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In-Vitro Antibacterial Effects of Laggera *Alata* and *Ehretia Cymosa* against *Staphylococcus aureus* and *Streptococcus aglactiae* Isolated from Bovine Mastitis

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Abstract

An experimental study was conducted from November 2011 to May 2012 to assess the *in-vitro* antibacterial effect of *Laggera alata* and *Ehretia cymosa* on bacteria isolated from bovine mastitis. The plants/herbs were collected from their natural habitats and processed and extracted with 80% methanol and 95% ethanol. In this study, *Laggera alata with both types of alcoholic extraction* had antibacterial activities against *Staphylococcus aureus* and *Streptococcus agalactiae* but *Ehretia cymosa* had no antibacterial activity against *S. aureus* and *S. agalactiae.* The methanol (80%) and ethanol (95%) crude extracts of *Laggera alata* inhibited the growth of *S. aureus* and *S. agalactiae* at all concentrations (0.63% to 20%). Of doubling concentrations for both types of extraction, there was a dose-dependent inhibition on the tested bacteria showing the greatest activity at the highest concentration of crude extracts. A wider zone of inhibition was observed from methanol extracts of *Laggera alata* at all concentrations than *Laggera alata* ethanol extract. The efficacy of 20% crude extracts of *Laggera alata* in both types of extraction was comparable with conventional antimicrobial agents like Gentamycin, Erythromycin, and Kanamycin. The findings suggest that there is a potential in the discovery of novel antimicrobial agents from medicinal plants and further study should be made to identify the active phytochemical constituents and on the toxicity of active plant principles to determine their safety use.

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Introduction

Ethiopia's livestock population is the largest in Africa. Currently, Ethiopian is estimated to have about 56.71 million heads of cattle, 29.33 million heads of Sheep, 29.11 million heads of goats, and 56.87 million poultry. The livestock sector in Ethiopia contributes 16.5% of the national GDP, and 47.7% of the agricultural GDP, 15% of the country's export earnings and 30% of agricultural employment [1]. Mastitis is an inflammation of the udder resulting from the invasion of pathogenic microorganisms. At least 137 infectious causes of bovine mastitis are known to date and in large animals, the commonest pathogens isolated from bovine mastitis include *Staphylococcus aureus, Streptococcus agalactiae*, other *Streptococcus,* and coliforms [2]. The disease causes irreversible damage to the udder tissue and can lead to the reduction of offspring to a given production system due to insufficient milk production resulting in starvation [3,4]. The cow udder is an ideal environment for microbial growth and under optimum udder conditions, Pathogenic organisms multiply highly and cause udder damage and trigger the response that is recognized as mastitis [3,5].

Mastitis continues to be among the expensive disease of the dairy industry. Among cattle diseases, bovine mastitis is a serious problem that affects the basic income of farmers depleting their dairy sources. It adversely affects milk production whereby losses due to subclinical mastitis are more severe than those due to clinical cases [6,7]. Milk contamination by zoonotic pathogenesis often natural but can also occur through handling milk in unhygienic conditions [8]. Due to the absence of modern animal health services particularly in rural areas, livestock owners frequently visit traditional healers to get solutions for their ill-health animals including mastitis problems [5,9,10].

Antibiotics were considered a magic bullet that selectively targeted microbes that were responsible for disease causation, but at the same time would not affect the host and multiple varieties of the antibiotics have been used for therapeutic purposes over time [11]. The emphasis of clinical mastitis treatment has been on antimicrobial therapy and currently, there are several conventional antibiotics with different degrees of spectrums that are used for the treatment of the disease. An important aspect of mastitis therapy is the alleviation of inflammation that can result in swelling and subsequent pain associated with clinical mastitis that can cause considerable discomfort to the cow in the udder. Then, the purpose of mastitis therapy is to assist the affected quarter to clear infection as rapidly as possible to enable a quick return of the cow to normal milk production. A multitude of mastitis therapy, which includes the use of frequent striping, herbal udder ointments, and oral preparations of massage and diet changes have been used before and after the advent of antibiotic therapy [2,12,13].

