Journal of Veterinary Science & Technology

Asaye, et al., J Veterinar Sci Technol 2015, S:13
DOI: 10.4172/2157-7579.1000S13-001

Research Article Open Access

The *in-Vitro* Antibacterial Effect of Three Selected Plant Extracts against Staphylococcus aureus and Streptococcus agalactiae Isolated from Bovine Mastitis

Habtamu Kinde[1], Fekadu Regassa[1], Mebratu Asaye[2*] and Ayana Wubie[1]

[1]*College of Veterinary Medicine and Agriculture, Addis Ababa University, Debre Zeit-34, Ethiopia*

[2]*Faculty of Veterinary Medicine, University of Gondar, Gondar-196, Ethiopia*

[*]**Corresponding author:** Mebratu Asaye, Faculty of Veterinary Medicine, University of Gondar, Gondar-196, Ethiopia, Tel: +251-111-239746; E-mail: mebratu.asaye@gmail.com

Rec date: Aug 11, 2015; **Acc date:** Sep 15, 2015; **Pub date:** Sep 17, 2015

Abstract

The present study was conducted from November 2013 to April 2014 in Addis Ababa University, College of Veterinary Medicine and Agriculture, Debre Zeit, Ethiopia on the *in-vitro* antibacterial activities of the steam bark of *Combretum molle*, the leaves of *Xanthium strumarium* and FR1 against *Staphylococcus aureus* and *Streptococcus agalactiae* isolated from bovine clinical mastitis. The *in-vitro* antimicrobial test was found with encouraging results against the test bacteria. Among the plant preparations which were tested the Mean Zone of Inhibition (MZI) of *Xanthium strumarium* was found with the widest zone of inhibition when compared with the other two with Mean Zone of Inhibition (mm) of 20.5, 18.5, 16, 15.25, and 13 to the 10%, 5%, 2.5%, 1.25%, and 0.625% concentrations, respectively against *Staphylococcus aureus*. The Mean Zone of Inhibition (mm) of FR1 was found to be 23.25, 21.25, 18.75, 16.5 and 13.75 to the 10 %, 5%, 2.5%, 1.25%, and 0.625% concentrations, respectively, which was the widest MZI when compared with the other two plant extracts of similar concentrations against *Streptococcus agalactiae*. The plant extracts were found with dose dependent inhibition zone against both bacterial isolates. The 10% preparations of the plant extracts were comparable with the standard antimicrobial used (Gentamicin) as positive control which show better zone of inhibition than Tetracycline. The result of the present study is indicative that these herbal preparations might be considered as an alternative option for the treatment of resistant isolates of clinical bovine mastitis after studying their safety of margin in-vivo.

Keywords: Antimicrobials; *In-vitro*; Mean zone of inhibition; Plant extracts; Sensitivity; *Staphylococcus aureus*; *Streptococcus agalactiae*

Introduction

Bovine Mastitis is the most frequent and costly disease of dairy cattle. Losses due to mastitis can be attributed to both subclinical and clinical disease. Clinical mastitis losses are generally readily apparent and consist of discarded milk, transient reductions in milk yield and premature culling. Mastitis is a result of complex interactions of infectious agents, the environment and the management practice. It is incriminated as an important disease constraint in dairy cow and is responsible for reduction in quality and quantity of milk and milk products [1]. The common isolates of bacteria from the clinical mastitic quarters were *St.agalactiae* (30%) and *St.dysgalactiae* (30%), while from sub-clinical cases were *S.aureus* (42.6%), *S.epidermidis* (22.1%), *St.agalactiae* (12.8%) and *St.uberis* (10.3%) [2]. The conventional drugs used for the treatment of mastitis are limited in types in developing countries in general and in Ethiopia in particular. Due to this and other factors the causative agents were also reported to have developed variable degrees of resistance to the commonly used antimicrobial agents [3].

Antibiotic resistance has become a global problem. Antibiotics have been of value in controlling many infections, but they depend on judicious use to minimize the incidence of resistance forms [4]. The worldwide problem of antibiotic resistance impacts negatively on antibiotic therapy thus making successful empiric therapy much more difficult to achieve. The emergence of drug resistance is an evolutionary process that is based on selection for organisms that have an enhanced ability to survive and reproduce in the presence of a drug [5].

Strategies to improve the current situation include research in finding new and innovative anti-microbial from plants [6]. Plants produce wide array of bioactive molecules, most of which probably evolved as chemical defense against predation or infection [7]. For example, the drug taxol, (paclitaxel), one of the most powerful anticancer drug known, first isolated from the bark of the yew tree *Taxus brevifolia* has yielded two approved drugs for breast and ovarian cancer [8]. A medicinal plant is any plant which is in one or more of its organ, contains substance that can be used for therapeutic purpose or which is a precursor for synthesis of useful drugs [9].

