample size

Pexp= expected prevalence

d= desired absolute precision

An expected prevalence of mastitis 40.2% in different production systems (Dagne and Abdicho, 2001), 5% acceptable error and 95%confidence level were used for sample size determination. Accordingly, the determined sample size was 369.

3.3.2. Sampling techniques

The study type was a cross- sectional study. Stratified random sampling was used to allocate sample size for prevalence study in the study areas. Simple random sampling method was to select samples from the study sites. Experimental study was used to efficacy test of plants. Plants were selected purposively to their importance in different survey conducted previously. Simple random sampling was used to select isolates.

3.4. Study methodology

3.4.1. Clinical examination

The productivity status of each cow and health status of the udder of each cow was recorded using a prepared format (Annex 1). During sampling, clinical mastitis was examined through palpation and inspection of each quarter for presence of swelling, pain, hotness and indurations. Inspection of milk was also performed for discoloration, consistency and presence of clots.

3.4.2. California Mastitis Test

The CMT was carried out to detect the presence of sub-clinical mastitis. It was conducted in each quarter milk sample immediately after collection. A squirt of milk, about 2ml from each quarter was placed in each of four shallow cups in the CMT paddle. An equal amount of the commercial reagent was added to each cup. A gentle circular movement was applied to the

mixture, in a horizontal plane for 15 seconds. The reaction was interpreted based on the thickness of the gel formed by the mixture, and the test result were scored as negative (0), trace (+), + (weak positive), ++ (distinct positive), and +++ (strong positive) according to Schalm *et al.* (1971) and Quinn *et al.* (1999) (Annex 3) and the results were recorded using a prepared format (Annex 1).

3.4.3. Plant efficacy test

Plant collection and preparation

Parts of plant, leaf and root, were collected from their natural habitat and washed with clean tap water to remove unnecessary materials. They are allowed to dry under shade in a clean paper and then chopped and grinded in to smaller particles using cleaned pistil and mortar. Powders of the plant were sieved by ordinary mesh and amount of each test materials were measured using a weighing balance, labeled and stored in a bottle.

Plant maceration and extraction

Laboratory bottles were rinsed with 80% methanol after they were rinsed with distilled water. About 75-250 gm of each test material was measured and added to the bottles. Measured powder of the plants in a bottle was macerated by mixing with appropriate amount of 80% methanol (1 part test material: 10 parts of the solvent). The mixture was stirred well with glass rod and the bottle was closed with aluminum foil and shacked for 30 minutes and kept at room temperature. After 1-2 days the macerated plant was filtered by chromatographic filter paper of number 3 and size 113, and the filtrate was evaporated using a Rota vapor. Then, crystals of the extracts were labeled and stored in a bottle after they were freeze dried (lyophilized) (Shihata *et al.*, 1983).

Impregnation of discs

Crystals of plant extract were diluted by mixing with Dimethyl Sulfoxanide (DMSO) into six serial concentrations. About 1-2 gm of plant extract crystals were mixed with the appropriate amount of DMSO (1 part of crystals: 2.5 parts of DMSO) to prepare 40% solution in the 1st test tube. 50% of the amount of DMSO used for the first concentration and 50% of amount of 40%

solution were mixed to prepare 20% solution in the 2nd test tube. The process continues until 1.25% solution was obtained (Shihata *et al.*, 1983).

Three drops from each concentration of the seven extracts were added to impregnate 12 mm size paper discs placed in a sterilized and labeled petridishes. Impregnated discs were dried at 40^0C for one hour in a dry oven and kept at room temperature until they were placed in a plate for efficacy test (1-2 days).

Bacteriological preparation

Muller- Hinton agar and 5% sheep blood supplemented Muller-Hinton agar plates were prepared for efficacy test of plants and antimicrobial sensitivity test of *Staphylococcus* and *Streptococcus* species respectively (Annex 4).

The top of 4-5 isolated colonies of the same morphological type from an agar plate were touched with a sterile wire loop and transferred to growth tube containing 4-5 ml of tryptic soy broth, then incubated at 37° C for 2-8 hours. Turbidity standard of incubated broth culture was adjusted by adding with saline water until it had similar turbidity with the0.5% McFarland turbidity standard when compared by placing against a white background with contrasting black lines. 0.5% McFarland turbidity standard was prepared by mixing 0.5 ml of 1.75% aqueous solutions of Barium Chloride and 99.5 ml solution of 1% H_2SO_4 and placed in thin glass tube (Quinn *et al.*, 1999) (Annex 4).

Within 15-30 minutes after adjusting the turbidity of the inoculums suspension, a sterile swab was dipped and rotated several times, passing firmly on the inside wall of the broth culture tube above the fluid level to remove excess inoculums from the swab. Finally the respective agar plates of bacteria for antimicrobial sensitivity test were inoculated by swabbing in three directions of the entire surface of the agar and around the rime of the petridishes.

