African Journal of Basic & Applied Sciences 7 (4): 219-222, 2015
ISSN 2079-2034

DOI: 10.5829/idosi.ajbas.2015.7.4.94151

In-vitro Antibacterial Activity of *Acacia etbaica* Against Staphylococcus Aureus and *Escherichia coli*

[1]*Belayneh Getachew,* [1]*Samrawit Getachew,*
[2]*Berhan Mengiste and* [3]*Abebe Mekuria*

[1]College of Veterinary Medicine, Mekelle University, Ethiopia
[2]College of Health Science, Debre-Birihan University, Ethiopia
[3]College of Health Science, Arsi University, Ethiopia

Abstract: This study was conducted to determine the *in-vitro* anti-microbial activity of *Acacia etbaica,* native plant to east African countries, against *Staphylococcus aureus* and *Escherichia coli.* To achieve this, methanol extract of leaf of *Acacia etbaica* was tested for its *in-vitro* antibacterial activity against *Staphylococcus aureus* and *Escherichia coli* using agar disc diffusion method at two different concentrations (500µg/disc and 1000µg/disc). The Minimum Inhibitory Concentration (MIC) of the plant crude extract was also determined using micro-dilution method in 96-well plates. *Acacia etbaica* showed significant antibacterial activity with mean zone of inhibition of 13.34 ± 1.04 mm and 11.13 ± 1.04 mm in diameter at a concentration of 1000µg of plant extract per disc against *S. aureus* and *E. coli* respectively. The MIC of the crude extracts of *Acacia etbaica* was determined to be 0.039mg/ml and 0.313mg/ml against *S. aureus* and *E. coli* respectively. The results suggest that the methanol extract of *Acacia etbaica* could be a rich source of antibacterial compounds. The results also provide an indication of merit in the *Acacia etbaica* ethnomedicine use by the local communities.

Key words: *E. coli* · *Acacia etbaica* · Minimum Inhibitory Concentration · *S. aureus*

INTRODUCTION

Antimicrobial resistance is a global challenge that makes effective treatment and control of infection difficult. It is an increasingly serious threat to the world public and animal health. Antimicrobial resistances have been documented in different species of bacteria in many countries of the world [1-3]. There must be an effort to search for an option to tackle this global problem. One of the options is searching for alternative antimicrobials from different sources like plants.

Acacia etbaica is a medicinal plant which occurs in dry bush land, thickets, semi-desert scrub and wooded grasslands. It is native to Eritrea, Ethiopia, Sudan, Somalia, Kenya, Uganda and Tanzania [4]. It has been reported that the plant was used to treat swelling, ring worm infection, hemorrhoids, scabies, fire burn, eye infection of livestock and anthrax by the community of Kilte Awulaelo District of Tigray Region, Ethiopia [5]. The bark of the plant is also chewed as a stimulant and for the treatment of gonorrhea. Though the plant is used to treat various diseases of human and animal diseases by the local communities for centuries, still there is limited information on the antibacterial activity of the plant scientifically. Therefore, the objective of this paper was to determine the *in-vitro* antibacterial activity of *Acacia etbaica*.

MATERIALS AND METHODS

Plant Collection and Crude Extract Preparation: The leaf of *Acacia etbaica* was collected from mekelle and around mekelle city which is located in Tigray region of Ethiopia. Then, the leaf of the plant was air dried under shed at room temperature before it was grinded with micro plant grinder machine. Crude extract of the plant was extracted using 80% methanol according to the procedure described by Shewit *et al.* [6].

Corresponding Author: Belayneh Getachew, College of Veterinary Medicine, Mekelle University, Ethiopia, P.O. Box: 234, Tel: +251-914158840.

Disc Preparation for the Experiment: 6 mm of discs were prepared from filter paper (Whatmann No 4 filter paper, Whatman Ltd., England). Then the discs were impregnated by the extract at two concentrations (1000µg and 500µg/disc). This was done by loading with 10µl of plant extract solution (100mg of plant extract per 1 ml of DMSO) which results in 1000µg of plant extract per disc and the other group was loaded with 5µl of the same solution which results in 500µg of plant extract per disc. Finally, the prepared discs were sterilized under UV for 30 minutes.