The medicinal plant of Ethiopia and the developing countries play major supplementary roles in the limited modern health care available [14,15]. In Ethiopia, traditional healers use several plants/herbs for the treatment of bovine mastitis and the efficacy of some of these plants/herbs have been tested against a range of causative agents of mastitis [2,16]. *In- vitro* study conducted by [2,6,10,17,18] on different plants indicates that there are growth inhibitory effects on different bacterial species. The various literature available shows the significant role of medicinal plants in primary health care delivery in Ethiopia where 70% of humans and 90% of livestock population depend on traditional medicine again similar to many developing countries particularly that of Sub-Saharan African countries [14].

The most important characteristics of traditional herbal therapies are their crude preparations. Herbs with different ingredients and properties are administered in their natural state in the form of water mixtures, boiled or unboiled, and are applied topically as pastes as well as other forms [19]. The pharmacological studies and clinical trials on different plants indicate that a significant proportion of indigenous remedies of plant origin have shown promising biochemical activities and clinical effects [10,20].

The conventional drugs used for mastitis treatments are of limited types, especially in Ethiopia. For this reason, herbal remedies remain the only option for many poor farmers as the main animals' health intervention. However, there are no enough documented data about traditional remedies to confidently recommend it for the treatment of livestock diseases. Therefore, the current study aimed to determine the *In-vitro* antimicrobial activities of leaf extracts from some traditional medicinal plants; namely *Laggera alata*, and *Ehretia cymosa* extracted using methanol and ethanol extract of *Staphylococcus aureus* and *Streptococcus agalactiae* isolated from bovine mastitis.

Materials and methods

Study area

The study of the antibacterial effect of some selected medicinal plants on bacterial pathogen was carried out from November 2011 to April 2012 in Bishoftu Town. Bishoftu is located at 47 km southeast of Addis Ababa. The area has an altitude of 1860 meters above sea level with an average annual rainfall of 866 mm. It has a bimodal rainy season; a main rainy season extends from June to September and a short rainy season from March to May. The annual average minimum and maximum temperatures are 11^{o}C and 26^{o}C respectively. Day length is fairly constant throughout the year (12-13 hrs) with about 6hrs of sunshine during the rainy season and 8 hrs to 10 hrs for the rest of the year. Humidity is about 50.9% [5].

Study design

An experimental on in-vitro antimicrobial efficacy on selected plants were conducted between November 2011 and May 2012 in Addis Ababa University, College of Veterinary Medicine and Agriculture, Bishoftu. Besides, an investigation was carried out through a field survey or searching of different areas in Bishoftu town where plants were found and an experimental study.

Herbal/plant materials used for the study

Laggera alata /kesebedeje

A robust much-branched pubescent herb; 60-75 cm tall stems are winged, Herbaceous, and rarely somewhat denticulate and continuous. Leaves oblong, 2.8 - 10 * .7-1.5 cm with the decurrent bases and denticulate margins, acute to sub obtuse densely covered with long hairs. It is a strongly aromatic stout herb with a persistent thymol-like and sweet odor. Its leaves yield an essential oil that is said to possess the odor of black current and may have some application in perfumery [16,21].

Ehretia cymosa/Du-tsho is a deciduous shrub or small to medium-sized tree up to 20 to 25 m tall; bole often low branching and crooked, up to 30 cm in diameter; bark surface grey to pale brown, with prominent lenticels, inner bark soft, white, spotted with orange-brown, quickly turning brown upon exposure; crown spreading, often with drooping branches; twigs short-hairy but soon becoming glabrous. Leaves arranged spirally,

simple and entire; stipules absent; petiole 1-3.5 cm long, slightly grooved; blade elliptical to ovate-oblong, 7.5-20 cm × 3.5-12 cm, cuneate to rounded or slightly cordate at base, acuminate at apex, thinly leathery, nearly glabrous, pinnately veined with 3-8 pairs of lateral veins.

Inflorescence an axillary or terminal, strongly branched panicle up to 15 cm × 15 cm, composed of scorpioid cymes, hairy. Flowers bisexual, regular, usually 5-merous, heterostylous, fragrant; pedicel up to 2-3 mm long, jointed at base; calyx campanulate, 1.5-2.5 mm long, lobes about as long as the tube; corolla campanulate, 4-8 mm long, white to yellowish or pinkish-white, lobes about as long as tube, often reflexed; stamens inserted at corolla, exserted; ovary superior, ovoid, c. 1 mm long, 2- or 4-celled, style 1-4 mm long, 2-branched at the apex. Fruit an ovoid to globose drupe 2-6 mm long, orange to red and eventually turning black, splitting into 4 pyrenes, each 1-seeded [21].

