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ORIGINAL RESEARCH

Antimicrobial Activity of Selected Ethnoveterinary Medicinal Plants of Southern Region, Ethiopia

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Background: For decades, Ethiopians have employed ethnoveterinary medicinal plants to cure both human and livestock ailments. Currently, few studies have been conducted on antimicrobial activity evaluation in Ethiopia. This study, therefore, is designed to evaluate the antibacterial activities of selected ethnoveterinary medicinal plants used in treating livestock ailments in the study area.

Methods: Ethanol extracts of plants obtained by maceration of roots and leaves of four medicinal plant species were studied for potential antimicrobial activity using a disc diffusion method against *S. aureus* and *E. coli*. Data obtained from experiments were analyzed using ANOVA and the significant test was set to $P < 0.05$.

Results: The antibacterial properties of four ethanol extracts of leaves of *Withania somnifera* L., *Becium obovatum, Ageratum conyzoides* L., and root of *Pentas lanceolata* (Forssk.) Defiers were evaluated in vitro and found to be effective against *S. aureus* but not *E. coli*. There was no significant difference between the studied plant species and concentrations ($p > 0.05$), according to the results. The four test extracts had minimum inhibitory concentrations (MICs) ranging from 6.25 to 25 mg/mL, with inhibitory potential ranging from 12.5 to 100 mg/mL. *Pentas lanceolata* (Forssk.) Defiers's antibacterial activity at a concentration of 100 mg/mL (18.67 3.78 mm) was comparable to the standard antibiotic (Gentamicin 20g per disc), which had a measurement of 23.08 ±0.9 mm.

Conclusion: This finding on the selected medicinal plants of Dawuro Zone supports the traditional claims of effective antimicrobial activity in the treatment of livestock health management. Hence, the study suggests further investigations need to be conducted to isolate and elucidate active ingredients in the plant materials tested.

Keywords: antibacterial activity, Dawuro zone, extraction, medicinal plant, maceration

Introduction

Traditional medicine is defined as the totality of knowledge and practices that can be formally explained or used in the prevention and treatment of physical, mental, and social imbalances, based solely on practical experience and observation passed down verbally or in writing from generation to generation.[1,2] Medicinal plants have been used as a source of medicine in practically all societies from the dawn of humanity. Traditional medicine and medicinal plants are widely used as a normative basis for the preservation of good health in most underdeveloped countries.[3] Farmers have acknowledged the indigenous knowledge of ethnoveterinary medicine and its implications through a course of experience spanning hundreds of years. For addressing veterinary illnesses, livestock rearers in rural areas still rely heavily on folk wisdom practices of plants and household medicines.[4]

Antimicrobial resistance is currently having a significant impact due to treatment failures linked with multidrug-resistant bacteria, and it has become a global public health concern.[5,6] Numerous bacteria, especially Staphylococcus aureus, and E. coli, among others, have an innate resistance to numerous antibiotic substances.[9,42,48] As a result, discovering new antibiotics is a singularly essential goal. Natural products continue to be a major source of novel medicinal compounds. Biological activity has been documented in extracts obtained from a variety of plants, including antibacterial and anti-inflammatory properties.[5,6]

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The Solanaceae family *Withania somnifera* L, has played a significant role in the Ayurvedic and indigenous medical systems. Numerous investigations on this plant revealed that it has antiserotogenic, immunomodulatory, hemopoietic, and rejuvenating qualities in addition to having a favorable impact on the endocrine, cardiac, and central neurological systems reported;[35,36] and for treatment of blackleg, bloat, and bloody diarrhea in animals.[8] *Withania somnifera* L. is utilized for its aphrodisiac, liver tonic, astringent, senile dementia, emaciation, sleeplessness, analgesic effects, memory-improving effects, antibacterial, and anti-fungal properties.[36]

Billy goat weeds, also known as *Ageratum conyzoides* Linn. (Family Asteraceae), are annual herbs having a long history of traditional medical usage in tropical and subtropical regions of the world. The plant's stems and leaves are completely covered in tiny white hairs.[32] Ageratum conyzoides Linn. Leaves, roots, stems, and flowers can all be used medicinally. The styptic properties of leaves help heal wounds, treat boils, and prevent tetanus. Eye lotion can also be made from leaf juice.[42]

