Selected ethno-medicinal plants from Kenya with in vitro activity against major African livestock pathogens belonging to the “Mycoplasma mycoides cluster”

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A B S T R A C T
Ethnopharmacological relevance: Members of ‘Mycoplasma mycoides cluster’ are important ruminant pathogens in Africa. Diseases caused by these Mycoplasma negatively affect the agricultural sector especially in developing countries through losses in livestock productivity, mortality and international trade restrictions. There is therefore urgent need to develop antimicrobials from alternative sources such as medicinal plants to curb these diseases. In Kenya, smallholder farmers belonging to the Maasai, Kuria and Luo rely on traditional Kenyan herbals to treat respiratory symptoms in ruminants. In the current study extracts from some of these plants were tested against the growth of members of Mycoplasma mycoides cluster.

Aim: This study aimed at identifying plants that exhibit antimycoplasmal activities using an ethnobotanical approach.

Materials and methods: Kenyan farmers of Maasai, Luo and Kuria ethnic groups were interviewed for plant remedies given to livestock with respiratory syndromes. The plant materials were thereafter collected and crude extracts prepared using a mixture of 50% of methanol (MeOH) in dichloromethane (CH2Cl2), neat methanol (MeOH), ethanol (EtOH) and water to yield four crude extracts per plant part.

The extracts were tested in vitro against five strains of Mycoplasma mycoides subsp. capri, five strains of Mycoplasma mycoides subsp. mycoides and one strain of Mycoplasma capricolum subsp. capricolum using broth micro-dilution assays with an initial concentration of 1 mg/ml. Minimum inhibitory concentration (MIC) of the most active extracts were determined by serial dilution.

Results: Extracts from five plants namely: Solanum aculeastrum, Albizia coriaria, Ekebergia capensis, Piliostigma thonningii and Euclea divinorum exhibited the highest activities against the Mycoplasma strains tested. Mycoplasma mycoides subsp. mycoides were more susceptible to these extracts than Mycoplasma mycoides subsp. capri and Mycoplasma capricolum subsp. capricolum. The activities of the crude extracts varied with the solvent used for extraction. The MICs mean values of the active extracts varied from 0.02 to 0.6 mg/ml.

Conclusions: The results suggested that these plants could potentially contain antimicrobial compounds that might be useful for the treatment of respiratory diseases in ruminants. Future work should focus on the isolation and identification of the active compounds from the plant extracts that showed interesting activities and evaluation of their antimicrobial and cytotoxic potential.

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1. Introduction

Bacteria of the genus Mycoplasma are cell wall-less bacteria and are severe pathogens to humans and animals worldwide. The so-called ‘Mycoplasma mycoides cluster’ comprises five ruminant pathogens including: Mycoplasma mycoides subsp. mycoides, Mycoplasma mycoides subsp. capri, Mycoplasma capricolum subsp.
capri pneuniae, Mycoplasma capricolum subsp. capricolum and Mycoplasma leachii (Fischer et al., 2012). Mycoplasma mycoides subsp. mycoides and M. capricolum subsp. capricolum are of highest economic importance as they are known to cause severe respiratory diseases namely: contagious bovine pleuropneumonia (CBPP) and contagious caprine pleuropneumonia (CCPP), respectively. These diseases substantially affect the agricultural sector especially in developing countries through losses in livestock productivity, mortality and international trade restrictions. They are trans-boundary diseases and constitute a threat to disease-free countries (Tambi et al., 2006). Better measures are needed for the progressive control of diseases like CBPP in sub-Saharan Africa (Jores et al., 2013).

The Mycoplasma are resistant to many antimicrobials as they lack a cell wall. Antimicrobials widely used to treat infections with Mycoplasma include tetracyclines, macrolide-lincosamide-streptogramin-ketolide antibiotic group and fluoroquinolones (Gruzon et al., 2005; Renaudin, 2005). Lately, very high resistance levels against macrolides have been reported for the human pathogen Mycoplasma pneumonia in China (Cao et al., 2010). This demonstrates the build-up of acquired resistance within the genus Mycoplasma within a relatively short time (Morozumi et al., 2008; Xin et al., 2009). Increasing resistance against antimicrobials calls for the development of new antimicrobial substances. An approach for the development of novel antimicrobials is the use of ethno-botanical information in order to characterize plant substances for their antimicrobial activity. Traditional medicine based on medicinal plants in Africa is not only used in the primary health care system for people living mainly in rural areas in developing countries (Njoroge et al., 2010; Owuor et al., 2012), but also to manage a wide variety of livestock diseases (Githiori et al., 2005; Ole-Minran, 2003).

