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Study on Ethno-Veterinary Use of Medicinal Plants among Traditional Healers and Their *In vitro*-Effect against Major Bacterial Pathogens in and Around Wolmera District of Oromia Region, Ethiopia

Abera Girma, Habtamu Taddele, Kenesa Teferi and Abraham Diriba

Mekelle University, College of Veterinary Medicine, P.O. Box: 2084, Ethiopia

Abstract: An ethno-veterinary survey was conducted from November 2017 to April 2018 in Wolmera district, Oromia Regional State of Ethiopia. The objective of the study was designed to document ethno-veterinary medicinal plants, their preparation and application methods used by traditional healers and local farmers in treating different animal diseases, in Wolmera district. A purposive sampling technique was carried out using a semi-structured questionnaire and field observation to document indigenous knowledge of 101 (50 local farmers and 51 traditional healers). In addition, the antibacterial activity of two selected medicinal plants (*Calpurnia aurea* and *Clematis simensis*) was carried out using disc diffusion method against *Escherchia coli, Salmonella typhi* and *Staphylococcus aureus*. A descriptive statistic was used to analyze the reported ethno veterinary medicinal plants and associated indigenous knowledge. The majority of the informants were male, 88 (87%). In this study about 48 medicinal plant species belonging to 35 families against a total of 31 animal diseases were identified. Cattle, equine, poultry, shoat and pet animals were the livestock categories mainly treated by ethno veterinary medicinal plants against different diseases such as anthrax, rabies, blackleg, bloat, mastitis, retained placenta and others. The principal sources of the medicinal plants were from wild, cultivated and both with the proportion of 88%, 10%and 2 %, respectively. The most common administration routes were oral (42.6%) followed by topical (31.7%) and nose (21. 8%).The major plant parts used were leaf (60.4%) followed by root (29.7%). *C. simensis* and *C. aurea* showed a promising antibacterial activity against the tested bacterial species with a better activity against *S. aureus*. In conclusion, the study revealed that participants had a good indigenous knowledge on ethno veterinary medicinal plant and this plays an important role for the treatment of different animal ailments in the study district. But the dose varies among the healers. Therefore, further study should be conducted to evaluate the efficacy and standardize the dose of medicinal plants in the study area. Moreover, further study should be conducted on the bacterial activity of *C. simensis* and *C. aurea* and other medicinal plants identified in the area.

Key words: Bacterial Pathogens · Ethno Veterinary Medicinal Plants · *In vitro* -Effect · Traditional Healers · Wolmera District

INTRODUCTION

In most developing African countries like Ethiopia, which has the huge livestock population, livestock production remains crucial and represents a major asset among resource-poor small holder farmers by providing milk, meat, skin, manure and traction. However, the economic benefits of livestock populations remain low due to prevailing livestock diseases which are among the principal bottle necks of livestock performance and cause of high economic losses of the resource poor farmers [1]. The majority of livestock raisers in Ethiopia are geographically far away from the sites of animal clinic stations; and those that are closer to the sites may not afford the fees for services [2].

Plants have played a vital role in combating many ailments in human and livestock in many indigenous communities, including Africa [3]. Traditional practitioners and particularly medicinal plant herbalists, in Africa have the deepest knowledge-base of traditional medicine,

Corresponding Author: Abera Girma, Mekelle University, College of Veterinary Medicine, P.O. Box: 2084, Ethiopia.

which is transferred orally from one generation to the next through professional healers, knowledgeable elders and/or ordinary people [4]. Several studies have been conducted across the globe describing the importance of traditional knowledge in veterinary care [5]. The application of traditional medicines to veterinary medicine has been termed as Ethno Veterinary Medicine (EVM). EVM has been defined in broad sense as an indigenous animal healthcare system that includes traditional beliefs, knowledge, skills, methods and practices of a given society [6].

In many poor rural areas, ethno veterinary medicine can play a great role in animal production and livelihood development and often becomes the only available means for farmers to treat ill animals [7]. The pastoral lifestyle of Indigenous communities is bringing them close to natural remedies for treating their domestic animals [5]. Besides, most modern drugs are expensive and not affordable by the majority of Ethiopian farmers and pastoralists, most of them rely on their traditional knowledge, practices and locally available materials (mainly plants) in the control of diseases of their domestic animals [8].

In Ethiopia plant remedies are still the most important and sometimes the only sources of therapeutics for nearly 80% of human and more than 90% in livestock population. A lot of traditional plants are used to treat and prevent livestock ailments and medicinal plants which have been used both for prevention and cure of various diseases of humans and animals from time immemorial occupy the largest portion [9].

In developing countries conventional veterinary services, despite its great role, have limited in coverage and development of anti-microbial resistance is another challenge [10]. Because of this reason livestock keeper particularly in rural areas frequently visit traditional healers to get solutions for their ill-health animals including clinical cases of skin, udder, blackleg, bloat, retained placenta and gastrointestinal tract infections. Developing a socially acceptable and effective remedy from inexpensive resources that can complement modern medicine would be an attractive option [9].

An estimated floras of 6 500 to 7 000 species of higher plants are of medically essential and out of these medicinal plants 12% are endemic to Ethiopia [4]. However, the efficacy of most of these plants has not been investigated scientifically. Although they play a central role in catering for the health of human and livestock population, large part of the knowledge of ethno medicinal plants is on the verge of irreversible loss and declining to deterioration due the oral passage of herbal heritage from generation to generation rather than in writings form [11].