The plant extracts impregnated discs and antimicrobial sensitivity test discs were placed on the surface of inoculated agar plates using sterile forceps. Each disc was gently pressed down to ensure complete contact with the agar surface and the plates were inverted and incubated at 35^0C. After 16-24 hours of incubation each plates was examined for the presence of inhibition (leven *et al.*, 1979). Diameter of zone of inhibition was measured on the back of the plate and

over the surface of the agar and the results was interpreted, and reported as susceptible, intermediate or resistant for the antimicrobials (Quinn *et al.*, 1999) (Annex 5) and recorded in prepared format (Annex 2).

3.6. Data management and analysis

Clinical and sub-clinical findings of udder health of individual and mean zone of inhibition plants and drugs were entered in and analyzed using SPSS version 15.5 for window. Descriptive statistics were used to summarize data. Associated risk factors for mastitis were calculated using Chi-square test. ANOVA were used for comparison of means zone of inhibition of plants, resistant penicillin G and oxytracyclinie.

4. RESULTS

4.1. Prevalence of mastitis

From a total of 369 lactating cows examined for mastitis case, an overall prevalence of 50.7% (16.0% clinical and 34.7% sub-clinical) and 39.8% (6.3 % clinical and 33.5 % sub clinical) were recorded at cow base and quarter level, respectively (Table1).

Table 1: Prevalence of mastitis

Type of mastitis	Cow base		Quarter level	
	Prevalence (%)	95% CI	Prevalence (%)	95% CI
Clinical	16.0	0.156-0.162	6.3	0.062-0.064
Sub-clinical	34.7	0.345-0.396	33.5	0.333-0.337
Overall	50.7	0.487-0.527	39.8	0.396-0.400

In this study, 32.5% (11.7% clinical and 20.8% sub-clinical) and 55.5% (17.1% clinical and 38.4% sub-clinical) prevalence at cow base, and 19.2% (4.9% clinical and 14.3% sub-clinical) and 45.2% (6.7% clinical and 38.5% sub-clinical) prevalence were observed from local zebu and cross breed cows, respectively (Table 2).

Table 2: Prevalence of mastitis in local zebu and cross breeds at cow base and quarter level

Factor	Type of mastitis	Local zebu (n = 77)		Cross breed (n = 292)	
		Prevalence (%)	95% CI	Prevalence (%)	95% CI
Cow base	Clinical	11.7	0.045-0.189	17.1	0.096-0.176
	Sub-clinical	20.8	0.118-0.298	38.4	0.250-0.356
Quarter level	Clinical	4.9	0.025-0.073	6.7	0.053-0.081
	Sub-clinical	14.3	0.104-0.182	38.5	0.358-0.412

From the total 45.9% prevalence at cow base observed from highland and midland areas, 53.2% was from highland and 39.5% was from midland areas; and 44.9% was from farms with cow number from 1-3 and 52.5% was from farms with cow number from 4-6. Of the 50.7% overall prevalence at cow base 19.4%, 57.6% and 72.7% were from extensive, semi-intensive and intensive production systems, respectively, 45.0%, 51.9% and 65.3% prevalence were from cows at first, second and third and above lactations respectively, and 61.0%, 42.5% and 44.80% ere from cows at early, mid and late stage of lactation, respectively (Table 3).

Table 3: Prevalence of risk factors for mastitis

Risk factors	Category	N	Number positive	Prevalence (%)	95% CI
Breed	Local zebu	77	25	32.5	0.200-0.430
	Cross breed	292	162	55.5	0.498-0.612
Agro-climate	Highland	141	75	53.2	0.450-0.614
	Midland	162	64	38.5	0.310-0.460
Cow size	1-3	263	118	44.9	0.389-0.509
	4-6	40	21	52.5	0.372-0.678
Production system	Extensive	93	18	19.4	0.113-0.275
	Semi-intensive	210	121	57.6	0.509-0.643
	Intensive	66	48	72.7	0.620-0.834
Lactation number	1	160	72	45.0	0.373-0.527
	2	160	83	51.9	0.442-0.596
	≥3	49	32	65.3	0.520-0.786
Lactation stage	Early	146	89	61.0	0.531-0.689
	Mid	80	34	42.5	0.317-0.533
	Late	143	64	44.8	0.367-0.529

Using binary logistic regression statistical analysis for the association of risk factors for mastitis, statistically intensive production system was statistically associated (or =10.727, p=0.000) with mastitis than extensive production system. Lactating cows at third and above lactation number showed a statistical association (OR: 5.48, p=0.001) than lactating cows at first lactation. And at second lactation (OR: 4.196, p=0.004) farms with herd size from 1 -3 were statistically associated (OR: 3.413, P=0.008) than farms with herd size from 4-6 (Table 4).