In Kenya, farmers such as those belonging to the Maasai, Luo and Kuria ethnic groups already rely on indigenous plants to treat livestock diseases. This is in part because antimicrobials are costly and not stocked or accessible in many regions of sub-Saharan Africa (Planta, 2007; Tsigemelak et al., 2016). Selected medicinal plants listed by these smallholder farmers belong to different families namely: Mimosaceae, Canellaceae, Oleaceae, Apocynaceae, Asteraceae, Ebenaceae, Fabaceae, Anacardiaceae, Rhamnaceae, Verbenaceae, Solanaceae, Clusiaceae, Cucurbitaceae, Lamancaceae, and Rutaceae. This study sought to identify plants that exhibit antimycoplasmal activities using an ethno-botanical approach.

Table 1
List of selected medicinal plants collected.

| Plant/Family/voucher number | Part used | Ethnic group | Local name |
|-----------------------------|-----------|--------------|------------|
| Acacia xanthophloeae/Mimosaceae/Fmk2012/01 | Stem bark | Maasai | Olerai |
| Albizia coriaria/Mimosaceae/Fmk2012/02 | Stem bark, roots and leaves | Luo, Kuria and Maasai | Ober, Lortalogo, Olerai |
| Warburgia ugandensis/Canellaceae/Fmk2012/03 | Stem bark | Maasai | Ol-sogonoi |
| Olea europaea subsp. africana/Oleaceae/Fmk2012/04 | Stem bark and root barks | Maasai | Ol-sogonoi |
| Ekebergia capensis/Comelaceae/Fmk2012/05 | Stem bark | Maasai | Ol-sogonoi |
| Carissa spinarum/Apocynaceae/Fmk2012/06 | Stem bark, roots and fruits | Luo, Kuria | Ochungua, Omyunyore |
| Tithonia diversifoila/Asteraceae/Fmk2012/07 | Stem bark | Luo, Kuria | Ochungua, Omyunyore |
| Euclia divinaria/Ebenaceae/Fmk2012/08 | Stem bark | Luo, Kuria, La, and Maasai | Ochungua, Omyunyore |
| Pilostigma thonningii/Fabaceae/Fmk2012/09 | Roots and fruits | Luo, Kuria, La, and Maasai | Ochungua, Omyunyore |
| Rhus vulgaris/Anacardiaceae/Fmk2012/10 | Roots, aerial part | Luo, Kuria | Ochungua, Omyunyore |
| Ziziphus abyssinica/Rhamnaceae/Fmk2012/11 | Leaves | Luo, Kuria | Ochungua, Omyunyore |
| Gutierrezia cordifolia/Asteraceae/Fmk2012/12 | Aerial part and/or the whole plants | Luo, Kuria, La, and Maasai | Ochungua, Omyunyore |
| Lantana trifolia/Verbenaceae/Fmk2012/13 | Fruits, roots | Maasai, Luo and Kuria | Oshigawa, Ochok, Ikibunora |
| Solanum aculeastrum/Solanaceae/Fmk2012/14 | Stem bark, roots | Luo, Kuria | Onjak |
| Tithonia diversifolia/Asteraceae/Fmk2012/15 | Roots | Luo, Kuria | Ombasa, Onombura |
| Tylolemus fassogadina/Fabaceae/Fmk2012/16 | Aerial parts | Luo | Ombora |
| Mormodona foetida/Cucurbitaceae/Fmk2012/17 | Aerial part | Luo, Kuria and Maasai | Nyabendra-kwach, Urumisia |
| Fuerstia africana/Lamiaceae/Fmk2012/18 | Stem bark, roots and leaves | Luo, Kuria and Maasai | Enduleles, Ochok, Iritototoro |
| Toddalia asiatica/Rutaceae/Fmk2012/19 | Fruits, roots and leaves | Luo, Kuria and Maasai | Enduleles, Ochok, Iritototoro |
| Solanum runcinatum/Solanaceae/Fmk2012/20 | Fruits, roots and leaves | Luo, Kuria and Maasai | Enduleles, Ochok, Iritototoro |

2. Materials and methods

2.1. Identification of medicinal plants used to treat respiratory live-stock diseases

An ethnobotanical approach (Cox PA1, 1994; Martin, 2000) was used, whereby farmers were interviewed on their ways of controlling respiratory diseases such as CBPP using medicinal plants. The questionnaire (Supplementary 1) and consent document (Supplementary 2) was translated into the local language of the farmers to be interviewed. Ethical clearance covering a period of one year (January 2014–January 2015) was obtained from the Ethical Review Committee at the Kenya Medical Research Institute, which is registered in Kenya (KEMRI/RES/7/3/1).