Becium obovatum (E. Mey. ex Benth. in E. Mey) N.E. Br is a species belonging to the Lamiaceae family. Children with gastrointestinal problems and pain are treated with enemas, which are warm water infusions of crushed roots and leaves of *Becium obovatum* (E. Mey. ex Benth. in E. Mey) N.E. Br in 1996 by Hutchings.[29]

The Rubiaceae genus of plants is one of the families. It has roughly 40 species, several of which are widely used as medicinal plants by African natives [61]. Out of 40 species, *Pentas lanceolata* (Forssk.) Defiers. was reported to be used as a folk treatment by African indigenous people including Ethiopia in treating some diseases such as lymphadenitis, abdominal cramps, ascariasis, snake poisoning, retained placenta;[49] and veterinary diseases such as anthrax, lice infestations, parasitism, and lumpy skin disease.[8]

Unlike conventional drugs, the units of measurement used to determine dosage are not standardized, and there are differences in the unit of measurement, duration, and time at which traditional healers take and prescribe remedies for the same types of health problems; lack of precision in dose determination has been noted in many studies.[9–13] While there have been few investigations on the therapeutic efficacy of herbal medicines in ethnoveterinary practices, there have been many studies have been found in the literature relating to the use of plants and plant materials in large animals.[9,10,14] Scientifically validating the efficacy of ethno veterinary medicinal plants can influence the preservation, conservation, and sustainable utilization of these species in livestock health management.

To our knowledge, in vitro, antimicrobial activity tests on ethnoveterinary herbs are uncommon in the Southern region. As a result, the purpose of this study was to evaluate the antibacterial activity of four different browsing species found in the study area: *Withania somnifera* L., *Becium obovatum* (E. Mey. ex Benth. in E. Mey) N.E. Br, *Ageratum conyzoides* L., and *Pentas lanceolata* (Forssk.) were chosen and tested for antibacterial activity on Gram-positive *S. aureus* and Gram-negative *E. coli* from the Dawuro Zone, Southern Ethiopia.

Materials and Methods

Geographical Location of the Plants

The present study was conducted in the Dawuro zone, which is one of the 14 zones of Southern Nation Nationalities and Peoples Regional State (SNNPRS). The zone is located between 6° 59'- 7° 34' N of latitude and 36° 68'-37° 52' E of longitude. The total surface area of the zone is estimated to be 4436 square km which shares 4.07% of the total area of the region.

The average lowest and maximum temperatures are 15.1 and 27.5 degree Celsius, respectively, at altitudes ranging from 1001 to 3000 meters above sea level. The average lowest and maximum temperatures are 15.1 and 27.5 degree Celsius, respectively, at altitudes ranging from 1001 to 3000 meters above sea level. The zone's annual rainfall ranges between 1201 and 1800 mm.[15] The map of the study area is presented in (Figure 1).

Plant Sample Collection and Preparation

The claimed medicinal plants reported for the treatment of livestock diseases were collected from Dawuro zone Tocha district, from November 2017 to September 2018. Field trips were made with local herbalists for the collection of the selected medicinal plants. Voucher specimens of medicinal plants were collected in the field by Dr. Tegegn Dilbato with the help of traditional healers and field assistants. Collected medicinal plants were dried, numbered, pressed, and labeled and were

