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Antimicrobial Activity of Condensed Tannin Extracts from Indigenous Fodder Plants of Gambella Region on Mastitis Pathogens

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Abstract: The aim of this study was to evaluate the antimicrobial activity of condensed tannin extracts from three indigenous fodder tree and shrubs (IFTS) species of Gambella region by in vitro disc diffusion method and to estimate the relative antimicrobial contribution of condensed tannin extracts against two mastitis causing pathogens *S. aureus* and *E. coli*. Commercially available penicillin-G disc was also used as positive control and distilled water as negative control. The data was subjected to ANOVA using the general linear model (GLM) procedure of statistical analysis system (SAS 2010 version 9.3). The proportions of extracted condensed tannin (CT) varied among IFTS (*T. indica, B. aegyptica* and *C. toka*) but were not statistically different at ($P< 0.05$). Generally, inhibition of these extracts increased in a dose dependent manner except for *C. toka*, which had similar inhibition zone at different dose levels for *S. aureus* pathogen. This study showed that CT extracts from IFTS exhibited a potential antimicrobial activity. The CT obtained from *B. aegyptica* and *T. indica* foliages had equivalent antimicrobial activity with the commercial penicillin. Further analysis should be done to identify which monomers from these CT are effective for the antibacterial effect.

Key words: Gambella · Mastitis · Pathogenic Bacteria · Tannins · Zone Of Inhibition

INTRODUCTION

Mastitis is characterized by physical, chemical and bacteriological changes in milk and pathological changes in the glandular tissue [1]. It is a complex disease occurring worldwide among dairy animals with heavy economic losses. It has been known to cause a great deal of loss or reduction of productivity to influence the quality and quantity of milk yield and to cause culling of animals at an unacceptable age [2]. Bacterial infections are considered the primary cause of mastitis in domestic animals. The causative agents are well defined and the available literatures indicate that *Staphylococcus aureus*, *Streptococcus spp*., *Micrococcus spp.*, *Streptococcus agalactiae*, *Staphylococcus epidermidis*, *Pasteurella haemolytica*, *Escherichia coli* and *Corynebacterium spp*. [3, 4] have been implicated as causes of mastitis in bovines. Similar results with high proportion has been reported by Duguma *et al*. [5] from crossbred dairy cows of Holeta research center in central highlands of Ethiopia.

The principal method to control mastitis has been antibiotic therapy since the advent of antibiotics for the treatment of infectious diseases. However, the emergence of resistance in bacteria causing mastitis pathogens to currently available drugs [6, 7] casts shadow on the control of this economically important disease. Moreover, during the treatment regimen, the accumulation of antibiotics in milk and milk products may lead to another potential hazard to the consumer. Given all these facts related to antibiotic therapy for mastitis, there is an urgent need to look for an alternative medication to control this disease. One potential area in the prevention and control of mastitis is the use of plant extracts like condensed tannins which is environmentally friendly unlike synthetic medication.

Extracts of condensed tannin from trees and shrubs had antibacterial properties and therefore represent a possible alternate option to control bacterial causes of mastitis. Improved small ruminant productivity can be attained with reduced mastitis pathogens through

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strategic supplementation of fodder mix. The tested fodder will be suggested for further investigation through in vivo experiments for reduction in mastitis pathogens as well as increases in animal productivity through strategic supplementation. The aim of this study was to evaluate the antimicrobial activity of condensed tannin extracts from three IFTS species of Gambella region by in vitro disc diffusion methodology and to estimate the relative antimicrobial contribution of condensed tannin extracts.

MATERIALS AND METHODS

Experimental Design: In this experiment, an *in vitro* study was conducted to compare potential antimicrobial activity of condensed tannin extracts from three different fodder tree species obtained from Gambella region and to assess the efficacy of different doses of these extracts (25, 50 and 100 % of extracts) against two mastitis causing pathogens *S. aureus* (Gram-positive) and *E. coli* (Gram-negative).

Plant Sampling Procedures: The specimens used for the study were collected from Gambella woreda, South Western Ethiopia. IFTS species selected for evaluation were Balanites *aegyptica, Tamarindus indica and Celtis.*The studied browse species have been selected by local farmers based on their accessibility to the farmers.