Livestock farmers belonging to the three ethnic groups namely Maasai, Luo and Kuria were interviewed in Nairobi, Kisierian, Narok, Kisumu, Migori and Kuria using a questionnaire. A total of twenty plants (Table 1) were identified and collected from different geographical regions (Fig. 1).

2.2. Plant preparation and extraction

Plant parts namely: leaves, roots, stems, stem bark or berries (about 1–2 kg per plant part) were sampled and immediately transported in open polythene bags from the collection site to the Department of Chemistry, School of Physical Sciences (SPS), University of Nairobi. A voucher number for each plant can be found at the School of Biological Sciences (SBS), University of Nairobi Herbarium. Plant parts were dried at room temperature without direct sunlight for three weeks and then ground into fine powder using a MM 20 grinder (Wiley laboratory mill, standard model No. 2, Arthur H. Thomas Company).

2.3. Extraction with selected organic solvents

The solvents used for the extraction process included MeOH, ethanol (EtOH) and a mixture of 50% MeOH in CH3Cl2. The plant material (200 g) was mixed with 500 ml of each of the above solvents in a glass beaker and soaked at room temperature for four hours. After decantation, the insoluble phase was discarded. The supernatant was transferred into a glass flask and concentrated to a volume of about 2 ml using a rotary evaporator (Buchi R114) at 35 °C. The mixture was then transferred into a 10 ml glass tube.
The glass flask was rinsed 2–3 times with approximately 1 ml of CH$_2$Cl$_2$ and the rinse was added to the 2 ml that were harvested before. The concentrated supernatant (about 4 ml) was covered with a perforated aluminium foil and stored to dryness at room temperature.

2.4. Extraction with water

Fresh plant material (200 g) was boiled for 15 min and cooled at room temperature. The mixture was transferred into a 50 ml flask and kept in a freezer at –20 °C for 3 days. After three days, the mixture was removed from the freezer and lyophilized using a freeze-drier (Christ Beta 336 Osterode/Harz) for a maximum period of four days. The dried crude extracts were removed from the freeze-dryer and kept aside at room temperature.

2.5. Preparation of crude extracts for antimycoplasmal activity tests

Crude extracts (100 mg) from the organic solvents were reconstituted into 1 ml of dimethyl sulfoxide (DMSO) while the same amount of aqueous extracts were reconstituted into 1 ml of water (100%) to make a stock solution of 100 mg/ml. The mixture of each extract was prepared by vortexing to ensure homogenization of the solution.

2.6. Bacterial strains and culture conditions

All laboratory manipulations with Mycoplasma were carried out under BSL2 conditions (Mwirigi et al., 2016). Mycoplasma mycoides subsp. capri, Mycoplasma mycoides subsp. mycoides and Mycoplasma capricolum subsp. capricolum (Tables 2 and 3) were cultured in Pleuropneumonia Like-Organism (PPLO) broth (Difco™ PPLO Broth) media prepared as follows: 21 g of PPLO was dissolved in 700 ml of distilled water and autoclaved for 15 min at 121 °C. The

Table 2
List of Mmc and Mcc strains used in this study.

| Strains designation | Species | Country of origin | Year of isolation | Host | Data Bank/Strain collection |
|---------------------|---------|-------------------|------------------|------|-----------------------------|
| Y- Goat             | Mmc     | USA               | 1979             | Goat | GenBank                     |
| G1313.94            | Mmc     | Germany           | 1994             | Sheep| MH                          |
| G1255-94            | Mmc     | Berlin            | 1994             | Sheep| MH                          |
| M-18                | Mmc     | Croatia           | 1988             | Goat | CS                          |
| 95010-C1            | Mmc     | France            | 1995             | Goat | JF                          |
| 6443-90             | Mcc     | France            | 1990             | Goat | JF                          |

Table 3
List of Mmm used in this study.

| Strains designation | Species | Country of origin | Year of isolation | Host | Data Bank/Strain collection |
|---------------------|---------|-------------------|------------------|------|-----------------------------|
| PG1                 | Mmm     | Africa            | 1931             | Cattle | JF                          |
| V5                  | Mmm     | Australia         | 1935             | Vaccine strain | JF                          |
| B237                | Mmm     | Kenya             | 1997             | Cattle | HW                          |
| Afade               | Mmm     | Cameroon          | 1968             | Cattle | JF                          |
| Gladysdale          | Mmm     | Australia         | 1953             | Cattle | JF                          |