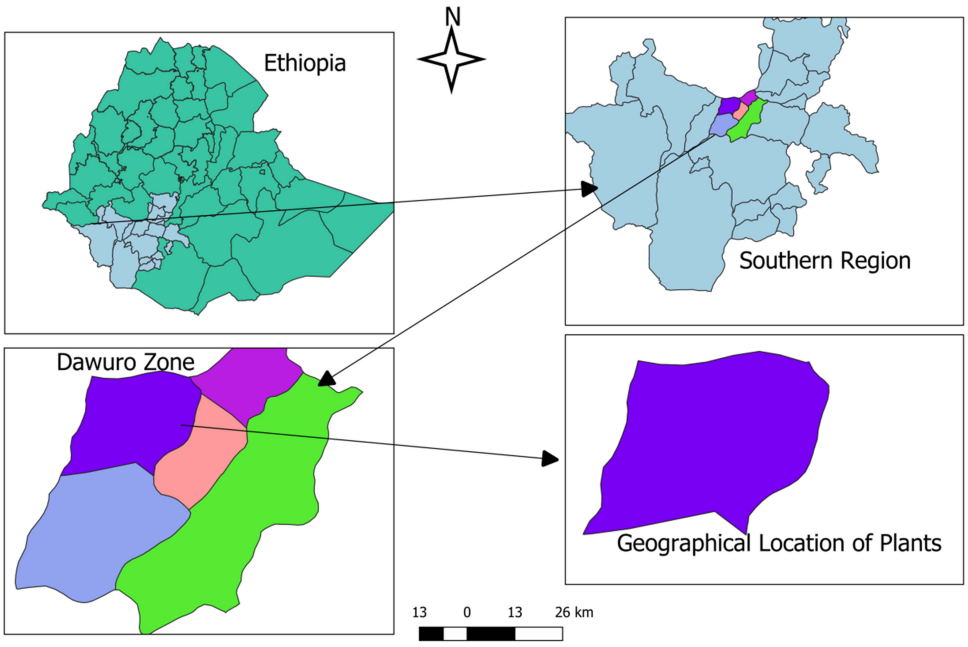

Figure 1 Map of the study area.

brought to the Ethiopian Herbarium for botanical classification. Specimen identification and confirmation were undertaken by using Flora of Ethiopia and Mr. Melaku Wondafrash who is a botanical taxonomist identified the ethnoveterinary medicinal plants. Finally, the specimens were deposited at the National Herbarium (ETH) at Addis Ababa University.

To eliminate soil particles and other debris, the fresh plant leaves and root samples were gathered and cleaned individually under running water. The leaves and roots were air dried for 15 days at room temperature in the laboratory. The dried samples were ground well into a fine powder in a pestle and mortar. Before maceration, the material was sieved and weighed. For further processing, the powder was kept in airtight polythene bags in the refrigerator at 4°C.

Preparations of Crude Extracts

The crude extracts were prepared according to the procedure described in.[9,16] About 25g of each test plant was weighed using a delicate balance and placed in flasks with 250 mL of 95% ethanol, which was added and mixed at maximum speed shaking for 30 minutes to aid in mixing and sufficient maceration of the plant components. For three days, the mixed substance was allowed to sit at room temperature. After 3 days, each sample was filtered using a qualitative filter paper (Whatman No 1 filter paper, Whatman Ltd., England) in separate flasks to obtain a solids-free solution. To remove the solvent, the solution is concentrated using a vacuum rotary evaporator at 50°C.

The percentage yields of each plant extract were calculated according to Kalayou et al and Tura et al.[9,17] The resulting concentrated extracts of each plant material were transferred to bottle bijou which had tight fitting cups and then labeled with respective plant name before being refrigerated at 4°C until tested for antimicrobial activity. The percentage yields of each plant extract were calculated as:

$$\text{Percentage extract yield}(\%) = \frac{\text{Weight of extract}}{\text{Weight of dried powder}} \text{X}100$$

A list of selected medicinal plants and their scientific name, ethnoveterinary use, preparation, route of administration; amount macerated, and yield obtained from maceration is summarized in Tables 1 and 2.

Test Microorganisms

In this study, two species of bacteria were used for in vitro evaluation of the antibacterial activity of the claimed medicinal plants. One Gram-positive, *Staphylococcus aureus (ATCC 25923)* and Gram-negative, *Escherichia coli (ATCC*

Table 1 List of Selected Ethnoveterinary Medicinal Plants Used by Traditional Healers for Livestock Health Management in the Dawuro Zone

