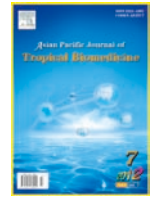




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In-vitro antimicrobial activity screening of some ethnoveterinary medicinal plants traditionally used against mastitis, wound and gastrointestinal tract complication in Tigray Region, Ethiopia

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ABSTRACT

Objective: To screen the antibacterial activity of nine ethnoveterinary plants traditionally used for the treatment of mastitis, wound and gastrointestinal complications. **Methods:** Hydroalcoholic extracts of medicinal plants namely, *Achyranthes aspera* (*A. aspera*) L. (Family Asparagaceae), *Ficus caria* (*F. caria*) (Family Moraceae), *Malvi parviflora* (*M. parviflora*) (Family Malvaceae), *Vernonia species* (*V. species*) (local name Alakit, Family Asteraceae), *Solanum hastifolium* (*S. hastifolium*) (Family Solanaceae), *Calpurinia aurea* (*C. aurea*) (Ait) Benth (Family Fabaceae), *Nicotiana tabacum* (*N. tabacum*) L. (Family Solanaceae), *Ziziphus spina-christi* (*Z. spina-christi*) (Family Rhamnaceae), *Croton macrostachys* (*C. macrostachys*) (Family Euphorbiaceae), were screened against clinical bacterial isolates of veterinary importance from October 2007 to April 2009. The antibacterial activity was tested using disc diffusion at two concentrations (200 mg/mL and 100 mg/mL) and broth dilution methods using 70% methanol macerated leaf extracts. **Results:** With the exception of *S. hastifolium* all plant extracts exhibited antibacterial activity. Among the medicinal plants tested *C. aurea*, *C. macrostachys*, *A. aspera*, *N. tabacum* and vernonia species (Alakit) showed the most promising antimicrobial properties. **Conclusions:** It can be concluded that many of the tested plants have antibacterial activity and supports the traditional usage of the plants for mastitis, wound and gastrointestinal complications treatment. Further studies into their toxicity and phytochemistry is advocated.

1. Introduction

Since ancient times plants have been indispensable sources of both preventive and curative traditional medicine preparations for human beings as well as livestock[1–3]. 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. Estimated floras of 6 500 to 7 000 species of higher plants are of medically important and out of these medicinal plants 12% are endemic to Ethiopia[4,5]. Despite their vital role in catering for the health of human and livestock population, large part of the knowledge of ethnomedicinal 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[6]. Environmental degradation, agricultural expansions, cultivation of marginal lands and urbanization are also posing a significant threat to the future wellbeing of human and animal populations that have relied on these resources to combat various ailments for generations[1,2,5] warranting urgent need to document and preserve the indigenous knowledge.

Many works that document the wealth of indigenous knowledge on the ethnobiology and ethnomedicine have been emerging[1,2,4,7–10]. However, very few information exists on the veterinary herbal medicines[10,11]. The available data indicate that plant remedies play a vital role in some of the resource-limited societies in the southern part of the country.

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In Ethiopia, animal diseases remain among the principal causes of poor livestock performance, leading to an ever increasing gap between the supply of, and the demand for, livestock products^[10]. Conventional veterinary services, despite its paramount role, have limited coverage in developing countries and development of antimicrobial resistance is another headache^[3, 12]. If at all, the usefulness of modern pharmacotherapy is still limited by the cost of treatment^[13].

Due to this reason livestock keepers particularly in rural areas frequently visit traditional healers to get solutions for their ill-health animals including clinical cases of skin, udder, teats and gastrointestinal tract infections. Developing a socially acceptable and effective remedy from inexpensive resources that can complement modern medicine would be an attractive option.

However, in most traditional healers the units of measurements to determine dosage are not standardized and there are variations in the unit of measurement, duration and time at which remedies are taken and prescribed by healers for the same kind of health problems. The precision, standardization and their toxic effect were not studied in the country which is as one drawback for the traditional health care system. To the best of our knowledge, *in vitro* antimicrobial trial on ethnomedicinal plants in Tigray region is rare.