Table 4: Summary results of binary logistic regression of risk factors for mastitis

Risk factors	Category	P-values	OR	95% CI for OR	
				Lower	Upper
Breed	Cross breed	0.316	1.466	0.694	3.098
	Local zebu*				
Agro-climate	Midland*	0.961	0.986	0.554	1.753
	Highland				
Cow size	4-6*	0.008	3.413	1.369	8.507
	1-3				
Production system	Intensive*				
	Extensive	0.000	10.727	4.576	25.149
	Semi-intensive	0.061	1.321	0.864	2.310
Number of lactation	3*				
	1	0.001	5.418	1.957	14.999
	2	0.004	4.196	1.571	11.203
Stage of lactation	Early*				
	Late	0.779	1.104	0.555	2.195
	Mid	0.042	0.496	0.252	0.976

*Reference category

4.2. Efficacy of plants

Result of mean zone of inhibition (mm) recorded from four of the seven plant species tested for efficacy on *Staphylococcus aureus* and *Streptococcus agalactiae* species were as presented in Table 5.

Table 5: Zone of inhibition (mm) recorded from six concentrations of four plant species on *Staphylococcus aureus* and *Streptococcus agalactiae*.

Plant species	Tested bacteria species	Concentration (%)					
		40	20	10	5	2.5	1.25
Bereza	*Staph. aureus*	21	20	19	18	17	16
	Strep. agalactiae	20	19	18	17	16	15
Commicarpus	*Staph. aureus*	33	31	30	27	22	18
pediculosus	*Strep. agalactiae*	32	31	28	27	23	19
Cyphostemum	*Staph. aureus*	20	14	-	-	-	-
adenocaule	*Strep. agalactiae*	18	16	14	-	-	-
Xanthium	*Staph. aureus*	27	24	22	18	16	15
stramarium	*Strep. agalactiae*	26	24	21	19	17	15

20% and 10% concentration of plants with positive result on screening test and two commercial drugs available for mastitis were tested on (n= 12) isolates of *Staphylococcus aureus* and recorded mean diameter of inhibition (mm) was presented in Table 6.

From isolates (n=12) of *Staphylococcus aureus*, 8(66.3%) were resistant (≤28mm) and 4 (33.7%) were susceptible to penicillin G, and 4(33.7%) were resistant (≤ 14mm) and 8(66.3%) were susceptible to Oxytetracycline.

Table 6: Mean zone of inhibition from 20% and 10% concentration of four plants, penicillin G and Oxytetracycline

Tested materials	Mean	95% CI for mean	
		Lower	Upper
20% BE	19.83	19.08	20.59
20% CO	29.0.	28.14	29.86
20% CY	15.67	15.04	16.29
20% XA	26.17	25.36	26.97
10% BE	18.92	18.23	19.61
10% CO	27.58	26.89	28.27
10% CY	9.92	5.24	14.60
10% XA	24.42	23.58	25.25
PG	19.13	14.02	24.23
OT	11.25	8.86	13.64

BE = Bereza CO = *Commicarpus pediculosus* CY = *Cyphostemum adenocaule*

XA = *Xanthium stramarium* PG = Penicillin G OT = Oxytetracycline

There was no significant difference observed between means of 20% and 10% concentration of a plant type but there is significant difference observed among plant types in each concentration.

There was a statistically significant difference between 20% CO and resistant OT, 20% XA and resistant OT, 10% CO and resistant PG and 10% XA and resistant PG at level of ($p < 0.001$) (Table 7-10) (Figure 1-6).

Table 7: Comparison of 20% CO mean zone of inhibition with resistant OT isolates of *Staphylococcus aureus*

Tested material	Mean difference	P-values	95% CI for mean difference	
			Lower	Upper
20% CO	17.750	0.000	14.14	21.36
Resistant OT				

Table 8: Comparison of 20% mean XA zone of inhibition with resistant OT isolates of *Staphylococcus aureus*

Tested material	Mean difference	P-values	95% CI for mean difference	
			Lower	Upper
20% XA	14.917	0.000	11.31	18.53
Resistant OT				

Table 9: Comparison of 10% CO mean zone of inhibition with resistant PG isolates of *Staphylococcus aureus*

Tested material	Mean difference	P-values	95% CI for mean difference	
			Lower	Upper
10% CO	8.458	0.000	5.60	11.31
Resistant PG				

Table 10: Comparison of 10% XA mean zone of inhibition with resistant PG isolates of *Staphylococcus aureus*

Tested material	Mean difference	P-values	95% CI for mean difference	
			Lower	Upper
10% XA Resistant PG	5.292	0.000	2.44	8.15