Studies conducted on traditional ethno veterinary medicinal plants in Ethiopia are very limited when compared with multi-ethnic cultural diversity and diverse flora of Ethiopia [12]. Even though traditional ethno veterinary knowledge of medicinal plant is very crucial to treat different animal diseases, there is no study conducted in Wolmera district on this regard. The present study was carried-out to document the ethno-veterinary medicinal plants, their preparation and application methods used by traditional healers in treating different animal diseases, in Wolmera district. Moreover, the study evaluated the *in-vitro* antibacterial activity of leaves of *Calpurnia aurea* (*C. aurea*) and *Clematis simensis* (*C. simensis*) against some microorganisms s*uch as E. coli, Salmonella typhi* and *S. aureus*.

MATERIALS AND METHODS

Study Area: The study was conducted from November 2017 to April 2018 in Wolmera district of Oromia special zone surrounding Finfine, Oromia regional state, central Ethiopia. Wolmera is located at 9 °07' N and 38°5' E with altitude of 2520 meters above sea level and the average temperature is 21°C and annual rainfall is 900-1100 mm [13] (Fig. 1). In this area the rainfall pattern is bimodal, with a short rainy period from February to April and a long rainy season from mid-June to September. The district is located at 34 kilometers west of Addis Ababa, the capital city of the country. Total area of the woreda is 674 km^2. Out of this 19510 hectare of land is suitable for irrigation. According to the population and housing census of 2007, the population of the woreda is estimated to be 83, 823 and out of this 42, 115 is male and the remaining 41, 708 are female [14]. The urban population is 3550 while the rural population is 80, 273. Agro ecologically majority of the woreda belong to the Dega and Woina Dega zones. Agriculture is the main sources of income in the district [13]. The research institutions in the town are helping the agricultural activities. Holeta Agricultural Research Center, Holeta Bee Research Center, Animal Production Centers and Holeta TVET College are among the institutes, which support agriculture sector. Crop farming is the main source of livelihood for most of the farmers in the woreda. Wheat, teff and barley serve as the major cash crop of the woreda. Nowadays, potato production is widely practiced as one source of income for smallholders of the woreda. The major livestock managed in the area include oxen, cows, goats, sheep, donkey, horse, mule and chicken.

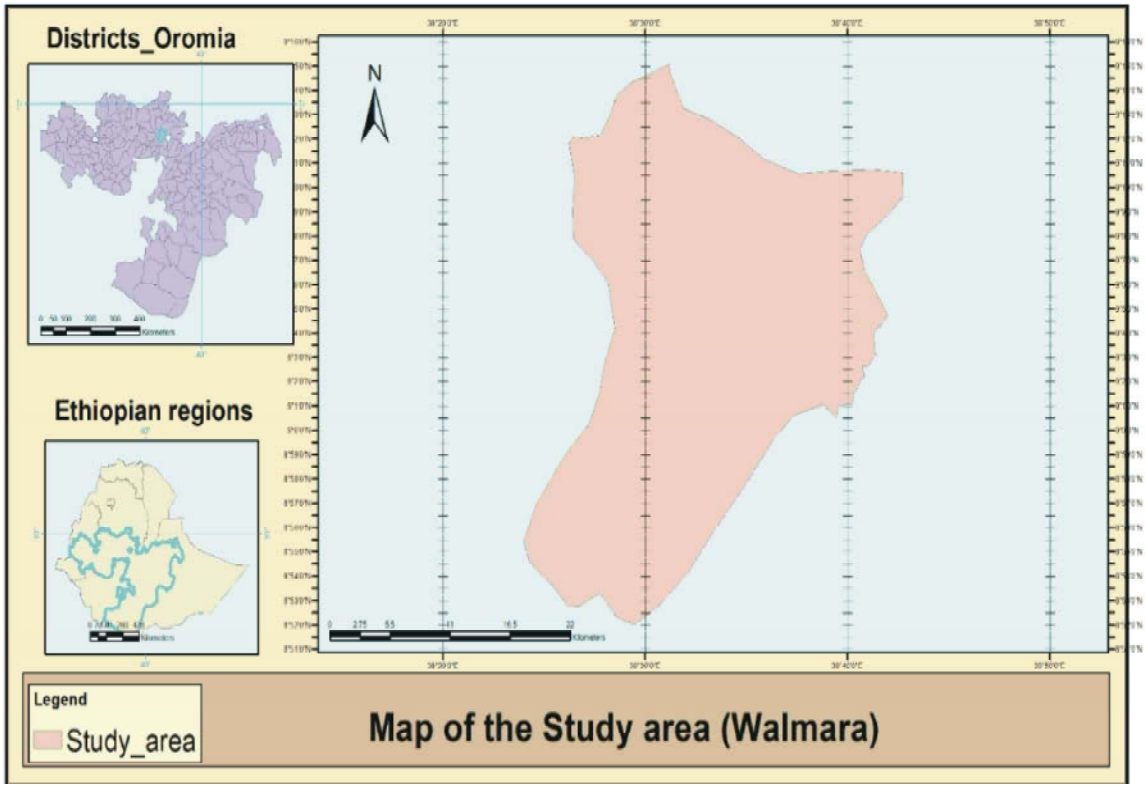

Fig. 1: Map of the study area (Wolmera District)

Study Population: The target populations for this study were 101voluntary traditional healers and local farmers who highly rely on traditional remedies. The study participants were selected purposively based on the recommendation of local authorities of the district.

Study Design: An ethno-veterinary survey was conducted from number 2017 to April 2018 to congregate information on the traditional usage of plants in the livestock health care system using a semi-structured interview and observation [15]. Local farmers and traditional healers who were highly dependent on traditional healings and possess indigenous knowledge were interviewed. Prior to the interview process, written permission was obtained from the office of the district and also permission was obtained from the administrator of each selected kebele. Following this, the purpose of the study was explained to each informant and verbal prior consent was obtained. This was done to clarify the purpose and build confidence of the respondents to provide reliable information without suspicion. Interviews and discussions were undertaken based on checklist of questions prepared in English and translated to local language 'Oromic language'.