*References about all these strains can be found in Fischer’s paper (Fischer et al., 2012).
mixture was cooled in a water bath to 55 °C and supplemented with phenol red (Carl Roth GmbH) to a final concentration of 3%, 200 ml horse serum (Sigma), 0.25% of glucose (Carl Roth GmbH), 0.15% of penicillin G (Carl Roth GmbH) and 0.25% of thallium acetate (Carl Roth GmbH). A forty-eight well plate was used to grow Mycoplasma strains where they were incubated at 37 °C for a minimum period of seven days. Growth of Mycoplasma cells was determined by color change from red to yellow (Stemke and Robertson, 1982), as a result of pH change due to the growth of Mycoplasma strains. Stock cultures of both Mmc, Mmm and Mcc liquid cultures were grown to a density of approximately 1–3 × 10⁶ cells per ml as measured by the colony forming units method (Stemke and Robertson, 1982) and cryopreserved at −80 °C.

2.7. Antimicrobial susceptibility testing

The broth microdilution method as described by Arjoon et al. (2012) was used to characterize the in vitro antimycoplasmal activity of plant extracts and to determine the minimum inhibitory concentrations employing serial dilutions of the plant extracts. Briefly, a stock solution of 100 mg/ml was prepared and 10 µ (1 mg) of the same was used as the initial concentration mixed with 1 ml of cell culture (1 mg/ml) for the antibacterial test. A control sample consisting of only the solvent included. After seven days of incubation, the cultures were checked for color change from red to yellow, which is an indication of Mycoplasma growth resulting from the change of pH. No color change meant that the crude extracts in the well prevented growth of Mycoplasma. Data were analysed using Graphprism software version 6.0.

2.8. Determination of the minimum inhibitory concentration (MIC)

Active extracts were serially diluted in order to determine the minimum inhibitory concentration (MIC), which was defined as the lowest concentration that inhibited the growth of different Mycoplasma strains for an incubation time of seven days to ensure complete inhibition. The MICs of all the active extracts were determined by broth microdilution as previously described by Arjoon et al. (2012) starting from 1 mg/ml to 0.000005 mg/ml. The experiment was repeated thrice for each assay and mean was recorded. The standard error of mean was obtained at P < 0.05.

3. Results

3.1. Interview

The results of the interview with 28 farmers from different parts of Kenya (Fig. 1) showed that most of the farmers rely on medicinal plants to treat ruminant respiratory diseases symptoms such as deep dry cough, extended neck, fever and weight loss. As a result of the interview 20 different plant species used as remedies were identified. The total number of farmers interviewed and that of plants collected are presented in Table 4.

Whilst the interview was based on remedies against livestock respiratory symptoms (Table 5), the farmers’ responses included human diseases treated with these plants (See supplementary table 3).

The current studies show that the most commonly used plants included; Carissa spinarum, Lantana trifolia and Solanum aculeastrum. These plants were administered orally in the form of concoctions, mostly in combination with other plants. The infusion of the roots, stem bark and fruits, of one of the active plants, Carissa spinarum were administered orally to the livestock, in combination with decoction of other plants such as Albizia coriaria (Johns et al., 1990; Owuor et al., 2012). The juice from the berries of Solanum aculeastrum, which showed interesting antimicrobial activities, was usually instilled into the cattle, goat or sheep’s nostrils (Owuor et al., 2012).

3.2. Antimicrobial susceptibility testing

The organic and aqueous extractions from the plant parts yielded a total of one hundred and fifty two extracts (Supplementary 4).

The antimicrobial activity of 152 extracts from the twenty plants (Supplementary 5) selected on the basis of their medicinal uses among the Maasai, Luo and Kuria farmers were evaluated against the growth of five Mmc, five Mmm and one Mcc strains. The results of the antimycoplasmal activity of the five most active plants are represented in Table 6.

The antimycoplasmal test of twenty ethno-medicinal plants used by these communities showed that only five (5) plants namely: Solanum aculeastrum, Albizia coriaria, Ekebergia capensis, Pilostigma thomningii and Euclsea divinorum inhibited the growth of all the strains tested in the current study. The activities of these extracts differed significantly depending on the plant part and the solvent used for the extraction process. The aqueous extracts from the stem of Solanum aculeastrum exhibited the highest antimicrobial activities with MIC values of 0.02 mg/ml at p ≤ 0.05. In general, Mmm strains showed that they were more susceptible to the active extracts from five active plants compared to Mmc and Mcc strains.

Furthermore, it was observed that all organic extracts ca 50% MeOH in CH₂Cl₂, neat MeOH and EtOH from the berries of Solanum aculeastrum inhibited all the Mmc, Mcc and Mmm strains used while the water extracts from the berries and the stem selectively showed inhibitory effect only on Mmm strains.