In the present study, nine species of plants, *Achyranthes aspera* (*A. aspera*) L. (Family Asparagaceae), *Ficus caria* (*F. caria*) (Family Moraceae), *Malva parviflora* (*M. parviflora*) (Family Malvaceae), *Vernonia* species (local name Alakit, Family Asteraceae), *Solanum hastifolium* (*S. hastifolium*) (Family Solanaceae), *Calpurinia aurea* (*C. aurea*) (Ait) Benth (Family Fabaceae), *Nicotiana tabacum* (*N. tabacum*) L. (Family Solanaceae), *Ziziphus spina-christi* (*Z. spina-christi*) (Family Rhamnaceae), *Croton macrostachys* (*C. macrostachys*) (Family Euphorbiaceae), having traditional claims for the treatment of mastitis, various wounds and gastrointestinal complications were investigated for their antimicrobial activities on Gram-positive and Gram-negative bacterial species. Prior to the *in-vitro* antimicrobial trial, inventory of ethnoveterinary data on the use of these plants was obtained directly from farmers and traditional healers.

2. Materials and methods

2.1. Study area

The study was conducted from October 2007 to April 2009 in selected sites of Tigray Regional State, Northern Ethiopia. These were Adigrat, Hawzien, Erob and Mekelle representing similar agro-climatic zone (high land to medium altitudes) and Kola Tembien and Tanqua Aberegele from low altitude.

Mekelle is the capital city of the region situated about 783 kms north of Addis Ababa at 38.5° East longitude and 13.5° North latitude at an altitude of 2748 meters above sea level (m.a.s.l.). Adigrat is situated 115 kms North of Mekelle at 14° North latitude and 39.5° East longitude and its elevation is 2 462 m.a.s.l. Hawzien is situated at 113 kms North West of Mekelle at 13.9° North latitude and 39.4° East longitude at an altitude of 2 258 m.a.s.l. Yechila (Tanqua Aberegele) is located 110 kms south west of Mekelle at 13.3° North latitude and 39° East longitude at an altitude of 1590 m.a.s.l. Abi-Adi (Kola Tembien) is located 13° 33' 39" North latitude and 38° 58' 26" East longitude.

2.2. Field survey and preparation of plant material

A survey was conducted to gather information on the traditional usage of plants in the health care system of livestock. Information of medicinal plants was obtained through direct interview with livestock keepers, local and religious healers. The interview was done based on a standard feedback questionnaire intended to address details on the types and characteristics of plant and their traditional preparation, method of application and other plants or substance used together. More than 245 individuals were involved in the interview. Specimens of plants that were mentioned by the traditional herbalists and farmers for treatment of livestock ailments were collected. Fifty nine plant species belonging to different families were gathered and documented in such a way that they should include the vegetative parts, leaves, floral, fruiting and/or the seed parts as it was appropriate for taxonomic identification.

The specimens were coded by their vernacular names and were transported by plastic bag to avoid drying. After collection and drying, voucher specimens were identified by botany specialists in Mekelle University and/or sent to science faculty of Addis Ababa University National Herbarium for botanical identification.

Based on the resources available, nine plant species which were most widely used in the study areas for treatment of bovine mastitis, skin diseases and gastrointestinal complications were selected for *in-vitro* antimicrobial screening. Plant species recognized as ethnoveterinary medicinal plants, their uses and properties is summarized in Table 1. Plant leaves from their natural habitat were collected and washed with tap water to remove unnecessary particles and shade dried at room temperature for about a month. Shade dried plant leaves were then separately grounded using a commercial blender at the pharmacognosy laboratory of school of pharmacy, Mekelle University.

Each plant was weighed using precision standard electronic balance before maceration. Aqueous methanol of 70% concentration was used as the solvent of extraction of the plant material.

2.3. Preparations of crude extracts

Preparation of crude extracts was conducted according to the method described previously^[4] with little modification. Maceration was carried out in beakers containing 70% methanol enough to cover all the plant powders with a continuous shaking with an orbital shaker at 100 rpm and an occasional stirring with a glass rod manually at 4 hours interval. After 72 hours the macerates of each plant leaves were filtered in separate flasks using a qualitative filter paper (Whatmann No 4 filter paper, Whatman Ltd., England). The marc (residue) of each plant was resoaked with a fresh 70% methanol, as mentioned before for about 48 hours. The procedure was repeated in a similar way for the third time for 24 hours. The residues after the third filtration process were discarded while the filtrate part was taken to a vacuum rotary evaporator machine.