In Ethiopia, plant remedies are still the most important and sometimes the only sources of therapeutics for nearly 80% of human and more than 90% of livestock population. Estimated floras of 6500 to 7000 species of higher plants are of medically important and out of these medicinal plants 12% are endemic to Ethiopia [10]. Despite their vital role for the health of human and livestock population, large part of the knowledge of ethno-medicinal plants is on the verge of irreversible loss and declining to deterioration due to the oral passage of herbal heritage from generation to generation rather than in writings [11].

In Ethiopia, animal diseases remain among the principal causes of poor livestock performance, leading to an ever increasing gap between

the supply of, and the demand for, livestock products [12]. Conventional veterinary services, despite its paramount role, have limited coverage in developing countries and development of antimicrobial resistance is another headache [13]. If at all, the usefulness of modern pharmacotherapy is still limited by the cost of treatment [14]. The importance of using medicinal plants can be attributed to affordability as well as the trust in herbal medicine as an out come from the witnessed positive results when applying herbs. Many medicinal plants are also used to treat cows, sheep, poultry, horses and pigs [10].

Due to this reason livestock keepers particularly in rural areas frequently visit traditional healers to get solutions for their ill-health animals including clinical cases of skin, udder, teats and gastrointestinal tract infections. Developing a socially acceptable and effective remedy from inexpensive resources that can complement modern medicine would be an attractive option [15]. However, in most traditional healers the units of measurements to determine dosage are not standardized and there are variations in the unit of measurement, duration and time at which remedies are taken and prescribed by healers for the same kind of health problems. The precision, standardization and their toxic effect were not studied in the country which is as one drawback for the traditional health care system [16].

Therefore the efficiency of some of these plants/herbs has been tested against a range of causative agents of mastitis at different times in Ethiopia. Regassa and Araya [17] screened some herbal preparation against mastitis causing pathogens and got promising results. Mengistu has screened six herbal preparations; namely, *Brucea antidysentrica*, *Combertum molle*, *Cyphostema adenacuale*, *Persicaria senegalensis*, *Plantago lanceolata* and *Zahneria scabra* on major isolates of bovine mastitis. Taddese et. Al. [18] has conducted *in-vitro antimicrobial effects of some selected plants on Staphylococcus aureus isolated from bovine clinical mastitis* and Mohamedamin [19] has conducted an *in-vitro* test of *Laggera alata* and *Xanthium strumarium* on *Staphylococcus aureus* isolate and observed encouraging result. Haile [20] has also conducted *in-vitro* sensitivity to determine and compare the *in-vitro* antimicrobial effects of two phyto preparations; namely *Xanthium strumarium* and Grewia bicolor juss on *Staphylococcus aureus* isolated from bovine clinical mastitis case and found a result which encourages further study. Sahlu [21] has also conducted study on antibacterial activities and preliminary phytochemical investigation of four selected medicinal plants namely leaves, stem bark and seeds of Combretum molle, stem bark of Bereza and leaves of *Xanthium strumarium* and *Laggora arota* aganist *Staphylococcus aureus*, *Streptococcus agalactiae* and Escherichia coli and got the result which supports the previous studies that these plants can serve as famous drugs for modern use.

Therefore, the main objective of this study is:

To evaluate ethanol extracts of *Combretum molle*, *Xanthium strumarium* and FR1 for their antibacterial activity against *Staphylococcus aureus* and *Streptococcus agalactiae* isolated from bovine mastitis.

Materials and Methods

Study area

Study of *in-vitro* antibacterial effect of some selected medicinal plants on bacterial isolates was carried out from November 2013 to April 2014 in Debre zeit, Ethiopia. Debre-Zeit is located 45 kms South East of Addis Ababa. The area is located at 9°N latitude and 40°E longitudes at an altitude of 1850 meters above sea level in the central high land of Ethiopia. It has an annual rainfall of 866 mm of which 84% is in the long rainy season (June to September). The dry season extends from October to February. The mean annual maximum and minimum temperatures are 26°C and 14°C, respectively with mean relatively humidity of 61.3% [22] [Figure 1].

Study design

Investigation of plants was carried out in different parts of Ethiopia based on past studies and there use traditionally and *in-vitro* antimicrobial sensitivity testing on selected bacterial isolates were performed from crude extracts of selected plants from November 2013 to April 2014 in Addis Ababa University College of Veterinary Medicine and Agriculture, Debre zeit, Ethiopia.

Herbal/plant materials used for the study: Combertum molle (Abalo" in Amharic, "Bika, Dadamata" in Oromiffa): Known with common name velvet leaved Combretum. This is a member of the family Combertaceae which is a small deciduous tree growing up to 15 meters high with an often-crooked trunk, commonly branching to the base (Annex 1a) . The bark is dark brown to black and deeply grooved in squares. The leaves are oppositely arranged, elliptic to lanceolate, large that covered with soft hairs, rounded at the base. The flowers generally appear before the leaves and the fruits yellowish, four-sided with wings [23]. The bark was used to assess the antimicrobial effect on bacterial isolates from mastitis cases.