Similarly, all organic extracts from the stem bark of Albizia coriaria showed inhibitory effects against the growth of all Mycoplasma strains used in this study while the polar aqueous extracts from the stem bark and leaves inhibited some Mmm strains (Table 6).

The extract of the stem bark Ekebergia capensis obtained using 50% MeOH in CH₂Cl₂ and 100% MeOH were active against the growth of almost all the strains of Mycoplasma tested, while those from the stem bark of Pilostigma thomningii obtained using MeOH in CH₂Cl₂ and 100% MeOH inhibited the growth of all the strains.

It was clear that the EtOH extracts of the active plants except for the stem bark of Euclsea divinorum and A. coriaria and from the berries of Solanum aculeastrum were inactive against all Mmm, Mmc, and Mcc strains used in this study. It is likely that the in-vitro activity of the EtOH extracts of most plants was attributed to the nature of the target compounds expected to be polar.

Six plants namely: Acacia xanthophloea, Warbugia ugandensis, Olea europeae, Rhus vulgaris, Solanum incanum and Tylosema fassogloses showed moderate antimycoplasmal activity that differed from one strain to another. The extracts from these plants inhibited almost half of all the Mmm, Mmc and Mcc strains with the exception of extracts from Tylosema fassogloses and Acacia xanthophloea, which were active against all Mmm, tested in this study.

The plants that had minimal to no activities including; Guterbergia cordifolia, Carissa spinarum, Zizyphus abyssinica, Garcinia buchananii, Lantana trifolia, Tithonia diversifolia, Toddalia asiatica, Fuerstia africana and Momordica foetida were excluded from further studies.

3.3. Minimum inhibitory concentration

The minimum inhibitory concentration (MICs) regarded as the lowest concentration of the active extract that inhibited the growth of Mycoplasma strains after 7 days of incubation were
Table 4
Results of the Interview.

| Interviewee Ethnic group | Collection site | Acacia xenthophloeae | Albizia coriaria | Carissa spinarum | Ekebergia capensis | Euclea divinorum | Fuerstia africana | Garcinia buchananii | Guternbergia cordifolia | Lantana trifolia |
|--------------------------|-----------------|----------------------|------------------|------------------|-------------------|-----------------|-------------------|----------------------|-----------------------|------------------|
| I                        | M 1             | X                    |                  |                  |                   |                 |                   |                      |                      |                  |
| II                       | M 2             |                      |                  |                  |                   |                 |                   |                      |                      |                  |
| III                      | M 3             | X                    |                  |                  |                   |                 |                   |                      |                      |                  |
| IV                       | M 4             |                      |                  |                  |                   |                 |                   |                      |                      |                  |
| V                        | M 5             |                      |                  |                  |                   |                 |                   |                      |                      |                  |
| VI                       | M 5             |                      |                  |                  |                   |                 |                   |                      |                      |                  |
| VII                      | K 6             | X                    |                  |                  | X                 |                 | X                 |                      |                      |                  |
| VIII                     | K 7             | X                    | X                |                  |                   |                 |                   |                      |                      |                  |
| IX                       | K 8             |                      |                  |                  | X                 |                 |                   |                      |                      |                  |
| X                        | K 9             |                      |                  |                  | X                 |                 |                   |                      |                      |                  |
| XI                       | K 10            |                      |                  |                  | X                 |                 |                   |                      |                      |                  |
| XII                      | K 11            |                      |                  |                  | X                 |                 |                   |                      |                      |                  |
| XIII                     | K 11            |                      |                  |                  |                   |                 |                   |                      |                      |                  |
| XIV                      | K 11            |                      |                  |                  |                   |                 |                   |                      |                      |                  |
| XV                       | K 11            | X                    |                  |                  | X                 |                 |                   |                      |                      |                  |
| XVI                      | L 12            | X                    | X                | X                | X                 |                 |                   |                      |                      |                  |
| XVII                     | L 12            | X                    | X                |                  |                   |                 |                   |                      |                      |                  |
| XVIII                    | L 13            | X                    |                   | X                | X                 |                 |                   |                      |                      |                  |
| XIX                      | L 14            |                      |                  |                  |                   |                 |                   |                      |                      |                  |
| XX                       | L 14            | X                    |                  |                  |                   |                 |                   |                      |                      |                  |
| XXI                      | L 14            | X                    |                   | X                | X                 |                 |                   |                      |                      |                  |
| XXII                     | L 15            | X                    |                   | X                | X                 |                 |                   |                      |                      |                  |
| XXIII                    | L 15            | X                    |                   |                  |                   |                 |                   |                      |                      |                  |
| XXIV                     | L 15            |                      |                  |                  |                   |                 |                   |                      |                      |                  |
| XXV                      | L 16            | X                    |                  |                  |                   |                 |                   |                      |                      |                  |
| XXVI                     | L 16            | X                    |                  |                  |                   |                 |                   |                      |                      |                  |
| XXVII                    | L 17            |                      |                  |                  |                   |                 |                   |                      |                      |                  |
| XXVIII                   | L 17            |                      |                  |                  |                   |                 |                   |                      |                      |                  |