The product was poured into evaporating dishes and was kept in dry oven at 40 °C for 3–4 days until the semi–solid material became completely solid and dry. Finally, the percentage yields of each plant extract were calculated. Scientific names of the plants used, amount, and yield obtained from maceration is summarized in Table 2. The resulting concentrated extracts of each plant material was 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.

2.4. Preparation of test bacteria

Clinical isolates of five bacteria species of equine in origin were obtained from National Veterinary Institution (NVI), Debre–zeit, Ethiopia. The bacteria were three Gram positive (*Staphylococcus aureus* (*S. aureus*), *Staphylococcus intermedius* (*S. intermedius*) and *Staphylococcus hycus* (*S. hycus*)) and two Gram negative (*Escherichia coli* (*E. coli*) and *Klebsiella pneumoniae* (*K. pneumoniae*)) originated from saddle sore. Three clinical bacterial isolates, *S. aureus*, *S. agalactiae* and *S. dysgalactiae*, were isolated from dairy cows/milk of Agazi dairy farm, Mekelle. One clinical bacterial isolate, *Dermatophilus congolensis* (*D. congolensis*), was from bovine skin scrapping diagnosed at Mekelle Veterinary clinic. For bacterial culture, identification and isolation standard microbiological techniques were followed [14].

2.5. Antimicrobial sensitivity tests

The antibacterial activity was tested using disc diffusion test and broth dilution methods.

2.5.1. Disc diffusion test

The disc diffusion method followed by the National Committee for Clinical Laboratory Standards^[15] protocol was used to evaluate antimicrobial activities. For susceptibility testing, two concentrations (200 mg/mL and 100 mg/mL crude extracts) were prepared in dimethyl sulfoxide (DMSO). Sterile antibiotic assay discs (Whatman, 6 mm) were impregnated with 100 µL of the reconstituted extract and were dried completely at 37 °C overnight. Each disc was gently pressed down to ensure complete contact with the agar inoculated with bacteria that was adjusted to the 0.5 McFarland standard equivalents to 10⁷ CFU/mL in a Muller Hinton molten agar. Extracts were tested in triplicate. DMSO saturated assay discs were used as negative control. Standard antibiotic disc, gentamycin (20 µg per disc), was used as positive control. The plates were then incubated at 37 °C for 18–24 h. Inhibition zones were recorded as the diameter of growth–free zones^[16].

2.5.2. Determination of minimum inhibitory concentration (MIC)

Broth dilution method was used to determine minimum inhibitory concentration (MIC). One mL of 24 h culture of test organisms (10⁷ CFU/mL) adjusted to McFarland turbidity standard were incubated in serial dilution ranging from 6.25 to 200 mg/mL of plant extracts in DMSO at 37 °C for 24 h. The concentration of the lowest dilution with no detectable bacterial growth was considered as MIC. Absence of growth was confirmed by absence of turbidity and by inoculating into agar^[17].

2.6. Data analysis

Data on zone of inhibition produced by each plant extract and MIC on various bacteria were stored in excel spreadsheet. Mean inhibitory zone of inhibition was determined using the statistical software SPSS v 15, 2002. Each experiment was done in triplicates.

3. Results

The results of antimicrobial screening of the extracts of test plants are shown in Table 3 and Table 4. Out of the nine plants tested, eight showed antibacterial activity by inhibiting one or more microorganisms. Among these plants *C. aurea*, *C. macrostachyus*, *N. tabacum*, *A. aspera* and *Vernonia* species (local name Alakit) showed promising activity against some of the common microorganisms of veterinary importance. There was a dose dependent inhibition on the tested micro–organisms. The antibacterial activity of these plants at a concentration of 100 mg/mL was comparable with that of the standard antibiotic (gentamycin 20 µg per disc). Results of the minimum inhibitory

Table 1

Plant species recognized as ethnoveterinary medicinal plants, their uses and properties plants collected for antimicrobial screening from selected sites of Tigray region, Northern Ethiopia.

