

# A Study On In-Vitro Antimicrobial Effects Of Some Selected Plants On Staphylococcus Aureus Isolated From Bovine Clinical Mastitis

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## Abstract

A study on in-vitro antimicrobial effects of some selected plants on Staphylococcus aureus from bovine clinical mastitis was conducted at Debrezeit, Faculty of Veterinary Medicine of Addis Ababa University from November 2007 to May 2008 with the objectives of determining and comparing of the in-vitro antibacterial effects of three preparations; namely, Combretum molle, Commicarpus pedunculatus and Lagerfloraria siceraria on Staphylococcus aureus. The plants were collected from central and northern parts of the country. Of the three candidate plants, in this study, Commicarpus pedunculatus and Combretum molle had antibacterial activity against the tested bacteria but Lagerfloraria siceraria had no any antibacterial activity against Staphylococcus aureus. The methanol (80%) crude extracts of the two plants inhibited the growth of Staphylococcus aureus at concentration of 2.5 %, 5 %, 10 % and 10 % for Commicarpus pedunculatus and from 1.25 % to 20 % of doubling concentrations for Combretum molle. There was a dose dependent inhibition on the tested bacteria showing greatest activity at highest concentration of crude extracts. A wider zone of inhibition was observed from methanol extracts of Commicarpus pedunculatus at higher concentrations than Combretum molle. The findings suggest that there is a potential in the discovery of novel antimicrobial agents from medicinal plants and further study should be made in order to identify the active phytochemical constituents and on toxicity of active plant principles to determine their safety use.

## INTRODUCTION

The conventional drugs used for the treatment of mastitis are of limited in types in developing countries in general and in Ethiopia in particular due to this and other factors causal agents have showed variable degree of resistance. Staphylococcus aureus are the bacterial causative agents which are all ready had developed resistance to certain antibiotics by experimental and practical application. In Ethiopia, the study finding reported on these bacteria from in-vitro sensitivity testing was proving this fact [1, 2, 6].

In Ethiopia, traditional healers use a number of plants for the treatment of bovine mastitis. The efficiency of some of these plants/herbs have been tested against a range of causative agents of mastitis. In-vitro study conducted by Sahle [3] indicated that Persicaria senegalensis, Cyphostema adenocaulum and Commisficifolius, have shown some degree of growth inhibitory effect. Markos [4] screened some herbal preparation against mastitis causing pathogens. Mengistu [5] has screened six herbal preparations; namely, Brucea

antidysentrica, Combretum molle, Cyphostema adenocaulum, Persicaria senegalensis, Plantago lanceolata and Zahneria scabra on major isolates of bovine mastitis and also Taddese [6] has conducted in-vitro tests of Combretum molle on Staphylococcus aureus isolate and observed encouraging results. Therefore the main objectives of the present study were to determine and compare the in-vitro antimicrobial effects of three phyto preparations; namely Commicarpus pedunculatus, Combretum molle and Lagerfloraria siceraria on Staphylococcus aureus isolated from bovine clinical mastitis.

## MATERIALS AND METHODS

### DESCRIPTION OF THE STUDY AREA

A study of in-vitro antimicrobial effect of phytopreparations was carried out in Debrezeit between November 2007 and May 2008. Debrezeit town is located at 47 km south-east of Addis Ababa. The area has an altitude of 1,850 meters above sea level with an average annual rain fall of 866 mm. It has a bimodal rainy seasons; a main rain season extends from the

month of June to September and a short rainy season from March to May. The annual average minimum and maximum temperature is 11 C and 26 C, respectively. Day length is fairly constant throughout the year (12-13 hrs) with about 6 hours of sunshine during the rainy season and 8 to 10 hours for the rest of the year. Humidity is about 50.9 % [7].

## **STUDY DESIGN**

An experimental study on in-vitro antimicrobial efficacies on selected plants was conducted between November 2007 and May 2008 in AAU, Faculty of Veterinary Medicine, Debrezeit.

## **HERBAL MATERIALS USED FOR THE STUDY**

1. Combretum molle (“Agalo, Abalo” in Amharic, “Bika, Dadamata” in Oromiffa) known with common name velvet-leaved combretum (Fig 1). This is a member of the family Combretaceae which is a small deciduous tree growing up to 15 meters high with an often-crooked trunk, commonly branching to the base. The bark is dark brown to black and deeply grooved in squares. The leaves are oppositely arranged, elliptic to lanceolate, large that covered with soft hairs, rounded at the base. The flowers generally appear before the leaves and the fruits yellowish, four-sided with wings. The leaves were used to assess the antimicrobial effect on bacterial isolates from mastitic cases.