Sample Size and Sampling Techniques: For this study five kebeles were selected from the study area using purposive sampling techniques because these areas typically possess intellectual healers and covered by different plant species that are used for traditional medication to treat different animal ailments. A total of 101 (50 local farmers and 51 traditional healers) individuals were purposively selected from 5 kebeles in and around the Wolmera district and were interviewed to gather all the relevant data.

Data Collection: Specimens of plants that were used by the healers for their animal ailments treatment were collected using the standard botanical methods together with the healers. Plant parts including the vegetative part, leaves and floral, fruiting and/or seed parts as it was appropriate for taxonomic identification were collected. The information gathered included types and characteristics of plant and their traditional preparation, parts used, route of administration, dose given by the local healers, duration of the treatment and other ingredients added. Moreover, information was collected about the way of passing the indigenous knowledge from generation to generation. The information collected also

included local name of the traditional medicinal plant, type (cultivated or wild), diseases treated and existing threats to medicinal plants.

Antibacterial Activity of Selected Medicinal Plants: An *in-vitro* experimental study with two plant extracts, one positive control (sulfamethazine-trimethoprim) and one negative control (DMSO). The experiments were repeated three times and the results were expressed as average value of zone of bacterial growth inhibition by each plant extract [16].

Plant Collection and Pre Extraction Preparation: The test plants were selected from survey conducted from November 2017 to April 2018 in and around Wolmera district based on its use traditionally by local farmers and traditional healers for the treatment of livestock diseases. After collection from their natural habitat the plants were taken to Holeta Agricultural Research Center Parasitology laboratory, `washed with tap water to remove unnecessary particles. Then dried under shade at a room temperature for about a month and grounded mechanically by mortar and pestle. The material was then sieved and weighted by sensitive balance before maceration.

Preparations of Crude Extracts: After the parts of plants were ground and sieved by fine mesh were weighted using sensitive balance and packed into a glass vial each plant was coded and labeled. Then taken to Addis Ababa public health institute for extraction by alcohol (methanolic extracts). Maceration of each plant was carried out in flasks containing methanol (80%) in a 1: 10 (solute: solvents) ratio enough to cover all the plant powders with a continuous shaking with an orbital shaker (Gemmy Industrial Corporation, VRN-480) at 140 rpm and left at room temperature for 72 hrs with frequent agitation and the resulting liquid was filtered (Whatman No. 1 filter paper, What man Ltd., England). Extraction was repeated three times and the filtrates of all portions were combined in one vessel. The organic solvent was removed by evaporation using rotary evaporator (BUCHI Rota-vapor R-205, Switzerland) at temperature not more than 40°C. The aqueous residue was then placed in a vacuum oven at 40°C for about a week to remove the water Gizachew *et al.* [17]. The resulting concentrated extracts of each plant material will be transferred to bottle bijou which had tight fitting cups and then labeled with respective plant name before refrigerated at 4°C until tested for antimicrobial activity [9].

Preparation of Test Bacteria: Bacterial species used in this study were obtained from Molecular Biotechnology Laboratory of College of Veterinary Medicine, Mekelle University. The specimens were originally isolated from animals and humans during disease investigation and kept lyophilized. These were *Salmonella typhi*, *Escherichia coli* and *Staphylococcus aureus*. Each bacterial isolate was homogenized with 3 ml of nutrient broth and a loopful of broth containing the bacteria was inoculated on nutrient agar. It was then incubated at 37°C for 24 hrs. The test bacteria were suspended separately into sterile universal bottles containing normal saline was added gradually to adjust the culture turbidity to that of McFarland turbidity standard, which corresponds to approximately (1.5×10^8 CFU/mL).

Antimicrobial Sensitivity Tests: The antimicrobial test was conducted using agar disc diffusion method. Muller-Hinton agar (Biotech UK) was used for antimicrobial sensitivity test. The media was prepared according to standard protocol. The top of 4-5 well isolated colonies of the same morphology were scooped using a wire loop from the nutrient agar and mixed using sterile normal saline and agitated with a vortex mixer. The turbidity of the bacterial suspension was adjusted by comparing with 0.5 McFarland turbidity standards. The swab was streaked in the three directions over the entire surface of the agar with objective of obtaining uniform inoculations and a final sweep with the swab was made against the agar around the rim of the petri dish. The inoculated plates were allowed to stand for not more than 15 minutes and the discs were placed on the agar surface using a sterile forceps. Each disc was gently pressed with the point of the sterile forceps to ensure complete contact with the agar surface. For this study, sulfamethazine-trimethoprim was used to compare their efficacy with herbal preparations and as a positive control, while DMSO was used as negative control. The appropriate crude extract (100mg/ml, 200mg/ml and 300mg/ml) impregnated discs and conventional antibiotic discs were applied at spaces of 24 mm apart from center to center and 15 mm away from the edge of the plates. This was made no later than 15 minutes after the inoculums has been added. The plates turned upside down, labeled and incubated at 37°C for 24 hrs. Diameter of zone of inhibition was measured using a caliper in mm [18]

Statistical Analysis: Some descriptive statistical methods, percentage, frequency, figures and tables were used to summarize the collected ethno-veterinary

medicinal data and associated indigenous knowledge. Mean zone of inhibition for the antibacterial activity was determined using the statistical software STATA version 15 package.

RESULTS

Socio Demographic Characteristics of the Informants: In this study, the majority of the study participants were male, 88 (87%) and the remaining 13 (13%) were females (Table 1). This may be due to local tradition that restricting such practices mostly to males whereas females were not allowed to be involved in outdoor activities rather than they look after babies and carry out domestic activities.