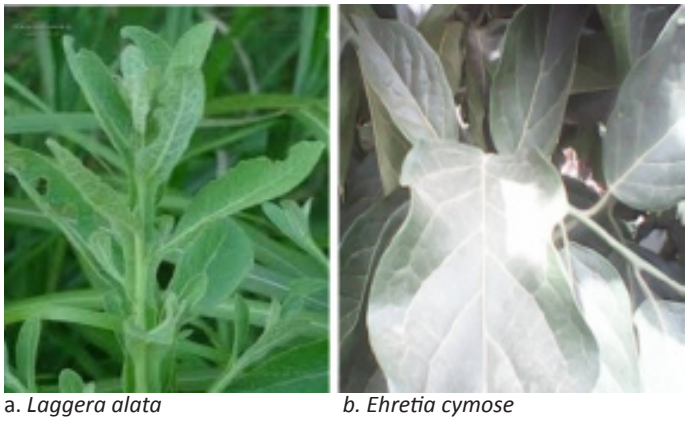
Bacterial organisms used to the study

Two bacterial species, *Streptococcus agalactiae* and *Staphylococcus aureus* isolated from bovine mastitis cases from the dairy farm of the College of Veterinary Medicine and Agriculture, AAU were used to the study.

Study methodology

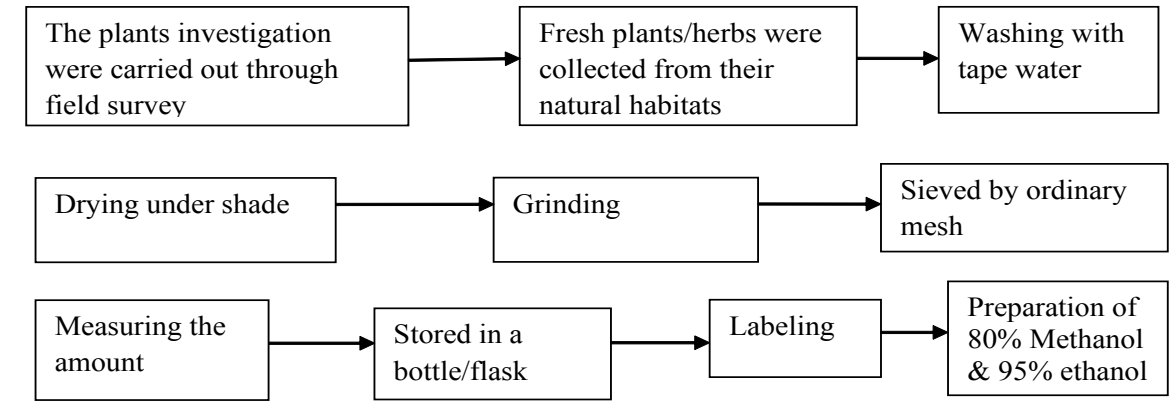
Plant collection

The plants were chosen based on the results showed by previous workers on the leaf of *Laggera alata* [17] and *Ehretia cymosa* was the first time. Both of the plants were collected from the Bishoftu area. The following chart shows the general information about the plant investigation to extraction.