2. Commicarpus pedunculatus (“Tihuan tila” in Amharic, “Sara” in Oromiffa) known with common name Chick weed (Fig 2). This is a member of the family Nyctaginaceae which is a prostrate or scrambling, glabrescent to pubescent herb with stems up to 3m long. Leaves ovate up to 7cm long, 4cm wide, the base shallowly cordate, truncate, or cuneate, apex acute to obtuse; margins entire to sinuate; petioles up to 2m long. Inflorescence a dense many-flowered head, on a long stout peduncle up to 23cm; flowers subsessile, but pedicels enlarging in fruit up to 4-10 mm long. The leaves were used to assess the antimicrobial effect on bacteria isolated from mastitic cases.

3. Lagenaria siceraria (“Kil” Amharic) known with common name bottle gourd (Fig 3) is belongs to the family Cucurbitaceae. The leaves were used to assess the antimicrobial effect.

## **BACTERIAL ORGANISMS USED FOR THE STUDY**

One species of bacterium, Staphylococcus aureus, coming from the clinical mastitis case and representing common

bacterial pathogens (from Department of microbiology of Faculty of Veterinary Medicine Debre Zeit) was used in testing.

## **STUDY METHODOLOGY**

### **PLANT COLLECTION AND PRE-EXTRACTION PREPARATION**

The plants were chosen based on the results showed by previous workers on the leaf of the plants [3, 5, 6]. Combretum molle was collected from its natural habitat, south Gondar and the other two plants (Commicarpus pedunculatus and Lagenaria siceraria) were collected from Debrezeit. After collection the plants were washed with tap water to remove unnecessary particles. Then dried under shade, chopped by knife and axe in to pieces to facilitate drying and grounded using pestles and wooden mortars. The material was then sieved and weighed before maceration.

### **PREPARATION OF CRUDE EXTRACTS FOR IN-VITRO EXPERIMENT.**

50 gm of each of the powdered herb leaves of the three plants were macerated in 80% methanol in a flask separately and mixed using a magnetic stirrer. Then after it is allowed to stand for 3 days at room temperature, each sample was then strained using a tea strainer to remove solids. The resulting filtrate was then further filtered using filter paper to obtain a solution free of solids (Fig 4). The solution was then concentrated in a rotary evaporator to remove the methanol. The plant extracts of the three plants were then taken out and put in evaporating dishes and kept in a dry oven at 40 C to remove the remaining solvent for 24 hours. The residues left after straining and filtration and were macerated again in the same way. The procedure was repeated in the same way for third time to have sufficient amount of extracts. The resulting concentrated extracts were weighed, transferred and labeled with the respective plant names and stored at + 40 until tested for antimicrobial activity. The flow chart from collection to extraction is shown below.

### **PREPARATION OF ANTIMICROBIAL DISCS FROM HERB EXTRACTS FOR IN -VITRO EXPERIMENT**

Six serial dilutions with different concentrations (20 %, 10 %, 5%, 2.5 % and 0.625 %) of each plant extract were prepared using Dimethylsulfoxide (DMSO) as described by Olila et al. [8]. In the first test tube 2ml of DMSO was added and each of the remaining five tubes was filled with 1ml of DMSO. 1ml of 20% solution from the first tube was

transferred to a second test tube to prepare 10% .The procedure continues by transferring 1ml of solution from the 10% preparation to a third test tube to get a 5 % concentration, and continued in a similar manner until a 0.625% concentration is reached. Discs of 12mm diameter were impregnated by adding three drops from each reconstituted solution and allowed to dry at 37 C over night. Dried discs were used to determine antimicrobial effects of the respective plant types. Each disc was gently pressed down to ensure complete contact with the agar and the plates were inverted and incubated at 37 C for 24 hours. The diameter of zone of inhibition was measured in millimeters.

### **ANTIMICROBIAL SENSITIVITY TEST**

The antimicrobial test was conducted using agar disc diffusion method. Staphylococcus aureus from clinical mastitis cases were used for this study. 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 (alternative method) and agitated with a vortex mixer [9].