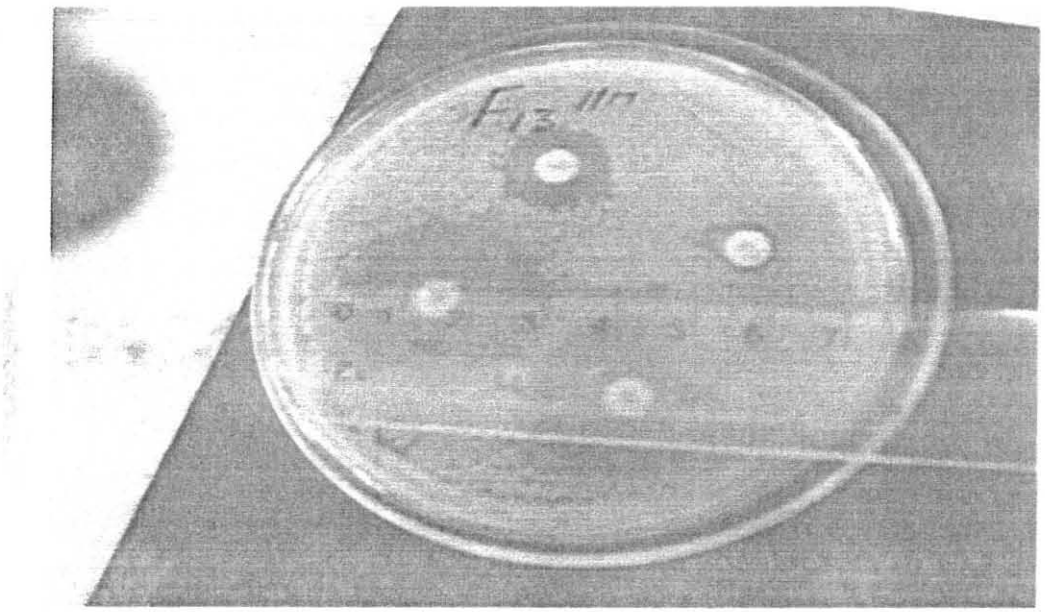

Figure 1: Inhibition zone observed from 40% (right), 20% (bottom), 10% (top) and 5% (left) concentration of *Commicarpus pediculosus* on *Staphylococcus aureus*

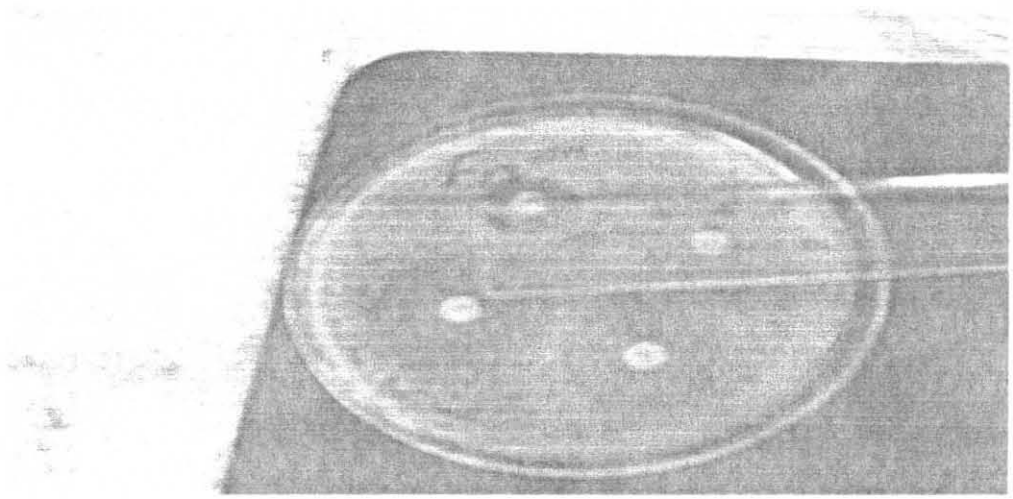

Figure 2: Inhibition zone observed from % (right), 20% (bottom), 10% (top) and 5% (left) concentration of *Xanthium stramarium* on *Staphylococcus aureus*