Ethno-veterinary Medicinal Plants for Treatment of Animal Diseases: In this study, a total of 48 plant species were used against 31 types of livestock diseases. Those plants were botanically distributed across 35 families (Table 2 and Annex I). Data from this study showed that the highest number of plant species was found in *Fabaceae* family followed by *Asteraceae, Lamacaceae* and *Ephorbaceae*.

The study showed that cattle, equine, poultry, shoat and pet animals were the livestock treated by ethno veterinary medicinal plants in the study area against different diseases such as anthrax, rabies, blackleg, bloat, mastitis, plant poison, retained placenta, dermatophilosis, parasite infestation, leech and others. This clearly indicated that the local people of the study area are concerned about the health of their livestock as their livelihood is highly associated with animals.

Source, Knowledge and Documentation Medicinal Plants: The study result indicated that most of the traditional healers acquired their knowledge from their fathers/families (48/51, 94.1%) and friends (3/51, 5.9%). The principal sources of the plants used by traditional healers were from wild (45/51, 88%), cultivated (5/51, 10%) and both (1/51, 2 %). In the study area the practices/knowledge, information on the ethno-veterinary medicinal plants, is not found in written form or not documented. All the 51 traditional healers participated in this study had no a written document (Table 3).

Dosage, Route of Administration and Plant Part Used: The most common routes of administration used in the study area was oral, 36 (35.6%) followed by topical, 34 (33.7%) and nose 28 (27.7%) (Fig. 2). Regarding with dosage the standard units of measurements were not used by traditional healers. But they used different techniques to determine dosage in the study area such as coffee cup/glass (71.3%), estimation (21.8%) and spoons (6. 9%).The major plant parts used in the study area were leaf (60.4%) followed by root (29.7%) (Fig. 3).

Antibacterial Activity of Selected Medicinal Plants: The experiments were performed in triplicate and the results are presented as mean values of the three measurements. The results of the antibacterial activity of methanol extracts of *C. simensis* and *C. aurea*using disc diffusion on three bacterial strains showed a maximum inhibitory effect on Gram positive bacteria, *S. aureus* (Table 4).

Of the two plant extracts tested, 80% methanol extract of *C. aurea* showed higher zone of inhibition (Table 5).

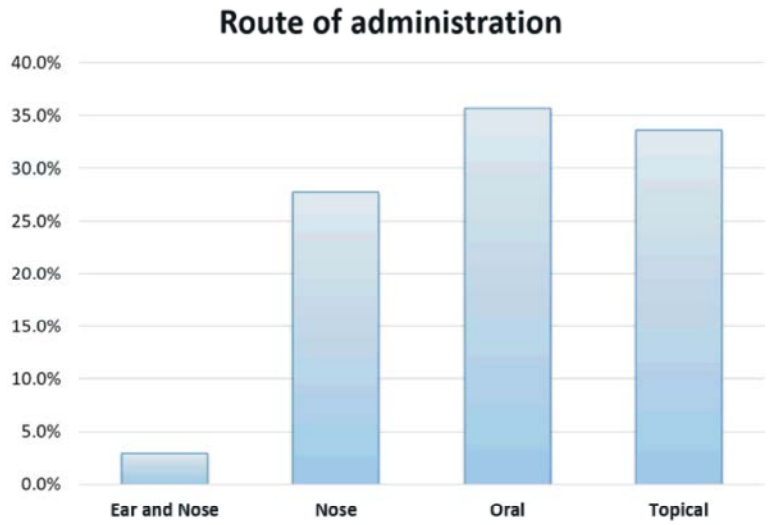

Fig. 2: Route of administration of medicinal plants for treatment of diseases

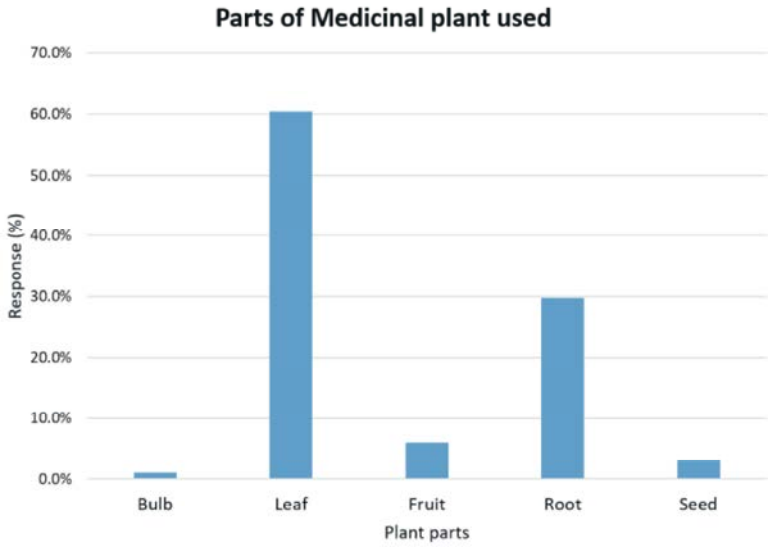

Fig 3: Plant parts used by the traditional healers for remedy preparation

Table 1: Socio demographic characteristics of the informants

	No. by Sex		
Informant types	Male	Female	Total frequency
Traditional healers	48	3	51
Local farmers	40	10	50
Total	88	13	101

Table 2: Animal species treated by different ethno-veterinary medicinal plant remedies against different ailments/ diseases conditions in the study area