No. of plant mentioned

| Mormondica foetida | Olea europaea | Pilostigma thonningii | Rhus vulgaris | Solanum aculeastrum | Solanum incanum | Tithonia diversifolia | Toddalia asiatica | Tylossema fassoglensis | Warbugia ugandensis | Ziziphus abyssinica | No of plants reported |
|-------------------|---------------|-----------------------|--------------|----------------------|------------------|-----------------------|-------------------|------------------------|----------------------|----------------------|-----------------------|
| X                 |               |                       |              |                      |                  |                      |                   |                        |                      |                      | X                     |

1–XVIII: Numbers of respondents; 1–17: Collection sites; X: Collected plant; M: Maasai; K: Kuria; L: Luo
| Plant/Family/voucher number | Part used | Ethnic group | Local name | Disease treated | Mode of preparation |
|-----------------------------|-----------|--------------|------------|----------------|---------------------|
| Acacia xanthophloea/Mimosaceae/ Fmk2012/01 | Stem bark | Maasai | Olerai | Unspecified livestock diseases and coughs | Infusion of the stem bark is administered to the sick animals |
| Albizia coriaria/Mimosaceae/ Fmk2012/02 | Stem bark | Luo, Kuria and Maasai | Ober, Lotologo, Olerai | Livestock respiratory diseases | Bark infusion for livestock used as treatment for livestock respiratory diseases |
| Warbugia ugandensis/Canellaceae/ Fmk2012/03 | Stem bark, roots and leaves | Maasai | Ol-sogunoi | Livestock respiratory diseases symptoms | Infusion of the stem and roots bark for livestock respiratory symptoms |
| Olea europeae/Oleaceae/Fmk2012/04 | Stem and root barks | Maasai | OL-orien | Contagious Bovine Pleuropneumonia | Root and stem bark infusion for CBPP |
| Ekebergia capensis/Meliaceae/ Fmk2012/05 | Stem bark | Luo, Kuria | Tido | Respiratory symptoms in livestock and remedy for worms | Stem infusion for livestock diseases |
| Carissa spinarum/Apocynaceae/ Fmk2012/06 | Stem bark, roots and fruits | Luo, Kuria | Ochuoga, Omunyore | Unspecified livestock diseases | An infusion of stem bark is treatment for livestock diseases |
| Tithonia diversifolia/Asteraceae/ Fmk2012/07 | Stem and leaves | Luo, Kuria | Maua makech, Irita kunguha | Management of weakness in livestock | An infusion of the stems is used as remedy for the management of livestock weakness |
| Eucleria divinianu/Ebenaceae/ Fmk2012/08 | Stem bark | Luo, Kuria | Ochond radhoo/Ochol, Ikimusi | Treat joint pain in livestock | Decoction of the stem back an pain killer for livestock |
| Pilostigma thonningii/Fabaceae/ Fmk2012/09 | Stem bark, roots, twigs and leaves | Luo, Maasai, Kuria | Otagalo/Ogalo, Olsagaram, Egekobure | Livestock respiratory symptoms | Infusion of the bark of stem and roots is used as remedy for livestock respiratory symptoms |
| Rhus vulgaris/Anacardiaceae/ Fmk2012/10 | Roots and fruits | Luo, Kuria and Maasai | Sangla maduonq, Sangla, Iki-nyororio, Engarani | Livestock weakness | Roots and fruit decoction for give strength to livestock |
| Ziziphus abyssinica/Rhamnaceae/ Fmk2012/11 | Roots, aerial part | Luo, Kuria | Lang'o | Contagious bovine pleuropneumonia | Decoction of the roots combined with leaves infusion is used to treat CBPP |
| Gutenbergia cordifolia/Asteraceae/ Fmk2012/12 | Leaves | Luo, Kuria | Akech, Ikiburu/Ikiburua | Livestock unspecified conditions, | Leaves are pounded and administered to livestock, cattle producing hard dung drenched infusion as remedy |
| Lantana trifolia/Verbenaceae/ Fmk2012/13 | Aerial part and/or the whole plants | Luo, Kuria | Nyabend winyo, Keheimbwe/Kebaris | Treatment for livestock join problems | Leaves infusion is used for livestock joint problems |
| Solanum aculeastrum/Solanaceae/ Fmk2012/14 | Fruits, roots | Maasai, Luo and Kuria | Osigawai, Ochok, Iri botoboto | Livestock respiratory symptoms and contagious bovine pleuropneumonia | Juice from the fruits is administered in drops to a sick animal |
| Carrusia buchanani/Clusiaceae/ Fmk2012/15 | Stem bark, roots | Luo | Onjak | Livestock respiratory symptoms | A decoction of the stem bark is used as a remedy for livestock respiratory symptoms |
| Tyloplea fassoglensis/Fabaceae/ Fmk2012/16 | Roots | Luo, Kuria and Maasai | Ombaras, Omombara | Fever and joint pains in livestock | Root infusion is used to treat livestock |
| Mormondica foestida/Curcubitaceae/ Fmk2012/17 | Aerial parts | Luo | Omobora | Coughs in livestock | A concoction is considered as remedy for livestock |
| Fuertia Africana/Lamiaceae/ Fmk2012/18 | Aerial part | Kuria | Ekebunga baare | Livestock unspecified conditions | Concoction of the aerial part is used as remedy for livestock |
| Toddalia asiatica/Rutaceae/Fmk2012/19 | Stem bark, roots and leaves | Luo, Kuria | Nyawlet-kwach, Utunisia | Livestock joint pains, coughs, fever | Roots and stem bark concoction is used to treat livestock |
| Solanum incanum/Solanaceae/ Fmk2012/20 | Fruits, roots and leaves | Maasai, Luo and Kuria | Endulelei, Ochok, Iritorotoro | Livestock unspecified conditions | Roots decoction used as remedy for livestock |
determined by preparing serial dilutions of the initial concentration. In general, lower MIC mean values were observed with Mmm strains compared to Mmc and Mcc strains. The extracts from the berries and stem bark of Solanum aculeastrum obtained using 50% MeOH-CH₂Cl₂ and water were the most active with MIC mean values of 0.02 mg/ml. The stem bark extracts from Albizia coriaria and Ekebergia capensis showed activity with the lowest MIC mean value of around 0.13 mg/ml against all strains while that of the extracts of Euclea divinorum was active with MIC mean value of 0.5 mg/ml.