Preparation of Test Bacteria: The test bacteria used to determine the antibacterial activity of *Acacia etbaica* were *Staphylococcus aureus* and E*scherichia coli* which were isolated from clinical cases. These bacteria were obtained from National Veterinary Institute, Ethiopia in lyophilized form. They were revived in nutrient broth before culturing them in their respective selective media i.e. *Staphylococcus aureus* and E*scherichia coli* were cultured on manitoal salt agar and Mckonkey agar respectively for confirmation. Finally the confirmed isolated were sub-cultured on nutrient agar aseptically and separated colonies were suspended in sterile salt solution within a test tube until the turbidity matches with 0.5 McFarland standards. All these activities were conducted by following the standard laboratory procedures.

Antimicrobial Susceptibility Test

Disc Diffusion Test: The disc diffusion method for antimicrobial susceptibility testing was performed according to EUCAST [7]. Briefly, the inoculums adjusted to 0.5 McFarland turbidity standards were spreaded evenly over the entire surface of the plate containing Muller -Hinton agar using sterile swabs. Then discs were applied immediately on the surface using sterile forceps. We used five discs (3 discs loaded with crude extract of a plant with equal concentration, one disc loaded with DMSO as a negative control and a chloramphenicol disc as positive control) per 90 mm diameter plate. The antimicrobial activity of each plant was tested at a concentration of 1000µg and 500 µg per disc. Then, the inoculated plates were incubated at 37°C for 20 hours. Finally zones of inhibitions were measured using electronic digital caliper in mm.

Determination of Minimum Inhibitory Concentration (MIC): The minimum inhibitory concentration was determined by micro-dilution method in 96 well plates according to Andrews [8] with slight modifications. Briefly, serial dilutions of the plant extracts were made in small test tubes. The first test tube was filled with 2ml of DMSO and the rest tubes were filled with 1ml of DMSO. 200mg of the plant extract was dissolved in the first tube and 1ml of the solution was transferred in to the second test tube. After thorough mixing, again 1ml of the solution was transferred in to the third test tube. This procedure was repeated up to test tube 10. Then, 25 µl of the crude extracts were transferred from each test tube to wells of 96-well plates i.e. the crude extracts of test tube 1, 2, 3,… were transferred to two wells of the first row, second row, third row,… of the 96 well plate respectively. Each wells of the plate was loaded with 25 µl of bacterial suspension (Adjusted to 0.5 McFarland standards) and 200 µl of broth except wells left for checking sterility. Chloramphenicol was used as a positive control, inoculated wells of antibiotic free broth were used as negative control and un-inoculated wells of antibiotic free broth were used to check sterility. Then the plates were covered with plate sealing tape and incubated at 37 °C for 20 hours. Finally, the lowest concentration of the plant extract that showed no visible growth was taken as minimum inhibitory concentration.

Data Analysis: Data on zone of inhibition produced by each disc on each bacteria were stored in excel spreadsheet. Then mean values for zone of inhibition and standard deviation were calculated using SPSS statistical software version 17. One way ANOVA was used to see any statistical differences among the mean values of the plant extracts at two concentrations and the negative control. Finally, post hock test (Using Bonferroni) was used to compare the mean values of the negative control with that of the plant extract at different concentrations.

RESULTS

For crude extraction from the leaf of *Acacia etbaica*, 80%methanol was used. 125.281gm of crude extract was found from 480gm of the plant powder which resulted in a total yield of 26.1%. The consistency of the crude extract was semi-solid and sticky. Using agar disc diffusion method, *Acacia etbaica* showed mean zone of inhibition of 10.95 ±1.49mm and 13.34 ±1.04mm at a concentration of 500µg and 1000µg respectively against *Staphylococcus aureus* (Figure 1 and Table 1). The plant also showed a mean zone of inhibition of 10.77 ± 1.18mm and 11.13 ±1.04mm at a concentration of 500µg and 1000µg respectively against *Escherichia coli* (Table 1).