Xanthium strumarium/Cockleba in Amharic: Is broad leaved, tap rooted herbaceous annual plant. Stems are erect, ridged, rough and hairy, and frequently branched, resulting in somewhat bushy plants from 8 to 59 inches (20-150 cm) tall (Annex 1b). It has small greed unisexual flower occurring in separate cluster at the end of the branches and main stem. The fruit is a brown, hard, woody bur from 0.4 to 0.8 inch long and covered with stout, hooked bristles. Its seeds are produced in a hard, spiny, globose or oval double chambered, single seeded bur. It's covered with stiff, hooked spines, which sticks to fur and clothing and can be quite difficult to extract. These remarkable burred seeds have allowed this plant to be carried all over the world by unsuspecting travelers. This plant reproduces only by means of its seed and weed easily dispersed through animals as the fruits have hooked bristles and 2 strong hooked beaks [24]. The leaf was used for the *in-vitro* antibacterial test.

Unidentified plant (coded FR1): It is a type of plant found dispersed in many areas in Debre zeit with a height of up to 1.5 meters and branched at the base and the leaves are wide (Annex 1c). This plant selected based on its use locally for the treatment of wound. It was collected in Debre zeit city and its leaf was used for the *in-vitro* antibacterial test.

Bacterial organisms used for the study: Two bacterial species, *Sterptococcus agalactiae* and *Staphylococcus aureus* isolated from bovine clinical mastitis cases of AAU/CVMA dairy farm were used. These organisms were selected based on their disease burden and increasing trend of antibiotic resistance in the developing world [25].

Study Methodology

Plant collection and pre extraction preparation: The study was an experimental trial *in-vitro* on selected medicinal plants against bacteria commonly isolated from bovine clinical mastitis.

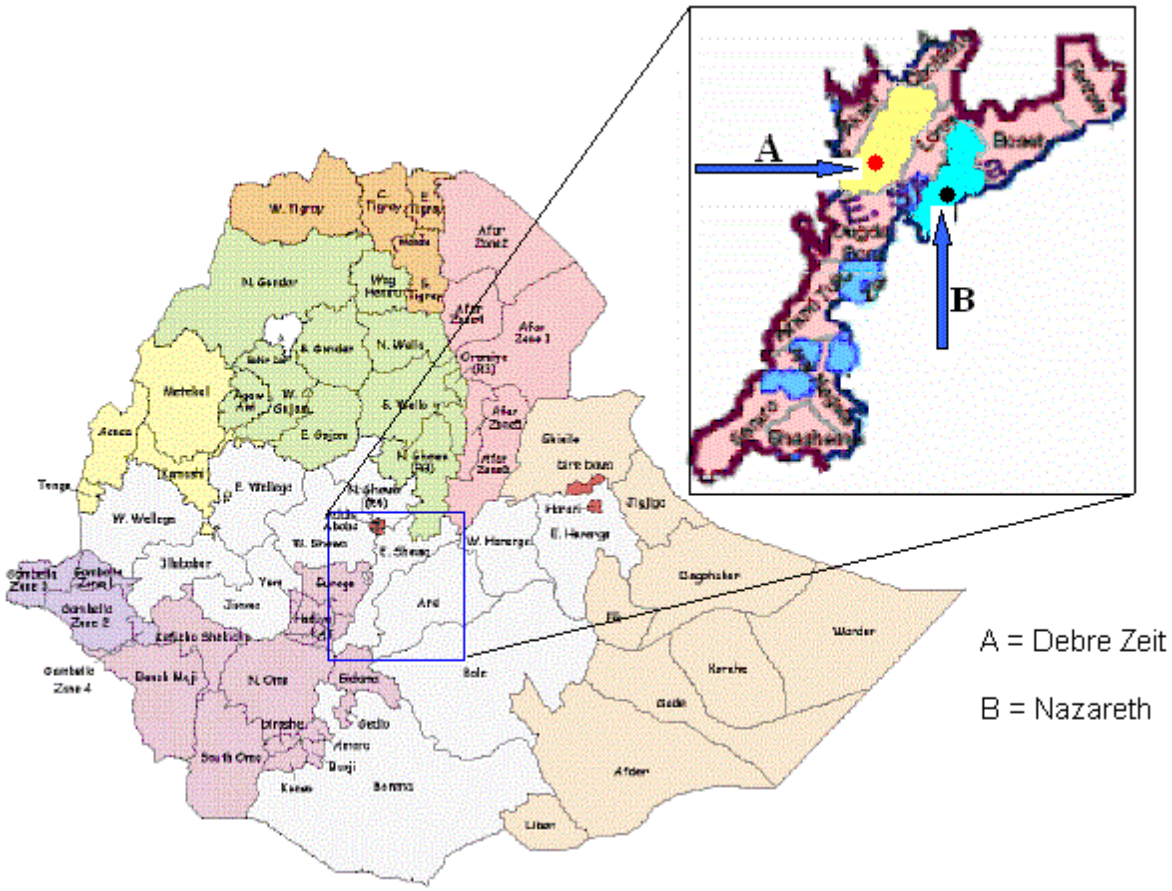

Figure 1: Map of the study area source: [21].