The MIC mean values of active extracts against Mmc strains ranged from 0.13 mg/ml to 0.60 mg/ml while those against Mmm strains ranged from 0.02 to 0.5 mg/ml (Table 7).

4. Discussion

It is interesting to note that all the extracts from the two plants frequently mentioned by farmers including: Carissa spinarum (10/28) and Lantana trifolia (8/28), were not potent against all Mycoplasma strains tested. Furthermore, this contradicts, previous reports that have shown that Lantana trifolia elaborated flavonoids such as umuhengerin (1) with good antimicrobial activity (Rwagabo et al., 1988). The inactivacies of the extracts of these two plants could probably be attributed to the fact that traditional medicine practitioners and farmers rarely use these plants singly but rather in combination with others. Carissa spinarum, for example, was mostly used in combination with plants like Albizia coriaria. Similarly, extracts of Lantana trifolia were frequently used in combination with those of Piliostigma thonningii.

Most of the plants with interesting in vitro antimycoplasmal activities against all strains tested including: Solanum aculeastrum (berries), Albizia coriaria (stem bark), Ekebergia capensis (stem bark), Piliostigma thonningii (stem bark) and Euclea divinorum (stem bark), have previously been studied for activities against other strains of bacteria such as; Staphylococcus aureus, Escherichia coli, S. typhi (Al-Fatimi et al., 2007; Munyendo et al., 2011; Owuor et al., 2012). However this is the first report of these plants against Mycoplasma.

All organic extracts from the berries of Solanum aculeastrum were active against all the strains, while the aqueous extracts showed inhibitory activities against only a few strains. Although farmers were not using the stem or leaves of this plant, extracts from these plant parts inhibited selected Mycoplasma strains. Previous investigations by Wanyonyi et al. (2003) showed that extracts from S. aculeastrum were active against E. coli and S. aureus. The ethno-botanical survey by Owuor et al. (2006) showed that this plant is traditionally used for the management of both livestock (respiratory symptoms) and human diseases such as gonorrhoea. In a separate report, Koduru et al. (2007) observed that the extracts from the berries and leaves of S. aculeastrum were active against S. aureus and S. aculeastrum were active against selected gram-positive and gram-negative bacteria with the concentration values ranging between 4.0 and 10.0 mg/ml, much higher than the initial concentration of 1 mg/ml, in the present study. Furthermore, this plant was also reported to exhibit antifungal activities Koduru et al. (2007). Nevertheless other bioactive alkaloids from this plant could also impact the observed activity in this study since this plant is known to be an alkaloid bearing plant.