Animal species	Diseases conditions	Ethno-veterinary medicinal plant remedies used
Bovine	Anthrax	*Asteraceae, Fabaceae, Euphobiaceae*
	Rabies	*Phytolacaceae, Salicaceae, Acanthaceae, Fabaceae*
	Bloat	*Brassicaceae, Euphorbiaceae, Salicaceae*
	Mastitis	*Fabaceae, Ranunculaceae, Simaroubaceae, Myrtaceae*
	Plant poison	*Convolvulaceae*
	Retained placenta	*Lineaceae, Urticaceae, Musaceae*
	Dermatophilosis	*Asparagaceae, Scrophulariaceae*
	Brucellosis	*Malvaceae*
	Black leg	*Ranunuclaceae, Meliaceae, Acanthaceae, Asteraceae*
	Diarrhea	*Asparagaceae, Asteraceae*
	Tape worm	*Myrsinaceae*
	Leech	*Fabaceae, Solanaceae*
	Wart	*Ranunculaceae*
	Ectoparasitosis	*Melianthaceae, Simaroubaceae*
Equine	Colic	*Solanaceae, Myrtaceae, Solanaceae, Asteraceae*
	Strangles	*Alliaceae*
	Glanders	*Ranunculaceae*
	Urine retention	*Rhamnaceae*
Pet animals	Rabies	*Acanthaceae, Salicaceae*
Shoats	Coughing	*Lamiaceae*
	Snake bite	Poaceae

Table 3: Knowledge, source and documentation of medicinal plants

Variable	Category	No. of respondents	Percent
Source of knowledge	Family	48	94.1
	Friend	3	5.9
Source of plants	Wild	45	88
	Cultivated	5	10
	Wild and cultivated	1	2
Documentation of medicinal plants	Documented	0	0
	Not documented	51	100

Table 4: Overall antibacterial activity of methanol extracts by the disc diffusion assay

Bacterial strain	Mean	Std. Err.	95%CI
E. coli	9.35	1.34	6.69-12.01
Salminella typhi	8.60	1.28	6.07-11.13
S. aureus	9.92	1.18	7.58-12.25

Table 5: Mean zone of inhibition by plant type

Plant types	Mean	Std. Err.	95% CI
C. aurea	10.16	0.40	9.35-10.96
C. simensis	7.03	0.15	6.73-7.33
Sulfamethathoxazole-Trimethoprim (24µg)	20.67	0.30	20.07-21.27

Table 6: Mean zone of inhibition of plants by concentration

Concentration	Mean	Std. err.	95%CI
100 mg/ml	7.78	0.38	7.01-8.55
200 mg/ml	8.61	0.51	7.60-9.62
300 mg/ml	9.38	0.61	8.16-10.59

Table 7: Mean zone of inhibition of plant extracts against bacterial strains

Plant type	Bacteria strain	Mean	SD	CV	Min	Max	P25	P50	P75
C. aurea	*E.coli*	9.81	0.770	0.08	8.75	11	9.25	9.75	10.25
	Salomella thyphi	8.22	0.78	0.10	7.1	9.54	7.85	8.05	8.65
	S. aures	12.43	1.71	0.14	9.95	14.95	11.5	12.75	13.56
C. simensis	*E. coli*	6.69	0.39	0.06	6.05	7.02	6.56	6.85	6.95
	Salomella typhi	6.44	0.34	0.05	6.04	6.9	6.12	6.45	6.75
	S. aures	7.95	0.50	0.06	7.28	8.64	7.68	7.8	8.5

Both of the plant extracts showed increase in zone of inhibition as concentration of extract increases. Both extract had higher zone of inhibition at 300mg/ml when compared with that of 200mg/ml and 100mg/ml (Table 6).

Mean zone of inhibition of bacterial growth by two plant extracts are shown in Table 7. From the two plant extracts tested, *C. aurea* showed more antibacterial activity against the three bacterial strains than *C. simensis*. *S. aureus* was more sensitive bacteria against both extracts. The anti-bacterial activity of the extracts of the investigated plants appears to be due to the presence of active ingredients naturally found in the parts of the plants.

DISCUSSION

In this study, the majority of the study participants were male (87%) and the remaining were females (13%). This study indicated that the men of the community had more knowledge about the ethno veterinary medicine practices because they are naturally selected during child hood to be apprentices of ethno veterinary practices [4]. In addition, in some regions, labour division makes women responsible for housekeeping only, while men take care of the animals [19]. The findings in the present study are in line with the findings of several researchers [20-23].

The study showed that cattle, equine, poultry, shoat and pet animals were the livestock treated by ethno veterinary medicinal plants in study area. This clearly indicated that the local people of the study area are concerned about the health of their livestock as their livelihood is highly associated with animals. Most of the plant species documented from Wolmera district were used to treat ailments of cattle and equine. The comparable study was conducted by Martinez and Luján [24] in Argentina who reported that veterinary ethno botanical knowledge is specialized and restricted to cattle. In this study, different livestock diseases such as anthrax, rabies, blackleg, bloat, mastitis, retained placenta, parasite infestation, leech and others diseases conditions were treated by the medicinal plants. This indicates that medicinal plants were widely used to treat different livestock diseases. Several research findings also indicated the potential application of medicinal plants for the treatment of different livestock diseases [23, 25]. The results of the study revealed that *Fabaceae*, *Astraceae* and *Laminaceae* families represented the dominant families in terms of number of species belongs to them. This result was in agreement with Chala *et al*. [23] who also indicated the wider use of these plants in Eastern Ethiopia. Similar results were also observed in different part of Ethiopia [22, 25, 26]. In the present study, 94.1% of traditional healers responded that they acquired their knowledge from their parents or families which is in line with study conducted by Gebremedhin *et al*. [27] who reported that 93.5% traditional healers acquired their knowledge from their parents or close relatives. This indicated that local healers have a very high intention to keep their ethno veterinary knowledge secrete and none of them were ready to transfer their knowledge either freely or on incentive bases to other people [28-30]. However, a study by Firaol *et al*. [31] from Dabo Hana District, West Ethiopia, stated that most of the traditional healers have willingness to transfer the knowledge to others who are interested to know.