Botanical name, Voucher and Family	Local name	Collection site	Mode of preparation, parts used and ethnoveterinary–medicinal uses
<i>A. aspera</i> (Asparagaceae) CVM25	Mechalo/Melhas ba'eray	Tanqua Abergelle, Quola Temben	The fresh root of an <i>A. aspera</i> is chopped and bounded together with a leaf of commicarpus podunculosus. This will be mixed with water and given orally for treatment of bovine mastitis. The leaves are also used as a remedy of wound
<i>C. macrostachyus</i> (Euphorbiaceae) CVM08	Aslamay	Freweyni	The leaf of this plant is chopped, boiled and the cooled decoction is used to wash the affected udder. The leaf is also used to treat dermatophilosis
<i>C. aurea</i> (Ait) Benth (Fabaceae) CVM43	Hitsawts	Yechila	The fresh leaves of <i>C. subdecandra</i> is grounded with small amount of water then applied to the skin affected by skin diseases and given for treatment of mastitis. It is also used to avoid lice and ticks.
<i>F. caria</i> L. (Moraceae) CVM59	Beles adgi	Adigrat, Erob	The fresh leaf of this plant is pounded with small water and applied topically to the udder as a treatment of mastitis.
<i>N. tabacum</i> L (Solanaceae) CVM57	Tambuk	Yechila	The leaf is grounded with small water and the juice is applied to udder for mastitis treatment, skin diseases and ectoparasites. Juices from the leaf of the plant is also infused through nostrils against leech infestation
<i>Z. spina-christi</i> (Rhamnaceae) CVM20	Gaba	Mekelle	The leaves are grounded and applied on the affected teat quarter for treatment of mastitis. Thickly grounded leaves are also applied in saddle sore in equines
<i>M. parviflora</i> L (Malvaceae) CVM30	Enkuftha	Quola temben	Squashes of fresh leaves are applied topically in the treatment of saddle sore in equine and camels
<i>S. hastifolium</i> Dunal. (Solanaceae) CVM03	Hintut	Mekelle	Juice from freshly grounded leaves is applied on fresh wounds
<i>Vernonia</i> species (Asteraceae) CVM32	Alakit	Dohan, Erob	Fresh leaves are grounded with small amount of water and the juice is applied topically in the treatment of eye infection and chronic open wounds. Freshly grounded leaves are also bandaged in cases of bone fracture.

Table 2

Scientific names, plant parts and amount used, consistency of extract and percentage yields of 70% methanolic extracted of test herbs.

Plant species and voucher	Plant part extracted	Amount	macerated (g)	Yield (g)	Yield (%)	Consistency of crude extract
<i>A. aspera</i>	Leaf	100	100	24.856	24.86	Soft
<i>F. caria</i>	Leaf	100	100	19.725	19.73	Solid and sticky
<i>M. parviflora</i>	Leaf	95	95	18.710	19.69	Solid and sticky
<i>V. species</i>	Leaf	400	400	115.896	28.97	Semi–Solid, greasy/sticky
<i>S. hastifolium</i>	Leaf	125	125	22.240	17.79	Solid and resinous
<i>C. aurea</i>	Leaf	100	100	16.100	16.10	solid
<i>N. tabacum</i>	Leaf	150	150	23.000	15.30	Solid and soft
<i>Z. spina-christi</i>	Leaf/Stem	160	160	27.600	17.30	solid
<i>C. macrostachyus</i>	Leaf	120	120	18.800	15.70	Solid and soft

Table 3

Antibacterial activity of hydro–alcoholic (70 % methanol) extracts of medicinal plants using disc diffusion method against skin wound and mastitis bacteria isolates.

Plants used	Concentration (mg/mL)	Mean zone of inhibition (mm) (\pm SE)			
		Sa	Sta	Std	Dc
<i>C. aurea</i>	200	17.7 (1.20)	17.0 (0.56)	18.0 (1.10)	17.8 (0.30)
	100	17.7 (0.76)	14.8 (0.73)	15.3 (0.33)	16.0 (0.47)
<i>C. macrostachyus</i>	200	22.0 (0.90)	19.0 (0.70)	19.7 (1.20)	20.3 (1.00)
	100	19.3 (1.95)	18.0 (1.81)	18.5 (0.52)	19.0 (0.58)
<i>N. tabacum</i>	200	20.0 (0.40)	19.0 (0.70)	19.8 (0.90)	19.1 (2.30)
	100	17.1 (0.89)	18.0 (1.91)	17.1 (0.62)	17.0 (0.48)
<i>Z. spina-christi</i>	200	16.3 (0.80)	14.6 (0.50)	16.0 (0.20)	–
	100	8.0 (3.21)	4.0 (1.82)	4.0 (0.33)	–
Gentamycin (Positive control)	20 μ g per disc	20.2 (0.42)	18.4 (0.21)	22.0 (0.20)	16.0 (0.14)
DMSO (Negative control)	–	–	–	–	–

Sa: *S. aureus*, Sta: *S. agalactiae*, Std: *S. dysgalactiae*, Dc: *D. congolensis*.