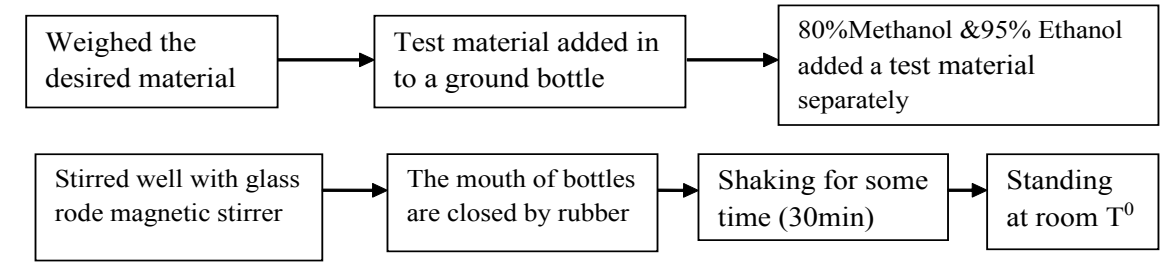


a. *Laggera alata* b. *Ehretia cymose*

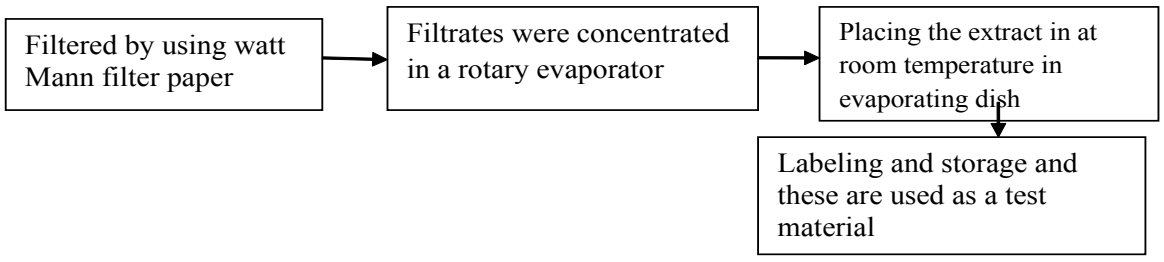
Step 1: Plant collection and preparation



Step 2: Maceration



Step 3: Extraction



Preparation of crude extracts for an in-vitro experiment

40 gms of each herb/leaves were weighed and macerated in 80% methanol and 95% ethanol in large ground bottle and mixing of content taking place at maximum speed for 30 minutes. Mixed content was allowed standing for five days at room temperature. Then after five days, each sample was strained using the strainer to remove the solids. The resulting filtrate was further filtered using filter paper to obtain a solution free of solids. The solution was then concentrated on a rotary

evaporator to remove methanol and ethanol. The plant extracts were taken out and put in evaporating dishes kept for 48 hours in an environmental temperature to remove the remaining solvent. The resulting concentrated extracts were taken out and labeled the respective plant names and store +4^{0}c until the test for antimicrobial activity.

Preparation of antimicrobial discs from herb extracts for an in-vitro experiment

Six serial dilutions at different concentrations (20%, 10%, 5%, 2.5%, 1.25% and 0.625%) of each plants extracts were prepared using Dimethyl Sulfoxide (DMSO) as described by [13]. Point eight grams of plant extracts were mixed with 4ml Dimethyl Sulfoxide (DMSO) in the first test tube to prepare a 20% solution according to [22,23]. In the second test tube, 2 ml of DMSO was added and each of the remaining four tubes was filled with 2ml of DMSO.

Two milliliters of 20% solution from the first test tube was transferred to the second test tube to prepare 10%. The procedure continues by transferring 2ml of solution from the 10% preparation to a third test tube to get a 5% concentration and continued similarly until a 0.625% is reached. Discs of 12mm diameter were impregnated by adding 3 to 5 drops from each reconstituted solution and allowed to dry at 37^{o}C overnight in a hot air oven. Dried discs were used to determine the 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^{o}C for 24 hrs. The diameter of the zone of inhibition was measured in millimeters.

Preparation of the test bacteria

The bacteria were isolated from bovine mastitis by collecting fresh milk samples aseptically and then culturing on different agar Media for testing of primary and secondary biochemical tests.

The in-vitro antimicrobial sensitivity test of the alcoholic extract

The antimicrobial test was conducted using the agar disc diffusion method. Bacteria from bovine mastitis cases were used for this study. The top 4 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 [13].

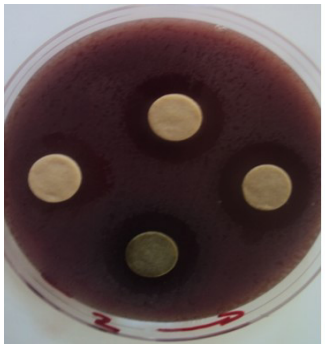
The turbidity of bacterial suspension was adjusted by comparing it with the 0.5 McFarland turbidity standard. Standard and the test suspension were placed in 10ml sized test tubes and compared against a white background with contrasting black lines until the turbidity of test suspension equates to that of turbidity standard. Adjustments of turbidity were made by adding saline or colonies depending on the degree of turbidity. A sterile swab was dipped into the standardized suspension of 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 3 directions over the entire surfaces of the agar to obtain uniform inoculations and a final sweep with the swap was against the agar around the rim of the Petri dish [24].

The inoculated plates were allowed to stand for not more than 15minutes and the discs were place 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 [24].