All of traditional healers participated in this study had no written document regarding the medicinal plant they use. This may be due to indigenous knowledge of ethno veterinary practice was passed from generation to generation in an oral manner rather than in writing form. The present finding was similar with study conducted by Tekle [22] in Amaro Special District, Southern Ethiopia who reported that no one had a written document out of the total herbalists participated in this study. Similar result was also reported by researchers from different corners of the country [11, 32].

Majority of the medicinal plants used for medicinal value by the traditional healers were obtained from wild. The probable reason for this could be the majority of farmers depend on wild environments where the plants are naturally grown rather than domestication by them is due to beliefs about medications and promote secrecy behind such practices [23, 24, 33, 34]. Study on route of administration revealed that oral route was the principal mode of administration followed by topical and nasal route, respectively. The choice of oral administration may be due to the use of some solvents or additives such as milk, local alcohol and feed that are commonly believed to serve as a vehicle to transport the remedies. Another reason could be due to its rapid physiological reaction with the pathogens and increasing the curative power of the medicines. The result was in line with finding of various researchers in Ethiopia who reported oral as the leading route of administration [28, 30, 35, 36]. Cups/glasses were used as units of measurements by most of traditional healers to estimate doses of traditional medicines. They estimate some doses of medicine which were difficult to be measured by glass/cup or spoon (e.g., for dematophilosis, fresh leaf of *Lippie adoensis* rubbed directly on affected skin; *Juniperarus proceral* is smashed and put on wound). The reason behind, majority of the traditional healers and farmers used glass or cup is because of they are suitable to dissolve remedies in water to produce complete extraction of active ingredients and comfort to administer remedies given by oral route. The dose depends on the patient's age, physical state and health conditions of the anima [9, 25]. However, there was no standardized dosage for the application of medicinal plants.

Leaves of plants were the preferred parts for treating diseases in animals. Plant leaves contain many active ingredients with characteristic anti-illness effects. This finding was in consistent with study of Tilahun, Gebrezgabiher *et al*. and Birhanu *et al*. [25, 33, 37] who reported leaves to be the most frequently used plant parts. A study by Lulekal *et al.* [38] reported that root to be the most used part. This difference could be due to the therapeutic value and concentration of active ingredients in each plant varied depending on climatic and edaphic factors. Harvesting of leaves for traditional remedies has less negative impact on the survival and continuity of useful medicinal plants and hence does not affect sustainable utilization of the plants.

The results of the antibacterial activity of methanol extracts of *C. simensis* and *C. aurea* using disc diffusion on three bacterial strains showed a promising effect. *S. aureus* was the most susceptible bacteria compared to

other strains. This could be suggestive for better activity of the plants against Gram positive bacteria. The present study revealed that *C. aurea* showed higher zone of inhibition on bacterial growth than *C. simensis* this may be due to active ingredients such asalkaloids and lectins present in the *C. aurea* which had antibacterial effect [39]. *C. aurea* was more effective against *S. aurea* and *E. coli* which could be due to the presence of alkaloids [17]. The study indicated that as concentration of extract increases mean zone of inhibition increases. This is due to increment in active ingredients of the plant.

CONCLUSION

The finding of present study suggests that there is a vast amount of indigenous knowledge on ethno veterinary medicinal plant and this knowledge plays an important role for the treatment of different animal ailments in the study district. The indigenous knowledge of ethno veterinary practice is passed from generation to generation in an oral manner. Without being properly documented this information could easily be lost or distorted. In general, plant parts, methods of preparation and source of such plants were also elaborately presented. In this study, 48 species and 35 families of ethno veterinary medicinal plants were identified and documented. The plants were mainly collected from the wild based on the consultation of traditional healers. This study indicates that, the fear of destruction of medicinal plants due to plant parts collected for the purpose of medicine is minimal as leaves were the leading plant parts sought in the area. The methanol extract of *C. aurea* and *C. simensis* against 3 bacterial strains showed a promising result. Gram positive bacteria (*S. aureus*) were more susceptible than Gram negative. *C. aurea* was more effective against all bacterial strains. As concentration of extract increases the mean zone of inhibition of bacteria also increases. Further research is needed on dosage determination and the preparations' concentration for sake of identifying the remedies side effects and also to dissect the active ingredients responsible for inhibiting growth of bacteria. The documentation and conservation of medicinal plants is highly recommended.

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7. ANNEX

Ethno veterinary medicinal plants, diseases treated, scientific name, family, local name, parts used (Pu), mode of preparation and route of administration.