Table 4

Antibacterial activity of hydro–alcoholic (70 % methanol) extracts of medicinal plants using agar well diffusion method against gastrointestinal complication bacteria isolates.

Plant used	Concentration (mg/mL)	Mean, zone of inhibition (mm) (\pm SE)				
		Sa	Si	Shy	Ec	Kp
<i>A. aspera</i>	200	17.80 (1.30)	18.20 (2.40)	18.20 (1.90)	18.40 (3.30)	19.20 (2.54)
	100	16.10 (0.37)	16.70 (0.22)	16.00 (0.24)	16.70 (1.71)	8.00 (2.65)
<i>S. hastifolium</i>	200	–	–	–	–	–
	100	–	–	–	–	–
<i>F. caria</i>	200	6.46 (0.22)	10.20 (0.41)	–	11.90 (0.33)	9.50 (1.42)
	100	–	6.30 (1.98)	–	8.51 (2.10)	6.80 (1.82)
<i>M. parviflora</i>	200	9.70 (1.10)	8.60 (0.96)	–	10.25 (2.20)	10.00 (0.80)
	100	6.40 (1.31)	7.40 (1.36)	–	7.10 (1.41)	7.40 (1.26)
Vernonia species–Alakit	200	22.00 (0.13)	16.40 (0.21)	17.10 (0.57)	16.40 (0.90)	–
	100	16.00 (1.76)	14.30 (1.68)	18.70 (1.73)	16.30 (1.90)	–
Gentamycin		18.10 (2.52)	17.00 (2.39)	16.80 (2.61)	20.20 (1.83)	14.50 (3.11)
DMSO		–	–	–	–	–

Sa: *S. aureus*, Si: *S. intermedius*, Shy: *S. hycus*, Ec: *Escherichia coli*, Kp: *K. pneumoniae*.**Table 5**

Minimum inhibitory concentration (MIC) of plant extracts against skin wound and mastitis causing bacterial isolates (mg/mL).

Test organism	Plant species	Extract concentrations (mg/mL)					
		6.25	12.5	25	50	100	200
<i>S. aureus</i>	<i>C. aurea</i>	–	–	+	+	+	+
	<i>C. macrostachyus</i>	–	+	+	+	+	+
	<i>N. tabacum</i>	–	–	+	+	+	+
<i>S. agalactiae</i>	<i>C. macrostachyus</i>	–	–	+	+	+	+
	<i>N. tabacum</i>	–	–	–	+	+	+
	<i>C. aurea</i>	–	–	–	–	–	+
<i>S. dysgalactiae</i>	<i>C. macrostachyus</i>	–	–	+	+	+	+
	<i>N. tabacum</i>	–	–	+	+	+	+
	<i>C. aurea</i>	–	–	–	–	+	+
<i>D. congolensis</i>	<i>C. macrostachyus</i>	–	–	+	+	+	+
	<i>N. tabacum</i>	–	–	–	+	+	+
	<i>C. aurea</i>	–	–	–	–	+	+

Table 6

Minimum inhibitory concentration (MIC) of plant extracts against gastrointestinal complication bacteria isolates (mg/mL).