The plants were chosen based on the results showed by previous workers on the bark of C. molle Taddese [18], Mengistu [26], on leaf of *X. strumarium* Mohamedamin [19], Haile [20] and Sahlu [21], but there is no information if work is done on FR1 or not because the scientific name was not known. It was mainly selected based on its use traditionally by farmers for the treatment of wound. After collection the plants were `washed with tap water to remove unnecessary particles. Then dried under shade and grounded mechanically. The material was then sieved and weighed before maceration.

Preparation of crude extracts for the in-vitro experiment: After the parts of plants which are grindined and sieved by fine mesh 25 gms of each test plant were weighted using sensitive balance and it was put to a bottle and 250 ml of 95% ethanol added and mixing takes place at maximum speed shaking for 30 minutes to help through mixing and enough maceration of the plant parts. Mixed content were allowed to stay at room temperature for 6 days. Then after 6 days each sample was strained using strainer to remove the solids. Further straining takes place using filter paper to obtain a solution free of solids. The solution was then concentrated using a vacuum rotary evaporator to remove the solvent (ethanol). Clean petri-dishes were prepared and weighed their initial weight using sensitive balance and labeled, and the concentrated plant extract were put on these petri-dishes and the weight were recorded and then the weight of the extract were recorded by subtracting final weight from initial weight of the petri-dish. The plant extracts were then kept in hot air dry-oven at 370C for 24 hours to help evaporate the remaining solvent. The resulting concentrated plant extracts then kept at room temperature until being tested for anti-microbial activity [27].

Preparation of antimicrobial discs from plant extracts for the in-vitro experiment: Five serial dilutions with different concentrations (10%, 5%, 2.5%, 1.25% and 0.625%) of each plant extract were prepared using Dimethylsulfoxide (DMSO) as described by Quinn PJ, et al. [28]. In the first test tube 2 ml of stock solution (10%) was added and each of the remaining four tubes was filled with 1ml of DMSO. 1 ml of 10% solution from the first tube was transferred to a second test tube to prepare 5%. The procedure continues by transferring 1ml of solution from the 5% preparation to a third test tube to get a 2.5% concentration, and the procedure continued in a similar manner until a 0.625% concentration is reached. In transferring from one concentration to another mixing were done by vortex mixer. Discs of 6mm diameter were impregnated by adding three drops from each reconstituted solution and allowed to dry at 370°C overnight. Dried discs were used to determine antimicrobial effects of the respective plant types on isolated and grown bacteria. Each disc was gently pressed down to ensure complete contact with the agar and the plates

were inverted and incubated at 370°C for 24 h. The diameter of zone of inhibition was measured in millimeters using caliper.

Antimicrobial Sensitivity Test: The antimicrobial test was conducted using agar disc diffusion method. Muller-Hinton agar (38 gm) (Biotech UK) medium was used for antimicrobial sensitivity test, and was mixed with one litter of distilled water, boiled to dissolve completely and autoclaved at 121°C for 15 minutes. The medium was later dispensed in to 90 mm sterile agar plates and left to set. The agar plates were incubated for 24 h at 370°C to confirm their sterility. When no growth occurred after 24 h, the plates were considered sterile and used for antimicrobial sensitivity tests.

The top of 4-5 well isolated colonies of the same morphology were scooped using a wire loop from the nutrient agar and mixed using sterile normal saline and agitated with a vortex mixer. The turbidity of the bacterial suspension was adjusted by comparing with 0.5 McFarland turbidity standards. McFarland turbidity standard was prepared by mixing 0. 05 ml of 1.175% aqueous solution of barium chloride (0.048NBCL2H2O) with 9.95 ml of 1% sulfuric acid (0.036NH2SO4). The standard and the test suspension were placed in a 10ml sized tests tubes and compared against a white back ground with contrasting black lines until the turbidity of the test suspension equates to that of the turbidity standard. Adjustments of the turbidity were made by adding saline or colonies depending on the degree of turbidity for *S. aureus*, while *St. agalactiae* was incubated overnight (18-24 h) in nutrient broth were standardized with McFarland 0.5 standard [29]. A sterile swab was dipped in to the standardized suspension of the bacteria and excess fluid was minimized by pressing and rotating the swab firmly against the inside of the tube above the fluid levels. The swab was streaked in the three directions over the entire surface of the agar with objective of obtaining uniform inoculations and a final sweep with the swab was made against the agar around the rim of the Petri dish. The inoculated plates were allowed to stand for not more than 15 minutes and the discs were placed on the agar surface using a sterile forceps. Each disc was gently pressed with the point of the sterile forceps to ensure complete contact with the agar surface.