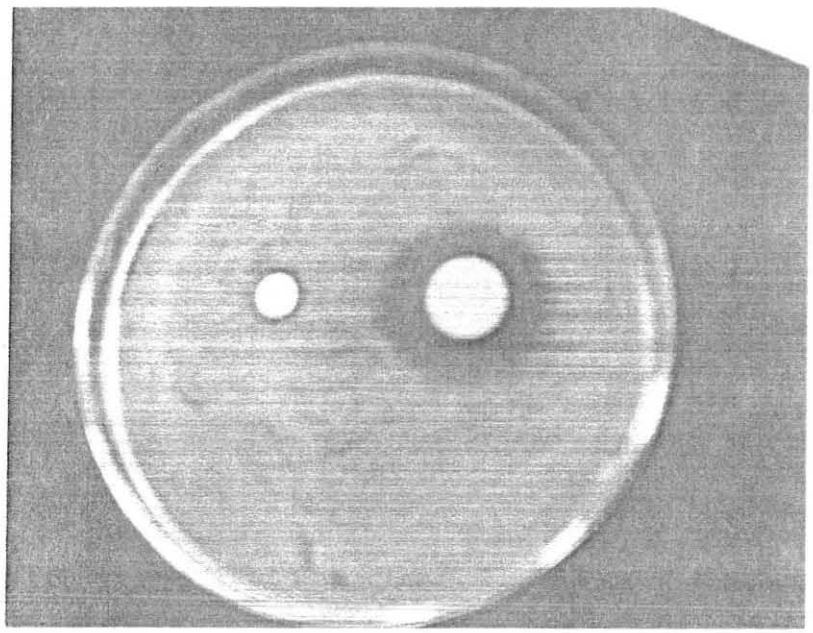

Figure 3: Inhibition zone observed from 20% concentration of *Commicarpus pediculosus* on OT resistant *Staphylococcus aureus*

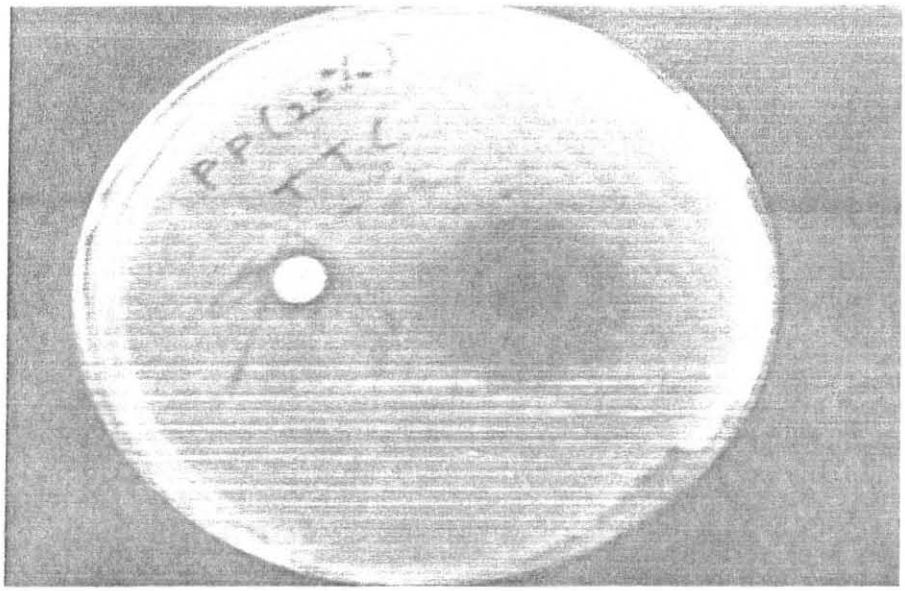

Figure 4: Inhibition zone observed from 20% concentration of *Xanthium stramarium* on OT resistant *Staphylococcus aureus*

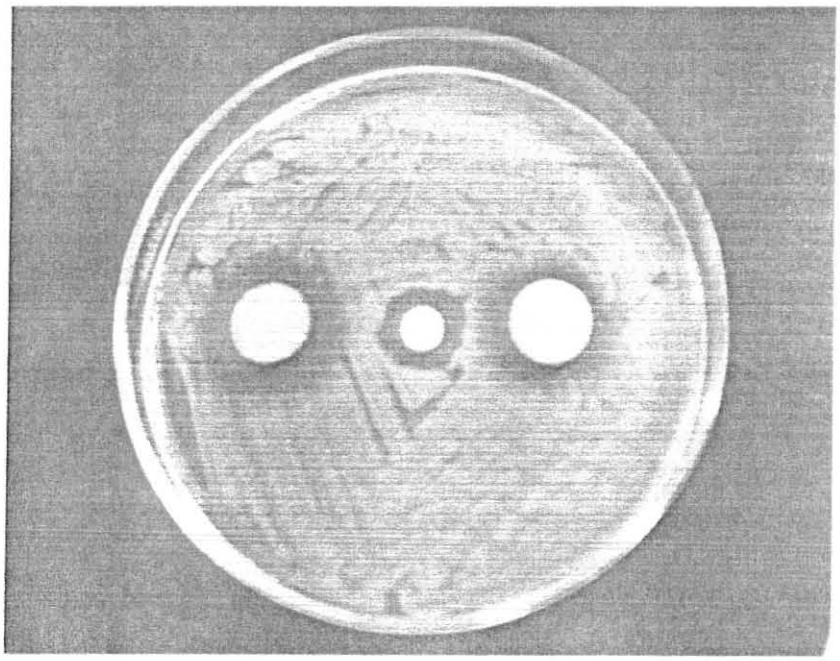

Figure 5: Inhibition zone observed from 10% and 5% concentration of *Commicarpus pediculosus* on PG resistant *Staphylococcus aureus*