Test organism	Plant species	Extract concentrations (mg/mL)					
		6.25	12.5	25	50	100	200
<i>K. pneumoniae</i>	Vernonia species	–	–	–	–	–	–
	<i>M. parviflora</i>	–	–	–	–	+	+
	<i>A. aspera</i>	–	–	–	+	+	+
	<i>S. hastifolium</i>	–	–	–	–	–	–
	<i>F. caria</i>	–	–	–	+	+	+
<i>S. intermedius</i>	Vernonia species	–	–	–	+	+	+
	<i>M. parviflora</i>	–	–	–	+	+	+
	<i>A. aspera</i>	–	–	–	+	+	+
	<i>S. hastifolium</i>	–	–	–	–	–	–
	<i>F. caria</i>	–	–	–	+	+	+
<i>S. hycus</i>	Vernonia species	–	+	+	+	+	+
	<i>M. parviflora</i>	–	–	–	–	–	–
	<i>A. aspera</i>	+	+	+	+	+	+
	<i>S. hastifolium</i>	–	–	–	–	–	–
	<i>F. caria</i>	–	–	–	–	–	–
<i>E. coli</i>	Vernonia species	–	–	–	+	+	+
	<i>M. parviflora</i>	–	–	–	–	+	+
	<i>A. aspera</i>	+	+	+	+	+	+
	<i>S. hastifolium</i>	–	–	–	–	–	–
	<i>F. caria</i>	–	–	–	+	+	+
<i>S. aureus</i>	Vernonia species	–	–	–	+	+	+
	<i>M. parviflora</i>	–	–	–	–	+	+
	<i>A. aspera</i>	+	+	+	+	+	+
	<i>S. hastifolium</i>	–	–	–	–	–	–
	<i>F. caria</i>	–	–	–	+	+	+

concentration (MIC) of plant extracts against the test bacteria are also shown in Table 5 and Table 6.

4. Discussion

The study was conducted with the objective of assessing traditionally used medicinal plants in livestock and evaluating the antibacterial activity of selected herbs on bacterial isolates from mastitic milk, wound and gastrointestinal complication. Nine herb leave extracts namely *A. aspera* (Mechalo or Melhas Ba'eray), *M. parviflora* (Enkufftha), *F. caria* (Adgi beles), *S. hastifolium* (Hintut), *Vernonia* species (Alakit), *C. macrostachyus* (Tambuk), *C. aurea* (Ait) Benth (Hatsawtse), *N. tabacum* L (Tambuk) and *Z. spina-christi* (Gaba) were screened for their antibacterial activities.

The result of the antibacterial activity for *C. aurea* is in agreement with previous reports in human pathogens [3,13]. In this *in-vitro* study against clinical bacterial isolates of mastitis and wound, it showed strong antibacterial activity. The MIC values indicate that the extract is potent against the test bacteria. The lowest value observed was 25 mg/mL on *S. aureus* while its highest MIC value was 100 mg/mL for *St. dysgalactiae* and *D. congolense* (Table 5). The plant's use is reported for the treatment of dysentery in livestock, Dermatophytes, control of ticks, wound and swelling [4,11,18].

The extracts of *C. macrostachyus* exhibited the highest growth inhibition zone against the Gram-positive bacteria, *S. aureus*, with MIC of 12.5 mg/mL. Available evidence from indigenous practices in different areas is in agreement with the present *in-vitro* study. The plant has been documented as a remedy for a wide range of human and livestock diseases, such as scabies and diarrhea[2] in Ethiopia, leaf infusions as anthelmintic and laxative[18] in Tanzania, as a remedy for major bacteria like *E. coli*, *B. cereus*, *Micrococcus lutea* and *P. auruginosa*[19] in Kenya, leaf for bloat and ringworm treatment in cattle[10] in Ethiopia.

Nicotiana tabacum extracts also showed an impressive antibacterial activity against all Gram-positive bacteria. Traditionally, juices of the leaves are topically applied in the treatment of mastitis and various skin diseases, such as dermatophilosis. Moreover, nostril-infusion of leave juices is used for leech infestation in cattle. Leaves of *N. tabacum* have also been used against ectoparasites and Foot-and Mouth Disease (FMD)[20]. The antibacterial activity of *Z. spina-christi* was relatively low at a concentration of 100 mg/mL. It has been reported to possess antihelminthic[21] and anti-dandruff properties[22].

Achyranthes aspera showed the most potent inhibitory effect against *S. aureus*, *S. hycus* and *E. coli* at both its graded concentrations with MIC of 6.25 mg/mL (Table 6). It is used to treat various ailments including skin eruptions and colic[23]. Its use has been widespread across different societies

of the world owing, partly, to its efficacy. The chemical nature of the leaf of *A. aspera* was determined using high pressure liquid chromatography (HPLC) using methanol. Phytochemical analysis has shown that the leaf contains alkaloids and saponins and it was demonstrated that the leaf of *A. aspera* had better inhibition zone against *S. aureus* and *E. coli* [24].