The turbidity of the bacterial suspension was adjusted by comparing with 0.5 McFarland turbidity standards. 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. A sterile swab was dipped in to the standardized suspension of the bacteria and excess fluid was expressed by pressing and rotating the swab firmly against the inside of the tube above the fluid 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 [9].

Muller-Hinton agar (38gm) (Biotech UK) medium was used for antimicrobial sensitivity test, and was mixed with 1 litter of distilled water, boiled to dissolve completely and autoclaved at 121c for 15minutes. The medium was later dispensed in to 90mm sterile agar plates and left to set. The agar plates were incubated for 24 hours at 37 C to confirm

their sterility. When no growth occurred after 24 hours, the plates were considered sterile and used for antimicrobial sensitivity tests.

Barium solution was used as standard to determine the bacterial concentration was prepared as 1% solution in 1% H<sub>2</sub>SO<sub>4</sub> solution. The preparation was kept in dark for the preparation of bacterial suspension. Colonies were picked from the culture under study and placed in 4ml sterile physiological saline and the culture was standardized by comparing with McFarland solution.

The appropriate crude extract impregnated discs and conventional discs were applied at spaces of 24mm apart from center to center and 15mm away from the edge of the plates. This was made no later than 15minutes after the inoculums has been seeded. The plates turned upside down, labeled and incubated at 37C for 24 hours. Diameter of zone of inhibition was measured using a ruler in millimeters and results were recorded as susceptible, intermediate or resistant by comparing with standard values for each conventional antibiotic disc [9].

### **DATA ANALYSIS**

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

### **RESULTS**

#### **ANTIMICROBIAL SUSCEPTIBILITY TESTING EFFECTS OF HERBAL PREPARATIONS**

Each plant extracts of the three plant species were tested at different concentrations levels (20%, 10%, 5%, 2.5%, 1.5%, 2.5%, 1.25%, and 0.625%) to see their inhibitory effects against Staphylococcus aureus (Fig 6). Of the three candidate plants in this study, two plants showed antibacterial activity against the tested bacteria and the remaining plant did not show any activity after alcoholic extraction (Table 1).

zone of inhibition in mm for each plant extracts at doubling concentrations ranging from 0.625% to 20% (Table 1). The inhibiting zone increased with increasing concentrations of the extracts for the two plant species (Commicarpus pedunculatus and Combertum molle). There are no inhibition zones for bacterial growth at the lower concentrations 1.25 % and 0.625 % of Commicarpus pendunculatus and 0.625 % Combertum molle respectively. A significant difference was observed against

Staphylococcus aureus inhibition by the leaf of both Commicarpus pedunculatus and Combretum molle at higher concentrations (Table 1 and Fig 5). The zone of inhibition for the leaf of Commicarpus pedunculatus against Staphylococcus aureus was higher than Combretum molle at concentrations 2.5% to 20 %, whereas Combretum molle has more effect than Commicarpus pedunculatus at lower concentration (1.25%) (Fig 5). The extracts of Lagenaria siceraria, however virtually demonstrated no activity at all concentrations against Staphylococcus aureus.

**Figure 1**

Fig 1. Combretum molle (velvet-leaved combretum)



**Figure 2**

Fig 2. Commicarpus pedunculatus ( Chick weed)



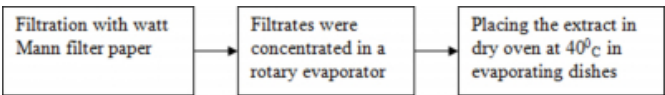
**Figure 3**

Fig 3. Lagenaria siceraria (bottle gourd)