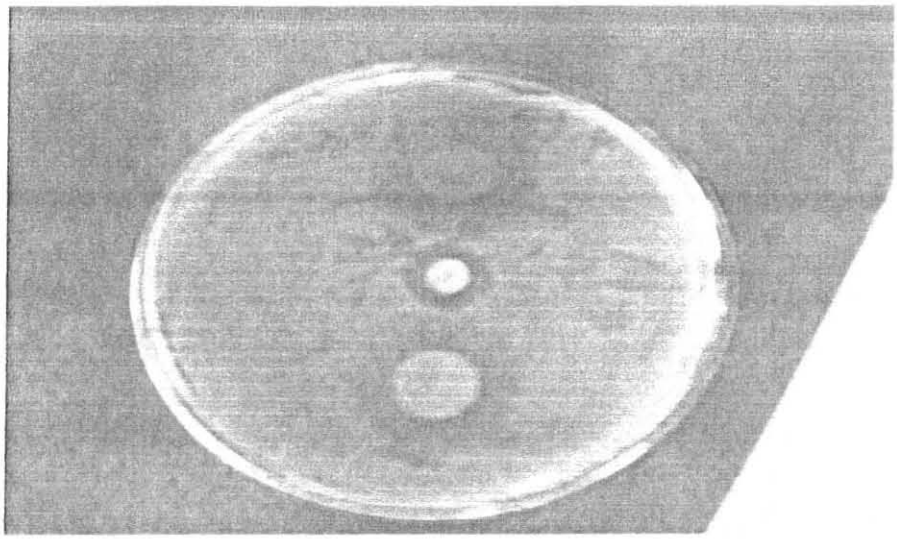

Figure 6: Inhibition zone observed from 10% and 5% concentration of *Xanthium stramarium* on PG resistant *Staphylococcus aureus*

5. DISCUSSION

This cross-sectional study showed the overall prevalence of mastitis in local and cross breed cows from small holder extensive and semi-intensive dairy farms in highland and midland areas, and in cross breed cows from large-scale intensive dairy farm.

When the 16.0% clinical and 45.0% sub clinical mastitis prevalence accounted at cow base in this study compared to some reports of 22.5% and 67.0% in Debre Zeit (GebreYohannes, 2008), 3.95% and 26. 36% in North Gondar (Nibret, 2007), 9% and 42.1% in Eastern part of Amhara region (Girum, 2005), 3.9% and 44.6% in Bahir Dar (Gizat, 2004) ,1.2% and 38.9% in Central highlands of Ethiopia (Tesfu *et al.*, 1999) , 5.3% and 34.3% in Addis Ababa(Bishi, 1998) and 1.9% and 5.3% (Hussien, 1997) clinical and sub clinical prevalence, respectively variations with most of clinical cases and some agreements were observed to the sub-clinical mastitis. The variability could be attributed to interactions of factors of management and environment to factors of animal and causative agents.

The higher prevalence observed in cross breed lactating cows than local zebu breed could be associated with variation in certain physiological and anatomical characteristics between the breeds. Cross breed cows have been found more susceptible to mastitis owing to position of teat, udder and anatomy of teat canal. Differences in genetic structure of the teat canal sphincter muscle, keratin in the teat canal or shape of teat end where pointed teat end are prone to injury (Seykora and Mc Daniel, 1985) which induces infection. In addition to physical barrier, differences in cellular immunity and fewer efficacies of phagocytic cells in higher yielding cross breed cows are factors for the observed higher prevalence.

Among the production systems compared for the prevalence of mastitis in this study, higher prevalence was observed in large-scale intensive dairy farm. This higher prevalence could be attributed to the presence of large number of cows which may result variation in herd management practices, in cow handling, nutrition, milking procedures, sanitation, housing and veterinary services which are predisposing factors for the individual animal as well as herds to mastitis.

Sub-clinical mastitis in both breeds had been higher than clinical mastitis and this could be due to defense mechanism of the udder, which reduces the severity of the disease and observation of clinical cases. The higher prevalence of sub-clinical mastitis, which reduces milk yield as much as 20% per infected quarter and high proportion of blocked teat (42.1%) from clinical cases, which reduces milk yield by 10% more will have a subsequent impact on food security and signifies the importance of the problem.

In this study, the higher prevalence of mastitis in the highland area than in midland area could be associated to the higher prevalence observed in cross breed cows than local and in semi-intensive than extensive production system. In highland area the proportion of cross breed cows and semi-intensive farms were higher than the proportion in the midland area (Annex, 6) which was the result of growing dairy production system in Central Ethiopian highlands (Lema *et al.*, 2001).