Solanum hastifolium's crude extract did not show any activity against all bacterial species used in this study. It also needs to be emphasized that *S. hastifolium* has never been evaluated for antibacterial activity elsewhere before. Failure to show antibacterial activity in this study might not, however, mean that the plant is devoid of active principles responsible for antibacterial medicinal use. It has been suggested that extraction technique and the choice of solvent could affect the extraction of active principle and, hence, affect the result[25–27]. Elsewhere, *S. hastifolium* has been used in the treatment of black quarter in cattle[28].

The traditional use of *Ficus caria*, for the treatment of diarrhea, wound, eye disease and its antibacterial and antifungal effect was previously reported [6]. In the present study, *F. caria* was totally ineffective against *S. hycus* but a MIC of 50 mg/mL was demonstrated against *E. coli*, *S. intermedius*, *K. pneumoniae*, and *S. aureus*. *Malva parviflora* was reported to possess anti-fungal protein[30] and antibacterial activity[4]. The antibacterial activity of the plant was relatively higher in Gram-positive than Gram-negative bacteria. An MIC of 50 mg/mL and 100 mg/mL was registered for Gram-positive and Gram-negative bacteria, respectively.

In conclusion, the present study was conducted by extracting of the crude substance of the herbal medicines and the result indicated that eight out of the nine test plants contain antibacterial activity. There was a dose dependent inhibition on the tested microorganisms suggesting towards the importance of in-depth study of these herbal medicinal plants and supports the knowledge of the herbalists. Moreover, a continuous and progressing researches need to be conducted to prove the biological ingredients and test the safety, efficiency and to determine the types of compounds responsible for the antibacterial effects of these medicinal plants.

Conflict of interest statement

We declare that we have no conflict of interest.