Scientific Name	Family Name	Local Name	Part Used	Preparation	Route	Veterinary Uses in the Study area	Medicinal Uses Reported from Other Parts of Ethiopia and Elsewhere	Voucher No.
Withania somnifera L.	Solanaceae	S'eemushsha	Leaf	Pounding, put the fresh leave on fire	Oral, Nasal, Fumes/ smoking	GIT parasitism, Diarrhea, Blackleg, Trypanosomosis (Equine)	Blackleg, bloody diarrheal (Lulekal et al2014), stomach problems (Bussmann et al 2011)	DZ155
Becium obovatum (E. Mey. ex Benth. in E. Mey) N. E. Br	Lamiaceae	Sa'a tuussaa/ C'am'ashiya	Leaf	Pounding and mixed with water, salt	Nasal	Listeriosis/ encircling disease, Mastitis, Blackleg, Diarrhea	Stomach ailments and abdominal pain (Hutchings et al 1996)	DZ31
Ageratum conyzoides L.	Asteraceae	Kirkissaa/ Puk'ak'iya	Leaf	Pounding	Oral	Diarrhea, Blackleg, Leech, Promote milk and butter yield	Bleeding wound (Bitew et al 2019), insecticide (Degu et al 2020)	DZ47
Pentas lanceolata (Forssk.) Defiers	Rubaceae	Shid'i bid'aa	Root	Pounding and mixed with water	Oral	Bloody diarrhea, abdominal pain	Calf diarrheal (Tekle, 2014), Anthrax, helminthiasis, Lumpy Skin Disease (Lulekal et al2014), lymphadenitis (Giday et al 2009); snake bite (Bekalo et al 2009)	DZ200

Abbreviation: DZ, Dawuro Zone.

Table 2 Scientific Names, Plant Parts, Amount Macerated, and Percentage Yields of 95% Ethanolic Extracted from Test Plants

Plant Species	Local Name	Plants Part Extracted	Amount Macerated (gm)	Yield (gm)	Yield (%)
Withania somnifera	S'eemushsha	Leaf	25	4.823	19.292
Becium obovatum	Sa'a tussa	Leaf	25	1.172	4.688
Ageratum conyzoides	Kirkissa	Leaf	25	1.02	4.08
Pentas lanceolata	Shiribid'd'a	Root	25	0.668	2.672

25922) strains were used. The standard strains were obtained from the Ethiopian Biodiversity Institute (EBI), Ethiopia, in May 2018. The bacteria strains were reactivated by sub-culturing in nutrient broth at 37°C and maintained on nutrient agar slant at 4°C for further use in Veterinary Microbiology Laboratory, Jimma University College of Agriculture and Veterinary Medicine. In addition, Gentamicin (20 μg per disk) was used to compare their efficacy with herbal preparations as a positive control, while DMSO-impregnated disc was used as a negative control.

Antimicrobial Susceptibility Test

Disc Diffusion Assay

The antimicrobial test was conducted using the disc diffusion method.[17–19] Muller-Hinton agar (38 gm) (Biotech UK) medium was used for the antimicrobial sensitivity test. The Agar media was prepared by mixing with one litter of

distilled water, boiled to dissolve completely, and autoclaved at 121°C for 15 minutes. The medium was later dispensed into 90 mm sterile agar plates and left to set. The agar plates were incubated for 24 hrs at 37°C to confirm their sterility. When no growths occur after 24 hrs, the plates were considered sterile and used for antimicrobial sensitivity tests.

The sterile filter paper discs (Whatman No. 1, diameter 6 mm) were soaked in 30 μL of plant extract for 30 minutes. The extract-soaked filter paper discs were then placed on the inoculated MHA plates, allowing prior incubation to stand for 30 minutes at room temperature to permit proper diffusion of the extract.[17,19] The top of 4–5 well-isolated colonies of the same morphology was scooped using a wire loop from the nutrient agar and mixed using sterile normal saline and agitate with a vortex mixer. The turbidity of the bacterial suspension is adjusted by comparing it with 0.5 McFarland turbidity standards (1.5×10^8 CFU/mL) and diffused on the Mueller Hinton agar (MHA) media with sterile swabs. McFarland turbidity standard is 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 are placed in 10 mL sized test tubes and compared against a white background with contrasting black lines until the turbidity of the test suspension equates to that of the turbidity standard. Adjustments of the turbidity are made by adding saline or colonies depending on the degree of turbidity for *S. aureus* and *E. coli*.[17,18,20] The appropriate crude extract-impregnated discs and conventional antibiotic discs will be applied at spaces 24 mm apart from center to center and 15 mm away from the edge of the plates. The plates turn upside down, labeled, and incubated at 37°C for 24 hr. After 24 hr incubation period diameter of the zone of inhibition (mm) was measured by using a caliper in millimeters. The experiment was done in triplicate.