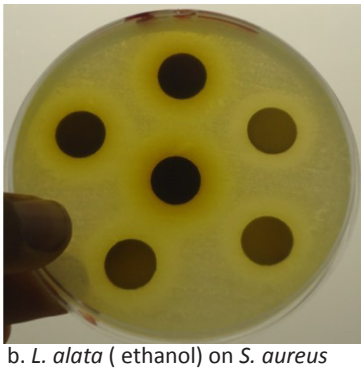
For this study penicillin, Gentamycin, Kanamycin, and Erythromycin were used to compare their efficacy of herbal preparations. The activities of the conventional antimicrobials were compared against 20% concentrations of each test plant. The solvents DMSO served as a negative control.

Muller-Hinton agar (38gm) medium was used for antimicrobial sensitivity test and was mixed with 1 litre of distilled water, boiled to dissolve completely and autoclaved at 121^{o}C for 15 minutes. The medium was later dispensing about 25 ml into 90 mm sterile agar plates and left to set. The agar plates were incubated for 24 hrs at 37^{o}C to confirm their sterility. When no growth occurred after 24 hrs, the plates were considered as sterile and used for antimicrobial sensitivity test for *streptococcus agalactiae* 5 to 10% of uncoagulated sheep blood was added [24].

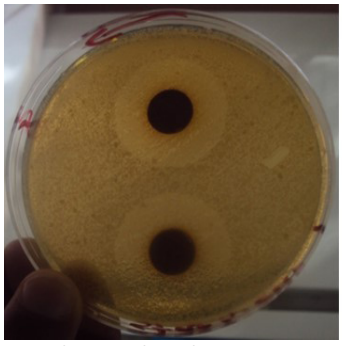
A barium solution was used as a standard to determine the bacterial concentrations that were prepared as a 1% solution in 10% H_2SO_4 solution. The preparations were kept in dark for the preparation of the bacterial suspension. Colonies were picked from the culture under study and placed in 4 ml sterile physiological saline and the cultures were standardized by comparing with 0.5 McFarland solution. The appropriate crude extract impregnated discs and conventional discs were applied at spaces of 24 mm apart from center to center and 15 mm away from the edge of the plates. The plates turned upside down, labeled and incubated at 37^{o}C for 24 hrs. Diameters of the zone of inhibitions were 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 [24].



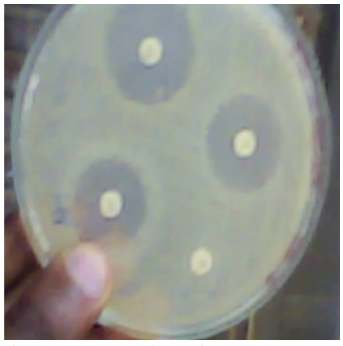
a. *L. alata* (methanol) extract on *S. aglactiae*



b. *L. alata* (ethanol) on *S. aureus*



c. *L. alata* methanol extract on *S. aureus*



d. Conventional antibiotic on *S. aureus*

Figure: Zone of inhibition conventional antibiotic discs and plants/herbs crude extracts.

Data management and statistical analysis

Data collected from laboratory were recorded in the format developed for this purpose and later on entered into Microsoft Excel 2016 and analysis was carried out using a standard statistical software program (STATA version 13). The data summarized using descriptive statistics and all values were expressed in Means and results were presented as tables and graphs for illustration.

Results

The effects of crude extracts

Each type of extracts of the two plant species were tested at different concentration levels (20%, 10%, 5%, 2.5%, 1.5%, 2.5%, 1.25%, and 0.625%) to see their inhibitory effects against *S. aureus* and *S. agalactiae.* Of the two candidate plants in this study, one plant (*L. alata*) both types of extraction (methanol and ethanol) showed antibacterial activity against the tested bacteria (Figure 3 & 4) and the remaining plant namely *E. cymosa* both types of extraction did not show any activity after alcoholic extraction.

The zone of inhibition in millimeters for each type of extract at doubling concentrations ranging from 0.625% to 20% were recorded (Table 1, 2, 3, and 4). The inhibition zone increased with increasing concentrations of the extracts for both types of extracts of the plant species (*L. alata*). There were inhibition zones for bacterial growth at all concentrations (0.625 % to 20%) of *L. alata* both types of extraction. The zone of inhibition for the leaf of *L. alata* extracted by methanol against *S. aureus and S. agalactiae* was higher than that of ethanol extracts *at* the same concentrations whereas *E. cymosa* has no effect on the tested bacteria at all concentration in both types of extraction. The Mean Zone of Inhibition (MZI) of *L. alata* methanol extractions were compared to ethanol extraction against *S. aureus* and *S. agalactiae* (Figures 1 & 2).