Scientific name	Family	Local name	Diseases treated	Pu	Mode of preparation	Route
Olea europaea L.	*Oleaceae*	Ejersa	Snake breathe	Leaf	Chewing the fresh leaf of *Olea europea* L. and pitting on the swelling area of the animals	Topical
			Diarreahoa	Leaf	Leaf of *Olea europea* L.is grinded, added to water and given to animals	Oral
Lippia adoensis	*Verbenaceae*	Sukaayee	Dermatophytosis	Leaf	Fresh leaf of *Lippia adoensis* is directly rubbed on affected area of cattle	Topical
Indiigofera tinctoria L	*Fabaceae*	Dingetegna	Blackleg	Root	The dried root of *Indiigofera tincoria* L. is powdered, dissolved in water and given to animals	Oral
			Anthrax	Root	Dried root of *Indiigofera tinctoria* is powdered, mix with grinded root of *E. kerebicho* added to water and given to animal	nose
Clematis simensis	*Ranunculaceae*	Hidda fiti	Mastitis	Leaf	Fresh leaf of *Clematis simensis* is squeezed and rubbed around inflamed udder	Topical
			Glander	Leaf	Fresh leaf of *Clematis simensis* is squeezed and the squeezed part of leaf is sealed in to the hole of wound	Topical
			Dermatophytosis	Leaf	Dried leaf of *Clematis simensis* is powdered, mixd with butter and saliva then painting on affected area of the skin of animal	Topical
			Mangimites	Root	Dried root of *Clematis simensis* is dried and powdered then painting on the skin of animals	Topical
			Warts	Leaf	The fresh leaf of *Clematis simensis* is crushe, mix with milk of *Euphorbia abyssinica* and rubbed on the affected part of skin	Topical
Justicia schimperiana	*Acanthaceae*	Dhumuga	New castle	Leaf	The fresh leaf of *Justica schimperina* is squeezed and the juice is added to enjera and given to checken	Oral
Millettia ferruginea	*Fabaceae*	Birbira	Leech	Leaf	The fresh leaf of *Millettia ferruginea* is crushed and dissolved in water then given to animal	Oral
			Rabies	Leaf	The leaf of *Millettia ferruginea* is dried and powder, mix with powder carcass of worm then added to water and given to cattle, added to milk and given to primates	Oral
Bersama abyssinica	*Melianthaceae*	Lolchisaa	Ectoparasite	Leaf	The leaf of *Bersama abyssinica* is powdered and painted on the skin of animals	Topical
Kalanchoe petitiana	*Crassulaceae*	bosoqee	Yoke tumor	Root	The fresh root of *Kaanchoe petitiana* is prepared in the form of cupsule, then put in the affected yoke of ox with piece of salt after the area is incised with blade	Topical
Croton macrostachyus	*Euphorbiaceae*	bakkanisa	Bloat	Leaf	After fresh leaf of *Croton macrostachyus* is dissolved in water it is given to animal	Oral
			Dermatophytosis	Leaf	The leaf of *Croton macrostachyus* is crushed and smashed then extract is creamed on affected part	Topical

Scientific name	Family	Local name	Diseases treated	Pu	Mode of preparation	Route
Urtica simensis Steudel	*Urticaceae*	Doobbi	Retained placenta	Root	Root of *Urtica simensis* is grinded and dissolved in water, then the solution is given to caw	Oral
			Rabies	Root	The root of *Urtica simensis* is grinded, dissolved in water and given to cattle. The powder is added to milk and given to dog	Oral
Datura stramonium	*Solanaceae*	hidhee	Plant poison	Leaf	The dried leaf of *Datura stramonium* is grinded, then the powder was added to milk and given to cattle	Left ear and nose
			Colic in horse	leaf	Leaf of *Datura stramonium* is dried and powdered; the powder is mixed with powder of root of *E. kerebicho* and dissolved in water, then given to horse.	Nose
Asparagus africanus	*Asparagaceae*	saaritii	diarreahoa	root	The root of *Asparagus africanus* is grinded and dissolved in water and the solution is given to cattle	Oral
			snake bite	root	Dried root of *Asparagus africanus* is powdered and mixed with powdered leaf of *Rumex nervosus* val, disolved in water and given to animal	Oral
Calpurnia aurea	*Fabaceae*	ceekaa	ectoparasitosis	leaf	The fresh leaf of *Calpurnia aurea* is dissolved in water and then washed the skin of animals	Topical
			mastitis	leaf	The leaf of *Calpurnia aureas* is dried and powdered, mixed with Vaseline and creamed on the affected udder of the cow	topical
Echinops macrochae	*Asteraceae*	sokoruu	Eye problem	root	The fresh root of *Echinops macrochae* is chewed and spitting to the eye of animals	ocular
Maesa lanceolata Forssk	*Myrtaceae*	burayuu	Equine colic	leaf	Fresh leaf of *Maesa lanceolata* forssk and leaf of *Rhamnus prinoides* L. are dissolved in one container and solution is given to horse	nose
			mastitis	leaf	The leaf of *Maesa lanceolata* Forssk is dried and grinded, then mixed with grinded root of *Brucea antidysenteri* and added to vesiline, creamed on the affected udder	topical
Rhamnus prinoides L	*Rhamnaceae*	geeshoo	urine retention in horse	leaf	The fresh leaf of *Rhamnus prinoides* L. is dissolved in water and the solution is given to horse	nose
Plantago lanceolata L.	Plantaginaceae	qorxobee	Snake bite	leaf	The fresh leaf of *Plantago lanceolata* L .is dissolved in water and the solution is given to animal.	Left ear and nose
			bleeding	leaf	Fresh leaf of *Plantago lanceolata* is squeezed, the juice is added to alcohol with sugar and dropped to the bleeding area	topical
Carissa spinarum L.	*Apocynaceae*	agamsaa	Warts	leaf	Fresh Leaf of *Carissa spinarum* is grinded, mixed with vesiline and painting on the affected part.	topical
Ruta chalepensis	*Rutaceae*	xenadami	Bloat	leaf	Fresh leaf of *Ruta chalepensis* is grinded, mixed with grinded seed of *Allium sativum* L. then added to water and given to sheep	oral
Juniperus procera L.	*Cupressaceae*	gatiraa	wound	leaf	Fresh leaf of *Junisperus procera* L .is is smashed and put on the wound part	topical
Ensete ventricosum	*Musaceae*	warqe dima	retained placenta	root	The root of *Ensete ventricosum* is grinded, dissolved in the water and the solution is given to cow	oral
Euphorbia abyssinica	*Euphorbiaceae*	adamii	Warts	leaf	milk like liquid from fresh leaf of *Euphorbiaceae* is dropped on the affected area	topical
Brucea antidysenteri	*Simaroubaceae*	qomonyoo	mastitis	root	Dried root of *Brucea antidysenteri* is grinded and mixed with grinded leaf of *Maesa lanceolata* Forssk, mixed with vesiline and the paste is creamed around the affected udder	topical
Buddleja polystachya Fresen	Loganiaceae	Qore baala waranti	Eye problem	root	Fresh root of *Buddleja polystachya*, *Fresen ichewed* and spitting to the eye of cattle	ocular
Echinops kerebicho M	*Asteraceae*	qerebicho	anthrax	root	The dried leaf of *Echinops kerebicho* is grinded, mixed with powdered root of *Indiigofera tinctoria* L and added to local alcohol and given to animals	oral
Allium sativum L.	*Alliaceae*	shunkurti adi	stranglus	seed	Seed of *Allium sativum* L. is mixed with root of *Echnops kerebicho* M grinded together and dissolved in water and given to horse	nose