Determination of in vitro Minimum Inhibitory Concentration (MIC)

The plants' Minimum Inhibitory Concentration (MIC) was determined using standard procedures described.[9,17,21,22] As a result, a stock solution containing 200 mg of plant extracts in 1 mL of DMSO was diluted using a two-fold serial dilution. This yields a series of test concentrations of 100, 50, 25, 12.5, and 6.25 mg/mL, respectively. The plant's antibacterial activity was tested by incubating each concentration of the extract for 24 hours with an inoculum containing 100μL of the microbial cell at 37°C. The MIC of the plants was defined as the smallest concentration that inhibited the growth of the test bacteria.[23,24] According to Bussmann et al,[25] strong activity was defined as MIC less than 5 mg/mL.

Inhibitory Potential of Plant Extract

This test is used to assess the in vitro killing ability of ethanolic plant extracts after exposure to test organisms. Bacteria for this test were cultured at logarithmic/exponential phase for 12 hrs of age at 37°C and serial dilutions of plant extract on the 10 mL test tubes were performed. Then after, add a single colony of test bacteria into the test tubes of plant extracts of each concentration and wait for 5 minutes. Aliquots (100 μL) of the cultures were taken after 5 minutes interval plated on freshly prepared Muller Hinton agar and thoroughly mixed and incubated at 37°C for 24 hrs. Following 24 hrs of incubation, the number of colonies was counted to determine the total viable bacteria number. Cell culture with DMSO was assayed as the control.

Data Analysis

An excel spreadsheet was used to keep track of the zone of inhibition caused by each plant extract, as well as the MIC and killing capabilities of two bacteria. The statistical program SPSS version 20 was used to calculate the mean inhibitory zone of inhibition. The mean inhibitory effects of the extracts were compared using ANOVA, with a significant test set at $P < 0.05$.

Results

The Extract Yields of the Plants

The leaves and roots of each plant were extracted using the same volume of ethanol. The method used for the extraction achieved varying extract yields ranging from 0.668 gm of *Pentas lanceolata* to 4.823 gm of *Withania somnifera*. The highest percent extract yield was obtained from *Withania somnifera* leaf (19.292%) and the lowest (2.672%) was from

Pentas lanceolata root. The list of selected ethnoveterinary medicinal plants for the treatment of livestock disease and yield obtained from maceration is summarized in Tables 1 and 2.

Antibacterial Activity of the Plant Extracts

All plant extracts showed good antibacterial activity by inhibiting *S. aureus*. Among these plants, *Becium obovatum, Ageratum conyzoides*, and *Pentas lanceolata* showed activity against *S. aureus* of the common microorganisms of veterinary importance at all the concentrations tested. The antibacterial activity of *Pentas lanceolata* at a concentration of 100 mg/mL (18.67±3.78 mm) was comparable with that of the standard antibiotic (Gentamicin 20 µg per disc), 23.08 ±0.9 mm. Generally, bacterial growth inhibition was seen to increase as the concentration of the extracts increased. Results of the mean inhibition zone and minimum inhibitory concentration (MIC) of plant extracts against the test bacteria are also depicted in Figures 2 and 3.

The results of ANOVA comparison of the zone of inhibition between plant species, different concentrations, and positive control (Gentamicin) were recorded. The results showed that there was a significant difference between plant