Table 1: Zone of inhibition conventional antibiotic discs and plants/herbs crude extracts.

Isolate	Zone of inhibition at different concentrations (mm)					
	20%	10%	5%	2.5%	1.5%	0.625%
1	25	24	20	18	16	14
2	26	25	23	18	16	14
3	28	27	26	24	22	20
4	24	22	19	17	16	14
Mean	25.75	24.5	22	19.25	17.5	15.5
95% CI	24.9-26.6	22.5-26.5	18.8-25.2	16.2-22.3	14.5-20.5	13.6-17.4

95% CI: 95 percent of the confidence interval

Table 2: Zone of inhibition of methanol extracts of *L .alata* against *S. agalactiae* at different concentrations.

Isolate	Zone of inhibition at different concentrations (mm)					
	20%	10%	5%	2.5%	1.5%	0.625%
1	24	22	21	20	19	17
2	25	23	22	21	20	18
3	23	22	21	20	19	17
4	26	24	21	21	20	18
Mean	24.5	23	21.5	20.5	19.5	17.5
95% CI	23.2-25.8	22-24	20.9-22.1	19.2-21.1	18.9-20.1	16.9-17.4

Table 3: Zone of inhibition of ethanol extracts of *L. alata* against *S. aureus* at different concentrations.

Isolate	Zone of inhibition at different concentrations (mm)					
	20%	10%	5%	2.5%	1.5%	0.625%
1	20	19	18	17	16	13
2	23	21	20	17	16	14
3	22	20	19	18	17	16
4	20	18	17	16	15	14
Mean	21.25	19.5	18.5	17	16	14.25
95% CI	19.6-22.8	18.2-20.8	17.2-19.8	16.2-17.8	15.2-16.8	13-15.6

Table 4: Zone of inhibition of ethanol extracts of L. alata against S. agalactae at different concentrations.

Isolate	Zone of inhibition at different concentrations (mm)					
	20%	10%	5%	2.5%	1.5%	0.625%
1	23	21	20	19	17	15
2	22	22	20	19	18	16
3	21	21	19	17	17	14
4	24	22	20	18	16	16
Mean	22.5	21.5	19.75	18.25	17	15.25
95% CI	21.2-23.8	20.5-22.5	19.3-20.3	17.3-19.2	16.2-17.8	14.5-16

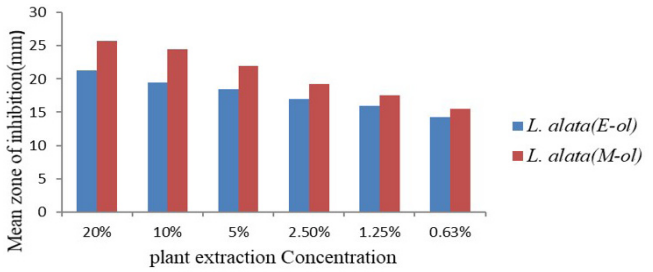


Figure 1: Mean zone of inhibition exhibited by different concentrations of crude extracts against *S. aureus*

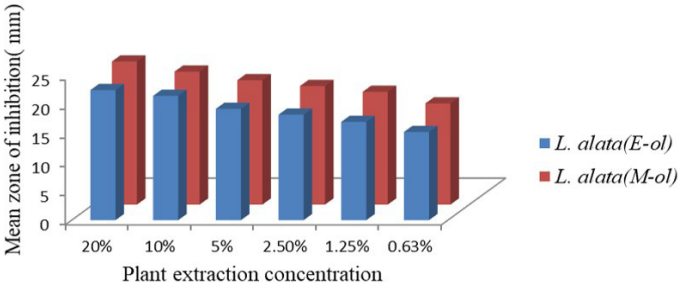


Figure 2: Mean zone of inhibition exhibited by different concentrations of herbal extracts against *S. agalactiae*.

The effect of 20% herbal extracts in comparison with commonly used conventional antibiotic discs

In general, the size of the diameter of inhibition zones exhibited by 20% concentration of both extracts (methanol and ethanol) was found almost comparable to those of noble antibiotic discs (Table 5 & 6). The DMSO impregnated disc hasn't shown any inhibition zone against the test organism which implies that the inhibition observed was exclusively by the crude extracts. Since the diameter of conventional antibiotic discs and crude extracts impregnated discs are 6mm and 12mm respectively for this reason 6mm was added to the zone of inhibition incurred by each antibiotic discs to make comparison easier.