Samples were collected at dry season, air dried and transported with plastic bag to JUCAVM micro biology laboratory and stored at -20°C until analysis of tannin content. Plant samples were ground to pass 1 mm mesh sieve for tannins extraction.

Preparation of Condensed Tannin Extracts: Leaf samples (200 mg) was extracted at 4°C in 10 ml aqueous acetone solution (Acetone/water: 7/3 v/v). After centrifugation (3000 rpm at 4°C for 10 min), the supernatants (total phenolics extract) was analysed for phenolic components (Total phenolics, non-tannin phenolics, total tannin phenolics and condensed tannins) as described by Makkar [8] using Spectrophotometer.

Contents of total phenolics was analysed using the Folin – Ciocalteu's reagent based on tannic acid standard. Total phenolics consist of simple phenolic compounds or non-tannin phenolics and pure tannins or total tannin phenolics. Polyvinylpolypyrrolidone has the property to bind tannins but not the simple phenolics. Two ml distilled (Triple glass) water and 2 ml total phenolics extract was added to the test tube containing 200 mg PVPP and vortexed twice and filtered through filter paper. The filtrate was used to estimate non-tannin phenolics, which was subtracted from total phenolics to obtain total tannins. The concentration of total phenolics and total tannins was expressed as tannic acid equivalent [9].

Three ml n-butanol – HCl (95:5 v/v) and 0.1 ml ferric ammonium sulphate (2%) in 2N HCl was added to the test tube containing 0.5 ml phenolics extract. The test tube was closed with a glass marble and heated in a boiling water bath for 60 min. The absorbance of the red anthocyanidin products (i.e., condensed tannin) was measured at 550 nm and condensed tannin was expressed as leucocyanidin equivalent [10]. All parameters were analysed in duplicate.

Isolation of Mastitis Causing Pathogens from Clinical Mastitis: Milk samples showing mastitis signs were subjected to bacteriological analysis [2, 11]. Briefly, mastitic milk was streaked on nutrient agar, blood agar and Mac Conkey agar. Morphological and cultural characters were used for identification of the organisms. This was followed by culturing on selective agar plates and subsequent staining and biochemical tests was performed to reach species level identification of bacterial strains used in this protocol. Bacterial strains used in this study were *Escherichia coli* and *Staphylococcus aureus*. Pure isolates of these organisms was cultured on nutrient agar at 37^0C for 24 hours and maintained in nutrient agar slant at 4^0C until used. Before testing, each inoculum was prepared and cultivated in trypticase soy broth for 24 hours at 37°C.

Antibacterial Activity: Susceptibilities were determined using the disc diffusion method described in Quinn *et al.* [11]. Discs of 6 mm in diameter were saturated with each plant extract solution and were aseptically placed on Mueller Hinton agar that has been spread with test bacteria. The agar plates for each test organism were incubated aerobically at 37°C overnight. Negative control discs were separately prepared by saturating with distilled water. For comparison, sensitivity of each bacterium was tested against a commercial Penicillin-G disc. Microbial growth was determined by measuring the diameter of the zone of inhibition of each bacterial isolate.

Statistical Analysis: The data was subjected to ANOVA using the GLM procedure of statistical analysis system of SAS [12]. Means were declared significant at $P < 0.05$ and separated using LSD.

RESULTS AND DISCUSSION

Composition of CT and zone of inhibition of IFTS: Condensed tannins concentration in the studied browse leaves varied among plant species, the mean extracted condensed tannin extraction of leaves of *T. indica, B. aegyptica* and C. *toka* were presented in Table 1. The proportions of CT didn't have statistically significant difference at (P<0.05) among the plants. Also C. *toka* had lower CT concentration. The lowest CT extract was investigated from the leaf of C. *toka* which contradicts with Pereira *et al*. [13] who reported that the minimum CT extract of the plant is 2 - 40% DM.

The concentrations of CT extracts from foliages of the studied fodder trees have stronge positive correlation with zone of inhibition *E.coli* (Table 2).

At 100 % concentration, CT extract of leaf of T. *indica* performed the largest zone of inhibition of E. *coli* than Penicillin G disc, where as B. *aegyptica* had no significant difference with both *T. indica* and penicillin G disc. As the result presented in table 2 the least inhibition of *E. coli* is measured from C. *toka* leaf extracts.