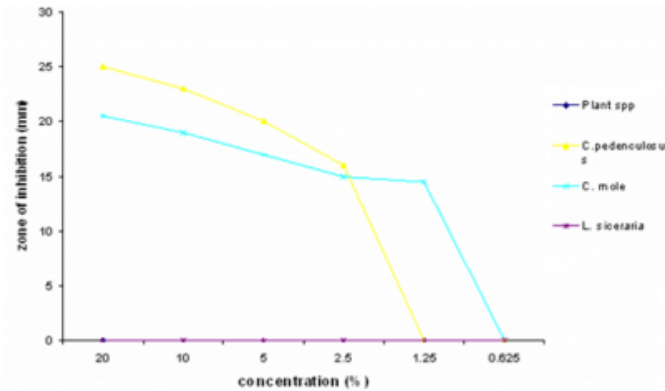
**Figure 4**

Fig. 4. Flow chart for extraction



**Figure 5**

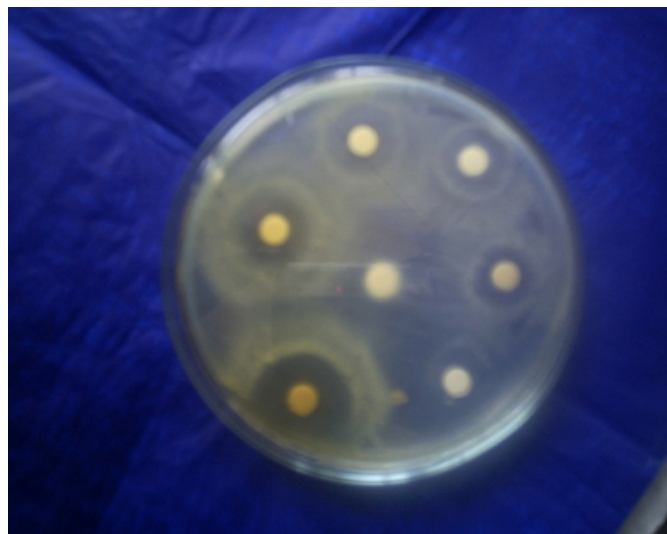
Fig. 5. In-vitro efficacy tests of 80% methanol crude extracts of leaf different plants on





**Figure 6**

Fig. 6. Inhibitory effects of against Table 1. In-vitro efficacy testes of 80% methanol crude extracts of leaf of different plants on .



## DISCUSSION

In this study an in-vitro antimicrobial efficacy test of three herbal preparations was performed on *Staphylococcus aureus* and the result of this study demonstrated that two plants; namely, *Combretum molle* and *Commicarpus pedunculatus* have shown a promising effect on *S. aureus*, indicating their potential value to treat mastitic infections caused by *S. aureus*. On the other hand extracts of *Lageria siceraria* were devoid of antimicrobial activity against the tested bacteria. Therefore it may not be of value in treatment of mastitic infections caused by *S. aureus*. This finding agrees with the previous study by Sahle [3] in which he reported *Commicarpus pedunculatus* used for bovine mastitis treatment traditionally. Conversely, disagree with Sahle [3] in which he reported the use of *Lageria siceraria* in traditional application for bovine mastitis.

Of the plant types tested for their efficacy in this study, the leaf part of *Commicarpus pedunculatus* had showed a wider zone of inhibition at higher concentrations as compared to *C. molle*. However, *Commicarpus pedunculatus* has a negative effect at its lower concentrations whereas *C. molle* showed a wider zone of inhibition at all concentration except at its least concentration. In this regards, *C. molle* has shown better activity at its lower concentrations than *C. pedunculatus* and *C. pedunculatus* has shown better activity at its higher concentration on *S. aureus*.

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. A wider zone of inhibition with increasing concentrations of methanol extracts of leaf of *Combretum molle* on *Staphylococcus aureus* agrees with the previous workers on this plant [5, 6] even if there is a variation in zones at each concentration levels. Mengistu [5] confirmed a good inhibitory effect of *Combretum molle* at all concentrations on *S. aureus* and also Taddese [6] in his in-vitro tests on different parts of *Combretum molle* was found an incredible result from root of this plant as compared to other parts and the result obtained from leaf was almost similar at some concentrations levels. Thus, the difference and similarities of this study with others may be due to the method of antimicrobial sensitivity test adopted, the solvent used to prepare the bacteria, extractions method, and storage condition.

## CONCLUSION

In this study the leaf of both *Commicarpus pedunculatus* and *Combretum mole* showed good antimicrobial activity against *Staphylococcus aureus* while the leaf of *Lageria siceraria* had no any activity. The demonstration of satisfactory growth inhibition against *Staphylococcus aureus* at different concentrations would seem to justify their future potential in the synthesis of new phytochemicals and their use in treatment of microbial infections. One way to control drug resistance problem is through the development of alternative drugs by screening and testing medicinal plants for their susceptible antimicrobial effects. Further studies should be made in order to identify the active phytochemical constituents and evaluate their effectiveness in-vivo and in-vitro so that they can be produced commercially. In addition, toxicity studies of the active plant principles should be done to determine their safety

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