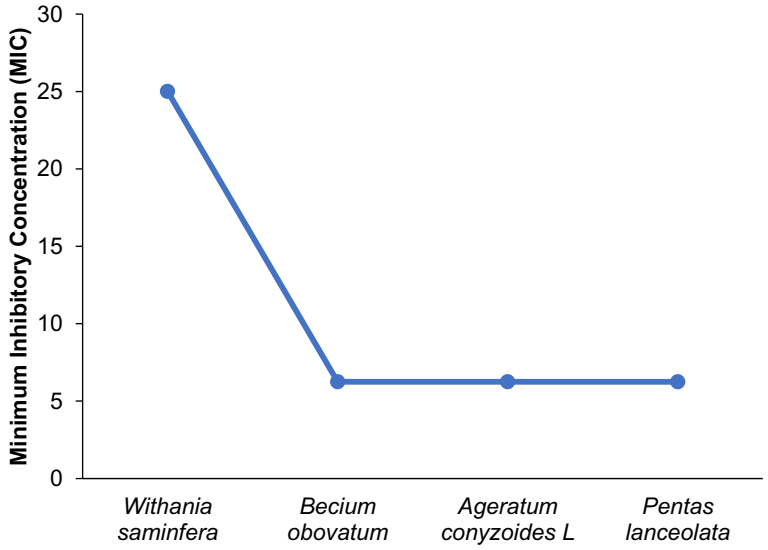

Figure 2 Zone of inhibition of plant extracts on *S. aureus*.

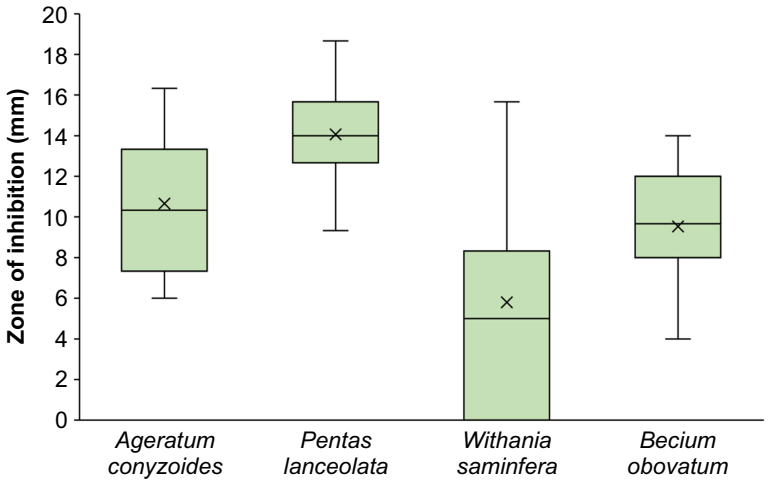

Figure 3 Minimum inhibitory concentration (MIC) of plant extracts.

extracts*Gentamicin (p = 0.00), but the difference between plant species* positive control was statistically non-significant (p = 0.191).

The inhibitory potential of the plant extracts was tested by culturing a colony of bacteria for different concentration of the plant extracts. A colony of the bacteria was treated in a solution of the extracts for 5 minutes after which it had been cultured on nutrient agar to recover viable bacterial cells. It showed that the bactericidal activity of the control, solvent DMSO, revealed that the colonies remained viable within the treated time. The test for the inhibitory ability of all extracts revealed a significant effect, in which the strains of *S. aureus* ATCC 25923 were completely inhibited in 5 minutes at 12.5 mg/mL to 100 mg/mL concentration for *Ageratum conyzoides* and *Pentas lanceolata*, 25 mg/mL to 100 mg/mL for *Becium obovatum* and 50 mg/mL to 100 mg/mL to *Withania somnifera*, respectively. For the inhibitory ability test against *E. coli*, all extracts even after 24 hrs of treatment, had not shown inhibitory potential.

Discussion

Natural products contain a wide range of lead compounds, which might be exploited to develop new antimicrobial drugs when standard antimicrobial treatments become ineffective owing to multidrug resistance. Secondary metabolites identified in medicinal plants may have antibacterial properties that help in resistance prevention.[9,10] The type and level of different biological activities exhibited by any plant extract, on the other hand, is dependent on a variety of factors such as geographical area, time of collection, soil conditions, harvesting time, moisture content, drying method, storage conditions, and post-harvest process. Furthermore, relatively high temperatures and humidity can be created during tissue grinding, which can denature chemical contents; the extractive solvent can also impact the quantity and composition of secondary active metabolites extracted, as stated by Lumpu et al.[27]

According to Gupta et al,[28] among modern methods of extraction, maceration is effective in extracting bioactive compounds at room temperature. These compounds contain broad-spectrum antibacterial agents acting against different species of bacteria.[29–32]

The present finding indicated that the highest percentage extract yield was obtained from *Withania somnifera* leaf and the lowest was from the root of *Pentas lanceolata*. The variations could be related to the concentration of the phytochemical constituents of the plant parts and species.