Table 5: MZI (mm) exhibited by leaf extract of *L. alata* 20% both methanol Ethanol extracts with compared to commonly used conventional antibiotic discs against *S. aureus.*

Type of diagnostic disc	The diameter of mean zone of inhibition (mm)
L. alata (E-ol)	21.25
L. alata (M-ol)	25.75
Penicillin	NI
Erythromycin	26
Gentamycin	27.66
Kanamycin	28.33
DMSO	NI

NI: No Inhibition

Table 6: MZI (mm) exhibited by leaf extract of *L. alata* 20% both methanol ethanol extracts with compared to commonly used conventional antibiotic discs against *S. agalactiae.*

Type of diagnostic disc	The diameter of mean zone of inhibition (mm)
L. alata (E-ol)	22.5
L. alata (M-ol)	24.5
Penicillin	NI
Erythromycin	26.75
Gentamycin	28
Kanamycin	27.25
DMSO	NI

NI: No Inhibition

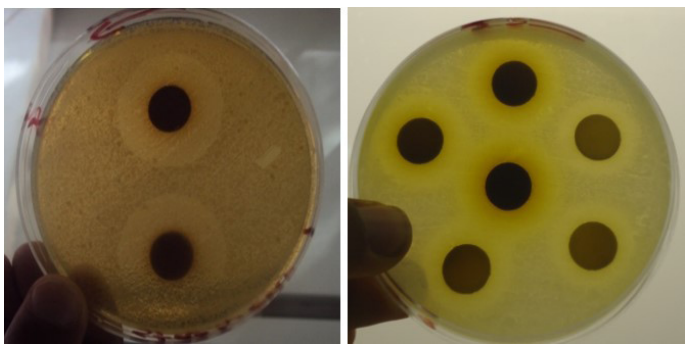


Figure 3: Shows *L. alata* (Methanol and Ethanol) zone of inhibition on *S. aureus,* respectively.

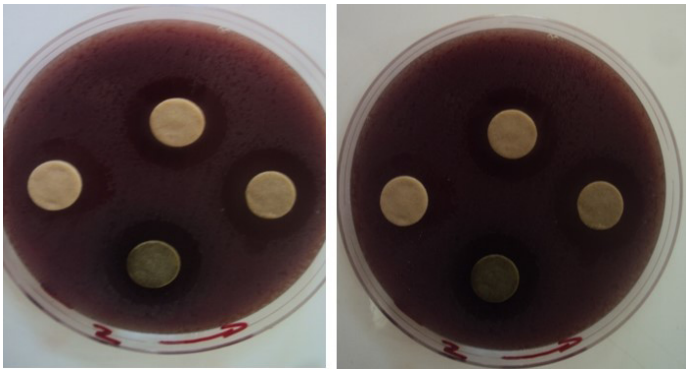


Figure 4: Zone of inhibition *L. alata* (methanol) *S. agalactiae*

Discussion

Mastitis is one of the most frequent diseases affecting dairy cattle among the causal agents *S. aureus* and *S. agalactiae* are the major ones responsible for the occurrence of the disease [5, 25]. The disease has high economic importance in the dairy industry and hence a wide variety of drugs have been used to treat the various forms of the disease. Antibiotic resistance of bacterial strains are increasingly emerging worldwide as a result, abuse or indiscriminate use of antimicrobial drugs that result in significant public health problems [26,27].

In this study, the antimicrobial susceptibility test was conducted on two species of bacteria most pathogenic to mastitis namely *S. aureus* and *S. agalactiae* isolated from bovine mastitis and two medicinal plants namely *L. alata* and *E. cymosa*. At different concentrations of alcoholic extraction of both methanol and ethanol extracts of *L. alata* and *E. cymosa* were used for the sensitivity test. Commercially available antibiotic discs (penicillin, Gentamycin, Kanamycin, and Erythromycin) were used to compare 20% of alcoholic extractions of these medicinal plants. The result indicated that *S. aureus* and *S. agalactiae* are resistant to penicillin and *Ehretia cymosa* has no antibacterial (antibiotic) effect on the tested bacteria. Evaluation of extracts from *L. alata* and *E. cymosa* were used against bacterial strains of mastitis-causing pathogens and according to [6,17], the alcoholic (methanol) extract of *L. alata* exhibited antibiotic effects against *S. aureus* and *S. agalactiae* and also the previous reports showed that the aerial part of *L. alata* was effective against *Bacillus cereus*, *Shigella dysentery* A, *Shigella flexineri* B, *Salmonella typhus* and *Salmonella typhimurium* [28].