Scientific name	Family	Local name	Diseases treated	Pu	Mode of preparation	Route
Verbascum sinaiticum Benth	*Scrophular-iaceae*	Gurraa harree	Urine retention in horse	leaf	Fresh leaf of *Verbscum sinaiticum* Benth is crushed dissolved in water and solution is given to horse	nose
			dermatophlosis	leaf	The fresh leaf of *Verbascum sinaiticum* benth is squeezed and rubbed to the affected area of the skin of animals	topical
Justicia schimperiana	*Acanthaceae*	sansalli	diarreahoa in sheep	leaf	The fresh leaf of *Justica schimperiana* is pounded dissolved in water and solution is given to sheep	oral
Cynodon dactylon	*Poaceae*	coqorsa	Snake bite	leaf	The fresh leaf of *Cynodon dactylon* is pounded, mixed with water and the solution is given to animal	oral
Salix subserrata Willd.	*Salicaceae*	alaaltuu	Bloat	leaf	The leaf of *Salix subserrata* is dried, powdered and mixed in the water then given to animals	oral
Satureja punctate	*Lamiaceae*	xosinyi	Coughing	leaf	The fresh leaf of *Satureja punctata* is pounded, dissolved in water and solution is given to animals	oral
Chlorophytum somaliense Ba	*Anthericaceae*	burii	Stomach ache	fruit	Fruit of *Chlorophytum somaliense* Ba is grinded, dissolved in water and given to animals	oral
Vigna membrancea L	*Fabaceae*	Hidda adi	Skin diseases	root	Root of *Vigna membrancea* L is grinded with root *Clematis simensis* and directly rubbed on the skin of animals	topical
Ipomoea cairica (L.)	Convolvul-aceae	kalaalaa	Plant poison	leaf	Fresh leaf of *Ipomoea cairica* (L) is chopped in piece mixed in water and solution is given to animals.	Left nose
Vernonia amygdalina	*Asteraceae*	ebicha	Leech	leaf	Fresh leaf of *Vernonia amygdalina* is crushed and mixed with crushed leaf of *Millettia ferruginea*, dissolved in water and given to animals.	oral
Embelia schimperi Vatke.	*Myrsinaceae*	hanquu	Tape worm	fruit	Fruit of *Embelia schimperi* Vatke is grinded, dissolved in water and solution is given to animals	oral
Rumex nepalensis	*Polygonaceae*	rooban jireeti	Stomach ache in lamb	root	Root of *Rumex nepalensis* is pounded, mixed in water and solution is given to lamb	
Ocimum lamii folium Benth.	*Lamiaceae*	Damakase	Trypanosomiasis	leaf	Leaf of *Ocimum lamii* folium Benth is pounded with leaf of croton macro stachyus and mixed with water, then solution is given to animals	oral
Stephania abyssinica	*Menisper-maceae*	hidda kalaalaa	Wound	leaf	Leaf of *Stephania abyssinica* is pounded and small amount is put on wound area	topical
Nicotiana tabacum L.	*Solanaceae*	tamboo	Leech	leaf	Leaf of *Nicotiana tabacum* L.is dried, powdered and mixed with water and solution is given to cattle	Left nose
Solanum dasyphyllum	*Solanaceae*	Hiddi warabeessaa	Colic	fruit	Fruit of *Solanum dasyphyllum* is powdered mixed in water and solution is given to horse	nose
Phytolacca dodecandra L. Herit	*Phytolacaceae*	andoodee	Anti-rabies	root	The root of *Phytolacca dodecandra* L. is dried. grinded and dissolved in water and given to animals before diseases was occurred	Left nose
Ekeberia capensis	*Meliaceae*	somboo	blackleg	leaf	Leaf of *Ekeberia capensis* and bulb of *Allium sativum* are powdered together and mix with water and given to cattle	oral
Ricinus communis L.	*Euphobiaceae*	qobboo	anthrax	fruit	Dried fruit of *Ricinus communis* L. powdered mix with water and given to cattle	Left nose
Lepidium sativum L.	*Brassicaceae*	feeccoo	Bloat	seed	Seed of *Lepidium sativium* L. and bulb of *Allium sativum* are pounded together and mixed in water and given to cattle	oral
Sida rhombifolia L.	*Malvaceae*	karabaa	brucellosis	root	Root of *Sida rhombi* folia L.is powdered and mixed with water and given to animals	oral
Thalictrum rhynchocarpum	*Ranunuclaceae*	Siraabuzuu	Black leg	root	Dried root of *Thalictrum rhynchocarpum* is powdered and mixed with water. then given to cattle	oral
Linum usitatissimum L	*Lineaceae*	talbaa	Retained placenta	seed	Seed of *Linum usitatissimum* L. is powdered and dissolved in water, then given to cow.	oral

Images of plants used for antibacterial activity

C. aurea

C. simens