The extracts were tested to determine their MIC values. *Becium obovatum, Ageratum conyzoides L, and Pentas lanceolata* all had MICs of 6.25 mg/mL. This finding is consistent with the findings of,[25,27] who found that the plants tested had strong antibacterial activity, with MIC values of less than 16 mg/mL. The relatively high values in *Withania somnifera* extract (25 mg/mL), imply only modest antibacterial effectiveness.

The antibacterial activity of *Pentas lanceolata* against *S. aureus* in all concentrations from highest to lowest is a promising example (9.33 mm to 18.67 mm). This plant has the largest inhibition zone (18.67 mm) against S. aureus ATCC 25923 and no inhibition against *E. coli ATCC 25922*. Similarly, no plant extracts demonstrated antibacterial efficacy against gram-negative *E. coli* species. Failure to demonstrate antibacterial action in this study, however, does not imply that the plant is devoid of active components responsible for antibacterial therapeutic efficacy on *E. coli* bacteria.[6,9] The variance might be attributed to the bacteria *E. coli*, which is previously known to be multi-resistant in nature, extraction techniques, solvent selection, and ecological variation of plant extracts examined.[33,34]

The antibacterial activity of *Withania somnifera* leaf extract against *S. aureus* was shown to be comparably good in vitro (5 mm to 15.67 mm). This result agrees with earlier results elsewhere[35,36] and also according to the report of Bokaeian et al,[44] the ethanol leaf extract of *W. somnifera* has a great potential as an antibacterial agent. Especially, the leaf extract of *W. somnifera* was to be more effective in inhibiting the antibiotic-resistant *S. aureus* strains as stated by Bokaeian et al.[44] But, the finding of this study disagrees with the conclusion of Jamal et al,[37] who stated that the ethanolic extracts of *Withania somnifera* leaf did not demonstrate action against *S. aureus*. In this study, the ethanol leaf extract of *W. somnifera* did not show any activities against *E. coli* and this agrees with the discovery of Jamal et al,[37] who concluded that the leaf extract of *W. somnifera* had no impact on *E. coli*.

Pentas lanceolata ethanol extracts had the largest growth inhibition zone against Gram-positive bacteria, *S. aureus*, with inhibition zones of 15.67 and 18.67mm at concentrations of 50 mg/mL and 100 mg/mL, respectively. This discovery is consistent with Matu et al[38]'s findings that ethyl acetate and methanolic root extracts of *Pentas lanceolata* were

effective against *S. aureus* species. However, the current findings disagree with the findings of Matu et al,[38] who found that ethyl acetate and methanolic root extracts of *Pentas lanceolata* were effective against *E. coli* species. The discrepancy might be attributed to the extractive solvent and the resistance of *E. coli*. The available data from indigenous practices in various places support the current study. Decoction of roots is taken as a remedy for gonorrhea, syphilis, and dysentery.[39]

At all dosages examined, ethanolic leaf extracts of *Ageratum conyzoides* demonstrated a significant growth inhibitory effect against Gram-positive bacteria, *S. aureus*. At a dosage of 100 mg/mL, the antibacterial activity was rather strong (16.33 mm). This result is consistent with the previous research findings[40,41] and even slightly higher diameter zone of inhibition (20.3 mm ± 1.3) *Ageratum conyzoides* extracts had been reported[45] from Indonesia and again Harjanti et al[46] reported that *Ageratum conyzoides* extracts have promising antibacterial activities against staphylococcus species.[46] However, this finding is not in line with previous findings by Lumpu et al,[27] Mitra[42,] and Trinh et al[47] who found that *A. conyzoides* had high antibacterial action against E. coli. The difference might be due to the bacteria E. coli, which is recognized for its multi-resistant characteristics, extraction procedures, and solvent selection. The difference may be attributed to a variety of factors such as environmental factors like geographical area, time of collection, moisture content, drying method, storage conditions, and post-harvest process, as stated by Lumpu et al.[27]