T. *indica* and *B. aegyptica* in this experiment showed significant difference in zone of inhibition of E. *coli* from C. *toka* only at 100 % concentration.

In agreement to other scholars Pereira *et al*. [13] theses experiment confirms the antibacterial activity of CT extracts against S. *aureus* and E. *coli* was bacteriostatic. Results in table 3 indicates that there is no significant difference between the studied fodder trees and penicillin G disc except C. *toka* have the least zone of inhibition of *S. aureus*. Also the negative control distill water doesn't have inhibition effect.

Zone of inhibition of (Table 3) S. *aureus* have strong positive correlation with doses of CT extracts of the studied fodder trees.

Results in table 3 indicate that foliages of T. *indica* have the highest zone of inhibition of S. *aureus* at 100% CT extracts followed by B. *aegyptica* and then pencilin disc.

Higher level of antimicrobial activity was observed for B. *aegyptica* and T.*indica* than C. *toka* at 100% concentration of CT (Table 2 and 3) for both E. *coli* and S. *auerus* strains of mastitis causing pathogen. This study suggests that sources of condensed tannins play a role in antimicrobial activity.

Inhibition of T. *indicus* and B. *aegyptica* increased in a dose dependent manner except for C. *toka* which had similar inhibition zone at different dose level for S. *aureus* pathogen. This is in agreement with the work of Min *et al.* [14] indicate that antibacterial activity was increasing with increase dose levels of tannins and this treatment effect was associated with type of tannins and bacteria, with *S. aureus* (Gram-positive) being more susceptible to plant tannin.

Table 1: CT extracts of the used plants

Fodders	CT (% DM)	P<0.05
T. *indica*	3.05[a]	>.0134
C. *toka*	1.2[a]	>.0134
B. aegyptica	3.07[a]	>.0134

CT – condensed tannin, DM- dry matter

Table 2: Effect of extract concentrations of fodder trees on zone of inhibition of E. *coli*

Dose (%)	*T. indica*	*B. aegyptica*	Penicillin G disc	*C. toka*	Distill water	SE	P<0.05
100	23.33[a]	21.67[ab]	20.33[b]	13.33[c]	0[d]	0.537	<.0001
50	10[b]	9[b]	15.33[a]	8.33[b]	0[c]	1.085	<.0001
25	8.33[a]	7[ab]	9.33[a]	5.33[b]	0[c]	0.516	<.0001

[a, b, c, d] means in a row with the same letter were not significantly different

Table 3: Effect of fodder trees on zone of inhibition of S. *aureus*

Dose (%)	*T.indica*	*B. aegyptica*	Penicillin-G disc	*C. toka*	Distill water	SE	P<0.05
100	17.33[a]	16.67[a]	15.67[ab]	8.67[b]	0[c]	1.62	<.0001
50	10.67[ab]	10[ab]	11.67[a]	7.33[b]	0[c]	0.86	<.0001
25	7.33[a]	7.33[a]	8.33[a]	5.67[b]	0[c]	0.3	<.0001

[a, b, c] means in a row with the same letter are not significantly different

Inhibition of E. *coli* by condensed tannins extracts of 50% dose varied among plant species and lower than that of penicillin-G disc (Table 2). Inhibition of penicillin-G disc of *E. coli* at 25 and 100% dosage was not significantly different with the CT extracts of *T. indica and B. aegyptica.*

Inhibition of S. *aureus* by condensed tannins extracts of the studied IFTS and penicillin-G disc at 25, 50 and 100% dosage was not significantly different except for C. *toka* extracts which had lower inhibition at 100% dose of extraction.

In summary, this study showed that condensed tannin extracts of *B.aegyptica, T. indica and C. toka* exhibited a potential antimicrobial activity. The condensed tannins obtained from the foliages of *B.aegyptica and T. indica* had equivalent or comparable antimicrobial activity with the commercial penicillin. Further analysis should be done to show which monomers of these condensed tannins (Monomers of CT are either catechin or epicathechin and their galloyl derivatives) are effective for the antibacterial effect of the mastitis causing pathogens.

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