The result indicated that the antimicrobial susceptibility test on *S. aureus* and *S. agalactiae* both types of alcoholic crude extracts of *L. alata* showed good inhibitory effects but *E .cymosa* did not have an antibacterial effect on the tested bacteria. On *S. aureus* and *S. agalactiae,* the effects of each type of extracts of the plant (*L. alata*) were assessed alone at different concentrations (20%, 10%, 5%, 2.5%, 1.25%, and 0.625%).

In the meantime, the DMSO used to the solvent to make different concentrations were used as a control and there was no inhibitory effect and the effect obtained from this study was purely related to the efficacy of each type of alcoholic extract of the Phytopreparation. In this study, the effect observed by the leaf extract of *L. alata* extracted by methanol and ethanol observed on *S. aureus* and *S. agalactiae* and the methanol extract of *L. alata* showed a good inhibitory effect than the ethanol extract of *L. alata* on tested organisms in all concentrations (20%, 10%, 5%, 2.5%, 1.25%, and 0.625%). It inhibited the growth of *S. aureus* and *S. agalactiae* at all concentrations and should a wider zone of inhibition than ethanol extract of *L. alata.* Of the types of extraction tested for the efficacy, it was the methanol extract of *L. alata* that had been studied for its antimicrobial effect have a better effect while there was antimicrobial effect in ethanol extracts of *L. alata*. In this study an inhibition zone of the growth at all concentrations were recorded against *S. aureus*, *S. agalactiae* isolates and the values were comparable to [6,17].

Twenty percent (20%) Phytopreparation of *L. alata* from both types of extracts were compared with conventional antimicrobial discs and the efficacy of these preparations at the mentioned concentration was satisfactory particularly methanol extracts of *L. alata*. The comparisons of herbal preparation with conventional antimicrobial discs were made only by the size of the mean zone of inhibition obtained by each test material impregnated discs against *S. aureus* and *S. agalactiae*. Its mean inhibition zone value on *S. aureus* was comparable to the inhibition zone of Gentamycin, Erythromycin, and Kanamycin and also on *S. agalactiae* almost similar to *S. aureus* but slightly lower than *S. aureus*.

On the ethanol extracts of *L. alata* is less inhibition zone than the inhibition zone of Gentamycin, Kanamycin, Erythromycin, and methanol extracts of *L. alata* on both of the tested bacterial species. This indicates that methanol extraction is better than that of ethanol extracts. The comparison among these test materials suggests that the herbal preparations do have a capacity to inhibit the growth of *S. aureus*, and *S. agalactiae* with a similar or different manner to that of conventional antimicrobial agents though there are no established standard formulae to judge the level of the zone of inhibition to say as a resistance, intermediate, and susceptible for the phytopreparation.

Conclusion and recommendations

Out of two herbs extracted by methanol and ethanol, tested *in-vitro* one of them, that is, methanol and ethanol extracts inhibit the growth of both bacterial isolates namely *S. aureus* and *S. agalactiae* at different concentrations. 20% crude extracts *L. alata* has a comparable antibacterial effect to conventional antibiotic discs. This result indicated that their future potential use in the synthesis of new medicaments. One way to control drug-resistant problems is through the development of alternative antimicrobials by screening and testing medicinal plants for their possible antimicrobial effects. Widespread use of antibiotics for the treatment of bovine mastitis has the potential to cause contamination of milk, which has become a subject of public concern, therefore medicinal herbs/plants are natural and safe approaches to alleviate the problems. Among the herbs/plants, the absolute methanol extract of *L. alata* performed well against test organisms. In conclusion, the current result indicates that this medicinal plant may have an effect against a wide range of pathogenic microorganisms that have veterinary and public health importance so; further researches should be undertaken to reveal such and other effects. Moreover, in parallel to testing the efficacy of this medicinal plant for antimicrobial activities, further detailed studies, like active principles of the plant, photochemistry, toxicity, cytotoxicity, etc. should be studied to determine the safety margin.

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