For this study Tetracycline and Gentamicin were used to compare their efficacy with herbal preparations and as a positive control, while DMSO was used as negative control. The anti microbial were chosen based on their frequent use for the treatment of clinical bovine mastitis. The appropriate crude extract impregnated discs and conventional anti biotic discs were applied at spaces of 24 mm apart from center to center and 15 mm away from the edge of the plates. This was made no later than 15 minutes after the inoculums has been added. The plates turned upside down, labeled and incubated at 370°C for 24 h. Diameter of zone of inhibition was measured using a caliper in millimeters according to [30].

Data analysis

Descriptive statistical methods were used for data analysis and results were presented as tables and graphs.

Results

Each plant extracts of the three plant species were tested at different concentrations levels (10%, 5%, 2.5%, 1.25%, and 0.625%) and all of the plant extracts showed strong anti-microbial activity mainly at higher concentrations against *Staphylococcus aureus* and *Streptococcus aglactiae* which is expressed in diameter of zone of inhibition (Tables 1-6). The inhibition zone increases with the increasing concentration of the extracts of the leaves of *Xanthium strumarium* and FR1 and the bark of *Combertum molle* against *Staphylococcus aureus* and *Streptococcus aglactiae*. All three plant extracts show wider zone of inhibition at all concentrations on *Streptococcus aglactiae* than *Staphylococcus aureus*. Bark of C.molle shows wider zone of inhibition on *Streptococcus aglactiae* than *Staphylococcus aureus* at all concentrations, which is also similar to the leaves of FR1 and *Xanthium strumarium*. Among the positive controls used Gentamicin show a good zone of inhibition to both bacteria's while Tetracycline was found with no inhibition and 10% plant preparations were comparable with Gentamicin. DMSO which was used as negative control was found with no inhibition zone. The plant extracts show a zone of inhibition which is concentration dependent which increases with increasing concentration.

Isolates	Zone of inhibition at different concentrations				
	10%	5%	2.5%	1.25%	0.625%
1	13	12	8.5	6	3
2	14	13	9	7	3
3	16	14.5	10	8	4
4	12	12	8	6	3
Mean	13.75	12.9	8.9	6.75	3.25

Table 1: zone of inhibition (mm) exhibited by ethanol extract of bark of *Combertum molle* against *Staphylococcus aureus*.

Isolates	Zone of inhibition at different concentrations				
	10%	5%	2.5%	1.25%	0.625%
1	19	16	15	13	11
2	20	16	15	13	12
3	20	17	16	14	13
4	19	16	14	14	12
Mean	19.5	16.25	15	13.5	12

Table 2: Zone of inhibition (mm) exhibited by ethanol extract of bark of *Combertum molle* against *Streptococcus aglactiae*.

Isolates	Zone of inhibition at different concentrations				
	10%	5%	2.5%	1.25%	0.625%
1	21	19	17	16	13
2	20	18	16	15	13
3	19	18	15	14	12
4	22	19	16	16	14
Mean	20.5	18.5	16	15.25	13

Table 3: Zone of inhibition (mm) exhibited by ethanol extract of leaf of *Xanthium strumarium* against *Staphylococcus aureus*.

Isolates	Zone of inhibition at different concentrations				
	10%	5%	2.5%	1.25%	0.625%
1	23	21	18	16	14
2	20	18	17	16	12
3	21	19	16	15	13
4	22	19	18	16	14
Mean	21.5	19.25	17.25	15.75	13.25

Table 4: Zone of inhibition (mm) exhibited by ethanol extract of leaf of *Xanthium strumarium* against *Streptococcus aglactiae*.

Isolates	Zone of inhibition at different concentrations				
	10%	5%	2.5%	1.25%	0.625%
1	15	14	10	8	6
2	14	12	10	7	5
3	14	13	08	6	4
4	13	10	08	7	4
Mean	14	12.25	9	7	4.75

Table 5: Zone of inhibition (mm) exhibited by ethanol extract of leaf of FR1 against *S. aureus*.

Isolates	Zone of inhibition at different concentrations				
	10%	5%	2.5%	1.25%	0.625%
1	23	21	19	17	14
2	24	22	19	17	14
3	23	21	18	16	14
4	23	21	19	16	13
Mean	23.25	21.25	18.75	16.5	13

Table 6: Zone of inhibition (mm) exhibited by ethanol extract of leaf of FR1 against *Streptococcus aglactiae*.

The effect of 10% plant extracts in comparison with commonly used conventional antibiotics

In general the zone of inhibition of the plant extracts were compared with commonly used conventional antibiotics for the treatment of clinical mastitis and they show better sensitivity than some antibiotics like Tetracycline. When compared with Gentamicin which is found to show better sensitivity to clinical mastitis 10% of FR1 was found with a comparable zone of inhibition than other plant extracts against *St. agalactiae* (Table 7), while 10% of *X. strumarium* found comparable with Gentamicin against *S. aureus* than other plant preparations (Table 8).