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References

- [1] Lulekal E, Kelbessa E, Bekele T, Yineger H. An ethnobotanical study of medicinal plants in Mana Angetu District, southeastern Ethiopia. *J Ethnobiol Ethnomed* 2008; **4**: 1–10.
- [2] Teklehaymanot T, Giday M. Ethno botanical study of medicinal plants used by people in *Zegie peninsula*, north western Ethiopia. *J EthnoBiol Ethnomed* 2007; **3**: 12.
- [3] Devi K, Karthikai GD, Thirumaran G, Arumugam R, Anantharaman P. Antibacterial activity of selected medicinal plants from Parangipettai coastal regions; Southeast coast of India. *World App Sci J* 2009; **7**(9): 1212–1215.
- [4] Tadeg H, Mohammed E, Asres K, Gebre–Mariam T. Antimicrobial activities of some selected traditional Ethiopian medicinal plants used in the treatment of skin disorders. *J Ethnopharmacol* 2005; **100**: 168–175.
- [5] Giday M, Asfaw Z, Woldu Z. Medicinal plants of the Meinit ethnic group of Ethiopia: An ethnobotanical study. *J Ethnopharmacol* 2009; **124**: 513–521.
- [6] Mesfin F, Demissew S, Teklehaymanot T. An ethnobotanical study of medicinal plants in Wona Woreda, SNNPR, Ethiopia. *J Ethnobiol Ethnomed* 2009; **5**: 28.
- [7] Taye B, Giday M, Animut A, Seid J. Antibacterial activities of selected medicinal plants in traditional treatment of human wounds in Ethiopia. *Asian Pac J Trop Biomed* 2011; 370–375. doi:10.1016/S2221–1691(11)60082–8
- [8] Wondimu T, Asfaw Z, Kelbessa E. Ethnobotanical study of medicinal plants around ‘Dheeraa’ town, Arsi Zone, Ethiopia. *J Ethnopharmacol* 2007; **112**: 152–161.
- [9] Weldegerima B, Abula T, Ragunathan M. Ethno–veterinary use of medicinal plants by traditional healers in Dabat district, Northwestern Ethiopia. *Pharm Mag* 2008; **4**: 93–99.
- [10] Sori T, Bekana M, Adunga G, Kelbesa E. Medicinal plants in ethnoveterinary practices of *Borana pastoralists*, Southern Ethiopia. *Int J Appl Res Vet Med* 2004; **2**: 220–225.
- [11] Yineger H, Kelbessa E, Bekele T, Lulekal E. Ethnoveterinary medicinal plants at Bale Mountains National Park, Ethiopia. *J Ethnopharmacol* 2007; **112**: 55–70.
- [12] Girish HV, S Satish. Antibacterial activity of important medicinal plants on human pathogenic bacteria—a comparative analysis. *World App Sci J* 2008; **5**(3): 267–271.
- [13] Sharma A, Verma R, Ramteke P. Antibacterial activity of some medicinal plants used by tribals against uti causing pathogens. *World App Sci J* 2009; **7**(3): 332–339.
- [14] Quinn PJ, Carter ME, Markey B, Carter GR. *Clinical veterinary microbiology*. London: Wolf Publishing; 1994.
- [15] National Committee for Clinical Laboratory Standards (NCCLS). *National Committee for Clinical Laboratory Standards*. Villanova: PA; 2000.
- [16] Bhattacharjee I, Chatterjee SK, Ghosh A, Chandra G. Antibacterial activities of some plant extracts used in Indian traditional folk medicine. *Asian Pac J Trop Biomed* 2011; S165–S169
- [17] Paul C, Arnold JV, Dirk VB, Louis M. Anti–infective potential of natural products: How to develop a stronger *in vitro* ‘proof–of–concept: Review. *J Ethnopharmacol* 2006; **106**: 290–302.
- [18] Adedapo AA, Jimoh FO, Koduru S, Afolayan AJ, Masika PJ. Antibacterial and antioxidant properties of the methanol extracts of the leaves and stems of *Calpurnia aurea*. *BMC Complement Altern Med* 2008; **8**: 53.
- [19] Wagate CG, Mbaria JM, Gakuya DW, Nanyingi MO, Kareru PG, Njuguna A, et al. Screening of some Kenyan medicinal plants for antibacterial activity. *Phytother Res* 2010; **24**(1): 150–153.
- [20] Nfi AN, Mbanya JN, Ndi C, Kameni A, Vabi M, Pingpoh D, et al. Ethno veterinary medicine in the northern provinces of Cameroon. *Vet Res Commun* 2001; **25**: 71–76.
- [21] Waterman C, Smith RA, Pontiggia L, DerMarderosian A. Anthelmintic screening of Sub–Saharan African plants used in traditional medicine. *J Ethnopharmacol* 2010; **127**(3): 755–759.
- [22] Yirga G. Assessment of traditional medicinal plants in Endrta District, South–eastern Tigray, Northern Ethiopia. *Afr J Plant Sci* 2010; **4**(7): 255–260.
- [23] Goyal BR, Goyal RK, Metha AA. Phyto–pharmacology of *Achyranthes aspera*: A review. *Plant Rev* 2007; **1**: 143–150.
- [24] Thilagavathi G, Kannaian T. Application of prickly chaff (*Achyranthes aspera* Linn.) leaves as herbal antimicrobial finish for cotton fabrics used in health care textile. *Nat Prod Radianc* 2008; **7**(4): 330–334.
- [25] Olila D, Olwa–Odyek, Opuda–Asibo J. Antibacterial and antifungal activities of extracts of *Zanthoxylum chalybeum* and *Warburgia ugandensis*, Ugandan medicinal plants. *Afr Hlth Sci* 2001; **1**(2): 66–72.
- [26] Kamazeri SA, Samah OA, Taher M, Susanti D, Qaralleh H. Antimicrobial activity and essential oils of *Curcuma aeruginosa*, *Curcuma mangga*, and *Zingiber cassumunar* from Malaysia. *Asian Pac J Trop Med* 2012; **5**(3):202–209.
- [27] Ponnusamy P, Natarajan V, Sevanan M. *In vitro* biofilm formation by uropathogenic *Escherichia coli* and their antimicrobial susceptibility pattern. *Asian Pac J Trop Med* 2012; **5**(3): 210–213
- [28] Ole–Miaron JO. The Maasai ethno diagnostic skill of livestock diseases: a lead to traditional bioprospecting. *J Ethnopharmacol* 2003; **84**: 79–83.
- [29] Taher M, Susanti D, Rezali MF, Zohri FSA, Ichwan SJA, Alkhamaiseh SI, Apoptosis FA. Antimicrobial and antioxidant activities of phytochemicals from *Garcinia malaccensis* Hk.f *Asian Pac J Trop Med* 2012; **5**(2): 136–141
- [30] Wang X, Bunker GJ. Potent heterologous antifungal proteins from cheeseweed (*Malva parviflora*). *Biochem Biophys Res Commun* 2000; **279**: 669–673.