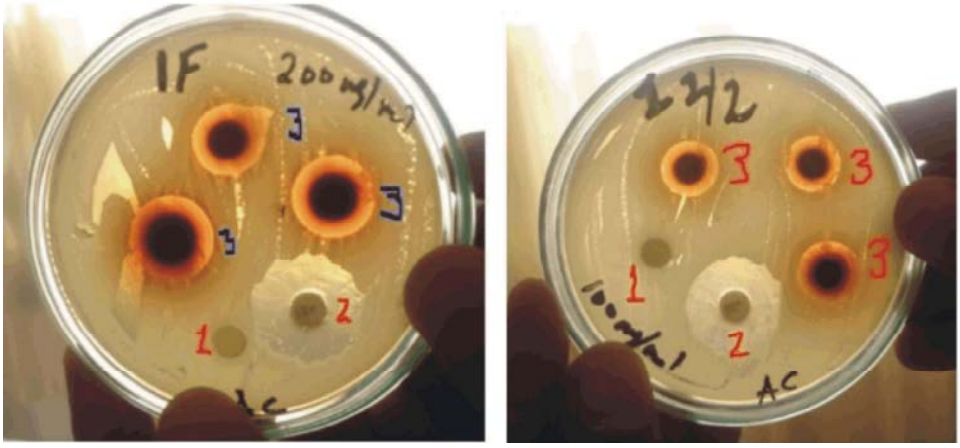

Fig. 1: Antimicrobial activity of *Acacia etbaica* against *Staphylococcus aureus*. Discs were loaded with 1000µg (*3 Blue*) and 500µg (*3 Red*) of 80% methanol extracts of *Acacia etbaica.*
1: Negative controls loaded with DMSO; 2: Positive controls which are standard chloramphenicol antibiotic discs

Table 1: Mean Zone of Inhibition of 80% methanol crude extract of *Acacia etbaica* against *S. aureus* and *E.coli*

	Mean Zone of Inhibition in mm ± SD	
Concentration	*S. aureus*	*E. coli*
500µg/disc	10.95 ± 1.49	10.77 ± 1.18
1000µg/disc	13.34 ± 1.04	11.13 ± 1.04
Positive control (Discs loaded with 30µg of Chloramphenicol)	21.19 ± 1.43	20.72 ± 1.62
Negative control (Discs Loaded with 10µl of DMSO)	6	6

Table 2: Minimum Inhibitory Concentration of *Acacia etbaica* crude extract against *S.aureus* and *E.coli*

	Extract Concentration (mg/ml)											
												MIC
Test bacteria	10	5	2.5	1.25	0.625	0.313	0.156	0.078	0.039	0.019	0.009	(mg/ml)
S. aureus	-	-	-	-	-	-	-	-	-	+	+	*0.039*
E.coli	-	-	-	-	-	-	+	+	+	+	+	*0.313*

-: No growth of bacteria +: There is growth of bacteria

To determine the minimum inhibitory concentration, micro dilution method using 96 well plates were used. Using this method, the minimum inhibitory concentration of *Acacia etibanica* crude extract was found to be 0.039mg/ml and 0.313mg/ml against *Staphylococcus aureus* and *Escherichia coli* respectively (Table 2).

DISCUSSION

Acacia etbaica at concentration of 500µg/disc and 1000µg/disc showed significant ($p< 0.05$) antibacterial activity against *Staphylococcus aureus* and *Escherichia coli* when compared with that of the negative control. The *Staphylococcus aureus* bacteria that we used for this study was found to be resistant to penicillin while we conducted antimicrobial susceptibility test for students in the laboratory. Thus, *Acacia etbaica* is potentially a promising plant for isolation of active compounds against resistant *Staphylococcus aureus* which is currently a big challenge in the globe. Potentially, the plant may also contain broad-spectrum antibacterial compounds, since it showed effect both on the gram negative and positive bacteria. *Acacia etbaica* is native to Eritrea, Ethiopia, Sudan, Somalia, Kenya, Uganda and Tanzania [4]. There is no previous report on the antibacterial activity of this native plant which makes this work the first report on this aspect.