Types of diagnostic discs	Diameter of MZI (mm)
X. strumarium	20.5
C. molle	13.75
FR1	14
Tetracycline	NI
Gentamicin	26
DMSO	NI

Table 7: MZI (mm) exhibited by 10% extract of three plants compared with commonly used conventional antibiotic discs and negative control against *S. aureus*. NI=no inhibition.

Types of diagnostic discs	Diameter of MZI (mm)
X. strumarium	21.25
C. molle	19.5
FR1	23.25
Tetracycline	NI
Gentamicin	25
DMSO	NI

Table 8: MZI (mm) exhibited by 10% extract of three plants compared with commonly used conventional antibiotic discs and negative control against *St. agalactiae*. NI= no inhibition.

Discussion

Mastitis is the most frequently bacterial infection causing morbidity in highly productive cows and cause great economic lost to dairy cattle industry [31]. Although a large number of antimicrobial agents have been discovered, pathogenic microorganisms are constantly developing resistance to these agents. In recent years, attempts have been made to investigate the indigenous knowledge to look for alternative drugs against infectious diseases for safe and effective therapy [31]. In this study of sensitivity test ethanol extracts of three plant species (*Xanthium strumarium* leaf, *Combertum molle* bark and FR1 leaf) were used against two common mastitis causing bacterial species (*Streptococcus aglactiae* and *Staphylococcus aureus*) which are known to develop considerable antimicrobial resistance to commonly used antibiotics and found with encouraging results (Tables 1-6).

Commonly used antibiotics for the treatment of clinical mastitis like Gentamicin and Tetracycline were used in this study as positive controls and also to compare their zone of inhibition with 10% phytopreprations zone of inhibition while DMSO was used as negative control. When the overall result analyzed all three plants have strong antibacterial activity against the test bacteria especially at higher concentrations (10% and 5%) which agrees with the previous reports of Regassa and Araya [17]; Taddese et al. [18]; Mohamedamin [19] and Haile [20]. When the positive controls effect against *Streptococcus aglactiae* and *Staphylococcus aureus* compared Gentamicin was found with good zone of inhibition on both bacteria, while Tetracycline was found resistant.

For all the three plant preparations *St. aglactiae* show more sensitivity than *Staphylococcus aureus* (tables 1-6), these may be due to the ability of *S.aureus* to produce ant-microbial chemical to defend it soon from attack by the plant chemicals, but this result is not in line

with the previous report of Sahlu [21] and Haile [20] in which they reported respectively as *C. molle* bark and *X. strumarium* leaf show wider zone of inhibition on *S. aureus* than *St. agalactiae*. The possible explanation for the difference between the reports should be the quality of the isolate in case of *St. aglactiae* might be better than *S. aureus*, variation in the stage of maturation of the plant used, the solvent used as dissolution and also due to the method of processing of plants. DMSO which was used as negative control does not show any sensitivity to both bacteria's implying that the zone of inhibition recorded was merely by the active antibiotic ingredients found in the plant parts.

When zone of inhibition of each ethanol extracts of different concentrations of plants compared *Xanthium strumarium* leaf is better than FR1 leaf, which in turn is better than *C. molle* bark when tested aganist *S. aureus* (Figure 2) and it is not in line with the previous report by Sahlu [21]. In case of their effects on *Streptococcus aglactiae* FR1 leaf has wider zone of inhibition than *X. strumarium* leaf which in turn has wider zone of inhibition than *Combertum molle* bark (Figure 3). The variations in the sensitivity against bacteria from plant to plant even if the solvent and the extraction process was the same should be due to the active chemical constituent of the herbs. When the anti-bacterial activity of the 0.63% concentrations *X. strumarium* and *C. molle* compared the lowest inhibition zone was recorded by the bark of *C. molle* which disagrees with the previous works by Sahlu [21]; Regassa and Araya [17], and Taddese et. al. [18].

In this study a direct relationship between concentration and zone of inhibition was observed. Therefore, in all cases of the test plants with antimicrobial activity, there was a dose dependent inhibition on the tested bacteria showing greatest activity at highest concentrations of the crude extracts which agrees with previous works by Haile [20] and Sahlu [21] even if there is a difference in zone of inhibition at different concentration levels.

Combertum molle bark sensitivity results were compared with Taddese et. al. [18] and disagree in that the present study found with narrow mean zone of inhibition. The antimicrobial effect of *X. strumarium* leaf against *S. aureus* was comparable with Haile [20] and Sahlu [21] ethanol extract of the plant. The antibiotic sensitivity results of present study on X. strumarium leaf and C. molle bark against St. aglactiae found with a little wider zone of inhibition when compared with Sahlu [21]. These differences may be due to the method of sensitivity test performed, bacterial isolate quality and also the type of solvent used to macerate the plants, as those plant extracts using methanol showed wider zone of inhibition than ethanol extracts as indicated by previous studies by Taddese et. al. [18] and Haile [20].