Becium obovatum is traditionally used to treat children with stomach problems and abdominal pain as enemas made from warm water infusions of pounded roots and leaves.[43] At all doses tested, the leaf extract of *Becium obovatum* exhibits antibacterial action against S. aureus. This finding agrees with Fawole et al's[29] findings, which reported that the extraction of *Becium obovatum* has good antibacterial activity against *S. aureus*. However, in the current study, *Becium obovatum* did not show any activity against *E. coli*. So, the antimicrobial of *Becium obovatum* against *E. coli* in this finding disagrees with the previous finding of Fawole et al[29] who reported that Becium obovatum has a good antimicrobial activity against gram-negative *E. coli* (40).

In general, the results showed that as the concentrations of the extractions increased, so did the zones of inhibition. The inhibitory zones formed by test bacteria exposed to *Withania somnifera* leaf extract varied from 5 to 15.67 mm against Staphylococcus aureus (ATCC 25923) at concentrations ranging from 25 mg/mL to 100 mg/mL. *Staphylococcus aureus* inhibition zones varied from 4 to 14 mm for *Becium obovatum*, 6 to 16.33 mm for *Ageratum conyzoides*, and 9.33 to 18.67 mm for *Pentas lanceolata* leaf and root extracts, respectively. This result is also consistent with findings from other regions of the world,[20,25] which discovered that the apparent antibacterial activity of the respective extracts may be affected by both concentration and nature of the extraction solvent used.

When tested against the same bacterial species, *S. aureus* ATCC 25923, the diversity within the various plant extracts and Gentamicin was considerable in this investigation. There was a significant difference across plant species at dosages ranging from 6.25 to 100 mg/mL for all investigated extracts with Gentamicin ($p < 0.05$). *Ageratum conyzoides* leaf extract displayed comparable antibacterial activity to *Becium obovatum* leaf extract, which was judged to be statistically insignificant ($p > 0.05$). Furthermore, there was no statistically significant difference across plant species and concentrations ($p > 0.05$). This might be owing to the existence of the same secondary metabolites, as well as the clinical strains' susceptibilities.

Furthermore, the in vitro findings of the plant extracts' inhibitory activity demonstrated that the control, solvent DMSO, revealed that the colonies remained viable during the treatment period. The inhibitory potential of all extracts was tested, and the strains of *S. aureus* ATCC 25923 were completely inhibited in 5 minutes at concentrations ranging from 12.5 mg/mL to 100 mg/mL for *Ageratum conyzoides* and *Pentas lanceolata*, 25 mg/mL to 100 mg/mL for *Becium obovatum*, and 50 mg/mL to 100 mg/mL for *Withania somnifera*.

Conclusion

The current study found that ethanol extracts of Withania somnifera L., *Ageratum conyzoides* L., and *Becium obovatum* (E. Mey. ex Benth. in E. Mey) N.E. Br leaves, as well *as Pentas lanceolata* root, have antibacterial action against Gram-positive bacteria, *S. aureus*, but not *E. coli*. The inhibitory potential of all extracts was tested, and the strains of *S. aureus* ATCC 25923 were inhibited in 5 minutes at concentrations ranging from 12.5 mg/mL to 100 mg/mL. This discovery of chosen medicinal herbs supports some of the traditional claims of effective antibacterial activity in animal health

management therapy. However, further investigations needed to be conducted to validate the biological ingredients and test these traditional medicinal plants' safety, efficacy, toxicity, and clinical evaluation.

Abbreviations

ANOVA, Analysis of Variance; ATCC, American Type Culture Collection; DMSO, Di-methyl-sulfoxide; DZFEDD, Dawuro Zone Finance Economic Development Department; FMD, Foot and Mouth Disease; SNNPRS, Nation Nationalities and Peoples Regional State; SPSS, Statistical Package for the Social Sciences.

Ethics Approval and Consent to Participate

Before conducting the study, verbal consent was obtained from all participants. No additional ethics approval was required.

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Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis, and interpretation, or in all these areas; took part in drafting, revising, or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

The authors declare that they have no conflicts of interest in this work.

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