The minimum inhibitory concentrations (MIC) of crude extract of *Acacia etbaica* were found to be 0.039 mg/ml and 0.313 mg/ml against *Staphylococcus*

aureus and *Escherichia coli* respectively. Ceftriaxone and cefoxitine inhibit the growth of *S.aureus* ATCC® 25923 and *E.coli* ATCC® 25922 at a concentration of 0.008mg/ml [9]. Even though *Acacia etbaica* showed higher MIC results than standard drug ceftriaxone and cefoxitine, it may be possible to get a comparable MIC result after separation and purification of the active compounds of the plant. In another study, Biswas and Roymoj [10] reported that MIC of cold methanol extracts of leaf of *Acacia arabica* was 0.6mg/ml against *E.coli* which is very closer to these results.

80% methanol extract of leaf of *Acacia etbaica* showed antibacterial activity against *Staphylococcus aureus* and *Escherichia coli*. The results suggest that the methanol extracts of *Acacia etbaica* could be a rich source of antibacterial compounds against *Staphylococcus aureus* and *Escherichia coli.* The results also provide a scientific basis for the traditional use of Acacia *etbaica* by the local communities. Therefore, further studies should be conducted to isolate or fractionate the active components of the plant having antibacterial effect. Moreover; i*n vivo* studies on this plant are needed to determine its effectiveness, toxicity and side effects.

ACKNOWLEDGMENT

This research partly received financial support from Ministry of Education (Ethiopia) with Grant No. CVM/RB/10/2013. The authors are very grateful to College of Veterinary Medicine, Mekelle University for allowing us to use all the necessary facilities to conduct this research and College of Medicine, Department of Pharmacy, Mekelle University for the extraction of the plant.

REFERENCES

1. Ma, P., K. Chan and C. Chou, 2006. Characterization of Bacterial Susceptibility Isolates in Sixteen Dairy Farms in Taiwan. Journal of Dairy Science, 89: 4573-4582.
2. Bedada, A. and A. Hiko, 2011. Mastitis and antimicrobial susceptibility test at Asella, Oromia Regional state, Ethiopia. Journal of Microbiology and Antimicrobial, 3(9): 228-232.
3. Sharma, D., S. Pradeep and A. Malik, 2011. Prevalence and Antimicrobial Susceptibility of Drug Resistant Staphylococcus aureus in Raw Milk of Dairy Cattle. International Research Journal of Microbiology, 2(11): 466-470.
4. Orwa, A., Mutua, R. Kind, R. Jamnadass and Anthony, 2009. Agroforestree Database: a tree reference and selection guide version, 4.0 (http://www.worldagroforestry.org/sites/treedbs/treedatabases.asp)
5. Teklay, A., B. Abera and M. Gidey, 2013. An ethnobotanical study of medicinal plants used in Kilte Awulaelo District, Tigray Region of Ethiopia. Journal of Ethnobiology and Ethnomedicine, 9: 65.
6. Shewit, K., H. Mekonnen, G. Gebremedhin, T. Tsegay, S. Samson, T. Habtamu and G. Mussie, 2012. In-vitro antimicrobial activity screening of some ethnoveterinary medicinal plants traditionally used against mastitis, wound and gastrointestinal tract complication in Tigray Region, Ethiopia. Asian Pacific Journal of Tropical Biomedicine, 2(7): 512-522.
7. EUCAST. European Committee on Antimicrobial Susceptibility Testing; Disc Diffusion Method for Antimicrobial Susceptibility Testing; European Society of Clinical Microbiology and Infectious Disease 2013, http:// www.eucast.org/antimicrobial_susceptibility_testing
8. Anderew, M., 2001. Determination of minimum inhibitory concentrations. Journal of antimicrobial chemotherapy, 48: 5-6.
9. CLSI, 2007. Clinical Laboratory Standards Institute, Performance standards for antimicrobial susceptibility testing, seventh information supplements, 27(1): 32-44.
10. Biswas, D. and G. Roymon, 2013. Search for in vitro antibacterial efficacy of phytoconstituents of Acacia arabica leaf extracts against various serogroups of E. coli associated with diarrheal infections in ruminants. Recent Research in Science and Technology, 5(2): 73-74.