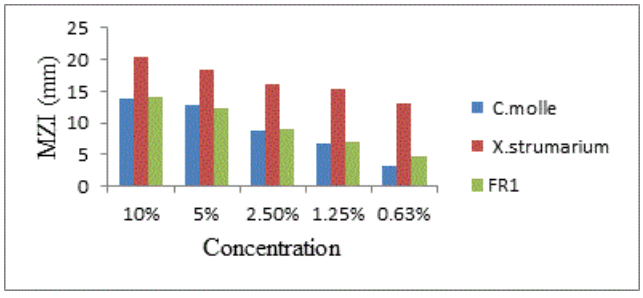

Figure 2: MZI of three different plant extracts on *S.aureus*.

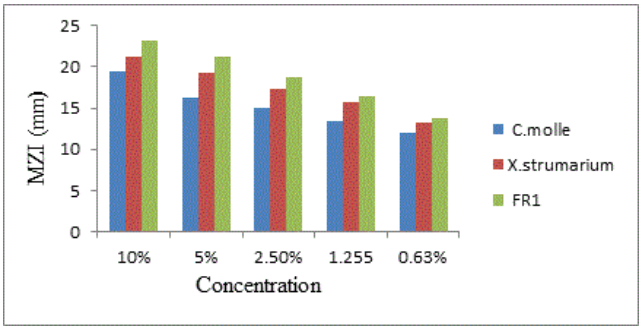

Figure 3: MZI of three plant extracts on *St. agalactiae*.

The antibacterial activity of 10% ethanol extracts of bark of *C.molle*, leaves of *X. strumarium* and FR1 were compared with standard antibiotic (Gentamicin) and appeared with a good antibiotic activity comparable with it which implies that these extracts may contain compounds with therapeutic potential comparable to the antibiotic. These *in-vitro* results told us as with some drugs, some of these plant extracts may be more potent in-vivo due to metabolic transformation of their components into highly active intermediates [31]. Therefore antimicrobial sensitivity results found in the previous and present studies may be a relief for the increasing antimicrobial resistance which frustrates the dairy industry and coming to be a major concern to the modern world animal and human health care.

Conclusion

Losses due to clinical mastitis cases are frustrating the dairy industry. It is not only because of the economic losses which comes by milk reduction and culling of high producing cows, but also because of the increasing resistance of the bacteria which causes mastitis for commonly used antimicrobial from day to day. Therefore the search for new antimicrobial is seen as saving the dairy sector. The present study was conducted on ethanol extracts of three selected medicinal plants and the results found against two bacteria species (*S.aureus* and *Streptococcus agalactiae*) which were isolated from bovine mastitis cases were very encouraging. The results found in this study are indicatives that these plants can be used as a source for the isolation of active compounds that may serve as leading compounds in antibacterial drug development and be a relief for the increasing problem of antibiotic resistance. These results might also confirm the great potential of plants of various countries for production of bioactive compounds for the treatment of many infections.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Acknowledgements

The authors are grateful to Addis Ababa University, College of Veterinary Medicine and Agriculture Microbiology Laboratory for provision of logistics, and the university staffs for material and logistic supports, and their cooperation to bring this research in to completion.

References:

1. Radostits M, Gay CC, Blood DC, Hinchllif KW (2007) Mastitis. In: Veterinary Medicine (9th edn) Haracourt Ltd, London Pp: 174-758.
2. Getahun K, Kelay B, Bekana M, Lobago F (2008) "Bovine mastitis and antibiotic resistance patterns in Selalle smallholder dairy farms, central Ethiopia", Tropical Animal Health and Production 40: 261-268.
3. Dego OK, Tareke F (2003) Bovine mastitis in selected areas of southern Ethiopia. Trop Anim Health Prod 35: 197-205.
4. Danso AAS, Vlas TDC (2002) Interpretation of low praziquanntel cure rates of Schistosomia monsoni. Trend in parasitology18: 95-143.
5. Suleiman MM, McGaw LJ, Naidoo V, Eloff JN (2010) "Detection of antimicrobial compounds by bioautography of different extracts of leaves of selected South African tree species". AJTACM 7: 64-78.
6. Freeman (1997) CD:"Anti-microbial resistance; implication for the clinician". JCCN 20: 21-35.
7. Samie A, Tambani T, Harshfield E,Green E (2010) Antifungal activities of selected venda medicinal plants against Candida albicans, Candidia krusei and Cryptococcus neoformans isolated from South African AIDS patients. AJB 9: 265-276.
8. Jagessar RC, Mars A, Gomes G (2008) "Selective Antimicrobial properties of Leaf extract of Phylanthus acidus against Candida albicans Staphylococcus aureus and E.coli using Stokes Disk diffusion, Streak plate and a dilusion methods". Nature and Science 6: 1545-0740.
9. Edwards R (2004) No remedy insights for herbal ransack. New science 181: 10-11.
10. Giday M, Asfaw Z, Woldu Z (2009) Medicinal plants of the Meinit ethnic group of Ethiopia: an ethnobotanical study. J Ethnopharmacol 124: 513-521.
11. Mesfin F, Demissew S, Teklehaymanot T (2009) An ethnobotanical study of medicinal plants in Wonago Woreda, SNNPR, Ethiopia. J Ethnobiol Ethnomed 5: 28.
12. Sori T, Bekana M, Adunga G, Kelbesa E (2004) "Medicinal plants in ethnoveterinary practices of Borana pastoralists, Southern Ethiopia". Ijarvm 2: 220-225.
13. Devi K, Karthikai GD, Thirumaran G, Arumugam R, Anantharaman P (2010) "Antibacterial activity of selected medicinal plants from Parangipettai coastal regions; Southeast coast of India". AJPS 3: 122-125.
14. Sharma A, Verma R, Ramteke P (2009) "Antibacterial activity of some medicinal plants used by tribals against uti causing pathogens". WASJ 7: 332-339.
15. Shewit K, Mekonnen H, Gebremedhin GE, Tsegay T, Samson S (2012) In-vitro antimicrobial activity of some ethno-vetrinary medicinal plants traditionally used against mastitis, wound and gastrointestinal tract complication in Tigray Region, Ethiopia. Apjtb 2: 516-522.
16. Regassa F, Araya M (2012) In vitro antimicrobial activity of Combretum molle against Staphylococcus aureus and Streptococcus agalactiae isolated from crossbred dairy cows with clinical mastitis. Tahp 44: 69-73.
17. Taddese T, Wagaye H, Regassa F (2009) A study on in-vitro antimicrobial effects of some selected plants on Staphylococcus aureus isolated from bovine clinical mastitis. Intjvm 8: 23-27.
18. Mohamedamin K (2011) An in-vitro antimicrobial effect of laggera alata and xanthium strumarium on Staphylococcus aureus isolated from bovine mastitis. DVM thesis, Addis Ababa University.
19. Haile W (2012) An in-vitro anti-bacterial effect of Xanthium strumarium and Grewia bicolor juss on Staphylococcus aureus isolated from bovine mastitis, DVM thesis, Addis Ababa University.
20. Sahlu T (2013) Antibacterial activities and preliminary phytochemical investigation of four selected medicinal plants, MSc thesis, Addis Ababa University. African Health Sciences 1: 66-72.
21. ADARDO (2007) Ada'a District Agricultural and Rural Development Office.
22. Rogers CB (1996) Chemistry and biological properties of the African Combretaceae. University of Zimbabwe Publications, Harare pp: 25-34.
23. Aghark SP (1991) Medicinal plants of Bomby presidency. Public Science Publishing. 1: 230.
24. Eloff IN, Famakin JO, Katerere DRP (2005) Isolation of antibacterial Stilbene from Combretum woodii (Combretaceae) leaves. African Journal of Biotechnology 4: 1167-1171.
25. Mengistu AK (2004) The effect of Herbal Preparation on Staphylococcus aureus and Strepto coccus agalatiae isolated from clinical bovine mastitis. MSC thesis, Addis Ababa University.
26. Olila D, Olwa-Odyek, Opuda-Asibo J (2001) Antibacterial and antifungal activities of extracts of Zanthoxylum chalybeum and Warburgia ugandensis, Ugandan medicinal plants. Afr Health Sci 1: 66-72.
27. Tanih NF, Okeleye BI, Clarke AM, Naidoo N, LMNdip (2010) Marked susceptibility of South African Helicobacter pylori strains to ciprofloxacin and amoxicillin: clinical implications. South African Medical Journal 100: 49-52.
28. Quinn PJ, Carter ME, Markey BK, Carter GR (1999) Clinical veterinary microbiology, London, Mosby International Limited, UK.
29. Jain PJ, Patil SD, Girase MV, Surana SJ (2010) Hypolipidemic activity of Moringa oleifera LamMoringaceae, on high fat died-induced hyperlipidemia in albino rats. Bjps, 20: 969-973.
30. Bastos HN, Antão MR, Silva SN, Azevedo AP, Manita I, et al. (2009) Association of polymorphisms in genes of the homologous recombination DNA repair pathway and thyroid cancer risk. Thyroid 19: 1067-1075.
31. Ndip RN, Malange Tarkang AE, Mbullah SM, Luma HN, Malongue A, et al. (2007) In vitro anti-Helicobacter pylori activity of extracts of selected medicinal plants from North West Cameroon. J Ethnopharmacol 114: 452-457.

This article was originally published in a special issue, entitled: **"Animal behaviour"**, Edited by Wael EL-Deeb