Lactation number and stage of lactation were found to affect mastitis prevalence significantly. Cows at their third and above lactation and cows at early stage of lactation were showed higher prevalence than other categories. The higher prevalence in cows at their third and above lactation could be due to the increasing ease of penetration of the teat duct by pathogens and accumulated previous infections. Absence of dry cow therapy regime could possibly be among the major factor contributing to high prevalence at early lactation. The high incidence of mastitis around calving is largely a consequence of high new infection rate in the dry period and a peri parturient suppression of host defense (Radostitis *et al.*, 2000).

In the efficacy test, seven medicinal plants were selected and tested for the effect on *Staph. aureus* and *Strep. agalactiae*. The efficacy of each plant was first assessed alone at six concentrations of 40%, 20%, 10%, 5%, 2.5%, and 1.5%. 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 phytopreparations.

From these plants, four of them showed visible zone of inhibition. Mean zone of inhibition seen from *Commicarpus pediculosus* (29.0mm) showed better result than (19.83mm) of Bereza, (15.67mm) of *Cyphostemum adenocaule* and (26.17mm) of *Xanthium stramarium* inhibition

zone, and also better than results of previously tested plants with mean zone of inhibition of (7mm) from *Brucea antidysentira* , (21mm)from *Combertun molle* and (11 mm) from *Periscaria senegalensis* results of Araya (2004) and Desta (1995) on *Staphylocens aureus* isolated from bovine mastitis.

Bereza, *Commicarpus pediculosus* and *Xanthium stramarium* were tested for the first time in this study. The result of *Cyphostemum adencocaule* agrees with 11 mm mean zone of inhibition made by the plant on *Staphylococcus aureus* (Araya, 2004). Of the three plants with no result of zone of inhibition, the result seen from *Plantago lanceolata* agrees with the result of Araya (2004) and varies with Desta (1995) and Belay (2003) results of mean zone of inhibition by the plant on *Staphylococcus aureus.*

When 20% and 10% concentrations mean result of zone of inhibition made from four plants with good zone of inhibition compared to resistant isolates penicillin G and Oxy tetracycline, A statistical significant difference ($p< 0.001$) were observed from 20% CO mean zone of inhibition when compared to mean of resistant isolates to PG 20% CO mean zone of inhibition were also showed a statistical significant difference ($p< 0.001$) when compared to mean of resistant isolates to PG and when compared to mean of resistant isolates to OT. A statistical significant difference ($p< 0.01$) were also observed from 20% of CY ($p< 0.05$) when compared to mean of resistant isolates to PG.

Although, the *in-vitro* antibiotic susceptibility does not purely reflect the *in-vivo* therapeutic value of the antibiotic (Radostitis *et al.*, 1994) the efficacy result of those plants will probably be an input for future preparation of antibiotic replacing the resistant drugs. When the antibiotic resistance of *Staphylococcus aureus* to penicillin G (66.3 %) and oxytetracycline (33.7%) in this result compared to antibiotic resistant reports of 45.3% to penicillin G and 20.6% to oxyteracycline (Nibret, 2007), 56.1% to penicillin G and 59.2% to oxytetracycline (Gebreyohannes , 2008) and 75% to penicillin G (Rediet, 2009) , it shows the gradual growing resistance of bovine mastitis causing organisms to commercial drugs.

As antibiotic use increases in veterinary medicine, the issue of bacterial resistance to antimicrobial becomes more worrisome. The emergence of increasing numbers of antibiotic resistant pathogens has implication for veterinary patients as well as humans (Hoffman, 2001)

From this result it is 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 AND RECOMMENDATIONS

This study indicated that mastitis was an important disease in dairy production of Ethiopia. From this study, sub-clinical mastitis was highly prevalent than clinical mastitis. This indicates absence of early treatment measures since the case was not easily noticed by farmers. In this study, the higher prevalence of mastitis in the highland area than in midland area could be associated to the higher prevalence observed in cross breed cows than local and in semi-intensive than extensive production system. In this finding, the higher prevalence observed from cross breeds and association of the disease in intensive production system was a major problem facing the dairy development program. The observed high percentage of drug resistance will have an effect on the treatment efficiency. This requires great attention to have alternatives replacing resistant drugs. Although, the *in-vitro* antibiotic susceptibility does not purely reflect the *in-vivo* therapeutic value of the antibiotic the efficacy result of those plants will probably be an input for future preparation of antibiotic replacing the resistant drugs.

Based on this the following recommendations are forwarded:

- Regular screening of cows for sub-clinical mastitis should be given on individual cows and herd levels in order to take appropriate control measures.
- Regular testing of antimicrobial susceptibility should be carried out giving emphasis to alternative use, supply and new formulation of drugs.
- Traditional knowledge for disease control which was the base for the development of modern medicine should be gathered from farmers, herbalists and other sources of the knowledge and be documented for future use.
- Plants which are the main components of the traditional treatment and are widely distributed in Ethiopia should be screened out for their use in treatment of disease, in particular to economically important disease like mastitis
- In addition to the efficacy test of plants on causative pathogens of mastitis repeatability and further detailed study, like phytochemistry, toxicity and cytotoxicity and scientifically accepted plants for their efficacy to treat a disease should be promoted by a responsible organization for plantation and use as input to pharmaceutical industries.

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8. ANNEXES

Annex 1: Record format for observation of clinical and sub clinical bovine mastitis

Zone: _____ Woreda _____ Kebele _____

S. No	Date	No. cows	Breed		Production	No of lactation	Stage of lactation			Diagnostic test result									
			Local	Cross-			Early	Mid	Late	Positive								Negative	Remark
										Clinical				Sub-clinical					
										Left front	Left back	Right front	Right back	Left front	Left back	Right front	Right back		

Annex 3: Interpretation of CMT

CMT score	Interpretation	Visible reaction
O	Negative	Milk fluid normal
T	Trace	Slight perception
1	Weak positive	Distinct precipitation but no gel formation
2	Distinct positive	Mixture thickness with gel formation
3	Strong positive	Viscosity greatly increased strong gel formation

Annex 4: Laboratory preparations of media and chemical

1. Preparation of muller-Hinton Agar
   - 38 gm of MHA mixed with 100 ml distilled water
   - Mixed auto claved at 1200C
   - Cooled at 370C water both
   - Both dispersed in a sterile environment in a sterile petridishes.
2. Preparation of McFarland O.S turbidity standard solution A (0.048 MBaCl2)
   = 1.179 Bacl2 + 100ml distilled water
   = Solution B (0.18 MH2SO4)
   = 1.0ml H2So4 + 100 ml distilled water

For standard: 0.5ml solution A + 99.5ml solution B.

3. Preparation of tryptic soy broth
   - 7.8gm of tryptic soy agar mixed with 1000ml of distilled water
   - Mixed auto claved at 120^0C
   - Cooled at 37^0C water both
   - Dispersed in a sterile environment in sterile pertridishes

Source: Quinn *et al.* (1999)

Annex 5: Zone size interpretations of some antimicrobials

Antimicrobial	Diameter of zone of inhabitation to nearest mm			
	Resistant ≤	Intermediate	Moderately susceptible	Susceptible
Amplicilin	13	-	14-17	18
Erythromycin	A13	14 – 22	-	23
Gentamycin	12	14 – 22	-	15
Pencillin G	28	-	-	29
Streptomycin	11	12 – 14	-	15
Tetracycline	14	15 – 18		19

Source: Quinn *et al.* (1994)

Annex 6: Proportion of local Zebu and cross breed cows, cow size, extensive and semi-intensive farms between high land and mid land areas

	High land	Mid land
Breed		
Local zebu	17	60
Cross breed	124	102
Cow size		
1 – 3	136	127
4 – 6	5	35
Production system		
Extensive	10	83
Semi-intensive	131	79

8. CURRICULUM VITAE

I. Personal back ground

Name: Mandefro Dargie Bezabih

Date of birth: Nov. 11, 1977 G.C

Place of birth: Gondar, Ethiopia

Nationality: Ethiopian

Address: phone n° 0913486737

II. Educational back ground

- ✓ Sep.1984 – June 1991: primary education in Hibret 1°school, Gondar Ethiopia
- ✓ Sept. 1991 – June 1993: junior secondary education in Atse Bekafa junior 2° Gondar, Ethiopia
- ✓ Sept. 1993 – June 1997: secondary education in Fasiledes 2° school Gondar, Ethiopia
- ✓ Sept. 1998 – June 2003: higher education in AAU,FVM,Debrezeit. Ethiopia
- ✓ Sept. 2008 – June 2010: post graduate study in AAU College of health science, school of Veterinary medicine, Debrezeit Ethiopia

III. Work experience

- ✓ Oct. 2003 – dec. 2008: instructor in Mizan teferi ATVET college, Benchmaji, SPNNRS, Ethiopia
- ✓ Jan 2008 – august 2008: instructor in Alagae TVET college, Alagae, Ethiopia

IV. Research work

- ✓ Nov.2002 – may2003: on cystic ovary observation in ewes in Addis Ababa municipal abattoir, Debrezeit Ethiopia
- ✓ Oct. 2009-April 2010: on epidemiology of bovine mastitis and efficacy test of plants on common isolates the disease, Debrezeit.

V. Skills

- Language – Amharic: spoken and written
  English: spoken and written
- Basic computer skills