The antimicrobial activities observed in this study could be attributed to the presence of compounds previously isolated from the genus Solanum such as flavonoid (tirosides, 2), alkaloids (solasodine, 3), steroidal alkaloids (capsimine, 4, solanudine, 5), steroidal alkaloids glycosides (solamargine, 6) (Murakami et al., 1985; Schakirov and Yunusov, 1990; Wanyonyi et al., 2003; Esteves-souza et al., 2003).
The organic extracts from *Albizia coriaria* were active against all the *Mycoplasma* strains used in this study. The presence of compounds such as saponins; albiziatrioside A and B, 7 & 8, alkaloids; macrocyclic spermine alkaloids, 9 and flavonoids; luteolin, 10, quercetin-3-O-α-L-rhamnopyranoside, 11 (Mar et al., 1991; Abbel-Kadder et al., 2001; Melek et al., 2011) from the genus *Albizia* could justify the antimycoplasmal activities observed in this study.

Water extracts from the stem bark of *Albizia coriaria* could not inhibit *Mmc* nor *Mcc* and were active only against few *Mmm* strains. Although farmers do not use the leaves, our study showed that extracts from this plant part inhibited selected *Mmm* strains. Previous studies by Owuor et al., (2012) and Faisal et al. (2012) reported evidence for antimicrobial activity of extracts from the genus *Albizia*, which could possibly also explain the
antimycoplasmal activities, observed in the current study. Moreover, secondary metabolites such as: saponins, flavonoids and alkaloids previously reported by Mar et al., (1991); Abbel-Kadder et al., (2001); Melek et al., (2011); Faisal et al., (2012) and Singab et al., (2015) on the genus Albizia are known to have antibacterial, antifungal and anticancer activities. The outcome of this study is an indication of the necessity of isolating individual compounds from this plant, which could then be used for the formulation of antimicrobial agents.

All organic extracts from the stem bark and the leaves of Ekebergia capensis inhibited the growth of almost all the Mmm strains used in this study while Mmc and Mcc strains were only susceptible to dichloromethane and methanol mixture or methanol extracts from the stem bark of this plant. Little is known on the antimicrobial activities of this plant, but Sewram et al., (2000) reported that compounds isolated from this plant showed uterotonic activities against both pregnant and non pregnant guinea pig uterine smooth muscle. However, compounds previously isolated from the genus Ekebergia such as terpenoids; oleanonic acid, 12, ekeberin A, 13, flavonoids; kaempferol-3-O-β-D-glucopyranoside, 14, quercetin-3-O-β-D-glucopyranoside, 15 (Irungu et al., 2014;) could be the reason why these extracts showed good antimycoplasmal activities against all the strains tested.

The extracts from Piliostigma thonningii (50% MeOH in CH₂Cl₂ mixture and neat MeOH) inhibited the growth of almost all the strains although not much is known about the antimicrobial properties of this plant. Previous reports on preliminary phytochemical screening of extracts from this plant by Ukwuani et al., (2012) showed that this plant contains compounds such as tannins, saponins, terpenoids, glycosides, flavonoids and alkaloids which could be responsible for the antimycoplasmal activity observed in the current study.

For Euclea divinorum, only ethanol extracts inhibited the growth of all the Mmc, Mmm and Mcc strains used in this study. This plant has previously been reported to have antimicrobial properties (Orwa et al., 2008), which bolsters our findings and its uses among Kenyan farmers.

Previous phytochemical reports showed that phenolic compounds such as; Eucleanal A and B, 17 & 18 from Euclea divinorum (Nishiyama et al., 1996; Ng’ang’a et al., 2012) which could be responsible for the observed antibacterial activities.

The extracts from the rest of the plants showed weak activities against some Mycoplasma strains. The low antimycoplasmal activities by these extracts could be due to the fact that the active principles were minor constituents in the extract. Alternatively, the constituent compounds could be acting antagonistically therefore reducing the resultant activities of these extracts.

Surprisingly, a plant such as Momordica foetida (whole plant) mentioned by farmers, was inactive against all Mmc and Mmm strains contrary to previous reports showing that related species such as; Momordica balsamina exhibited antimicrobial properties (Otimenyin et al., 2008). This could be due to the unique nature of Mycoplasma strains which unlike other bacteria strains lack a cell wall and have a small genome.

From the MIC values observed in this study, it is clear that extracts from the berries and stem bark of S. aculeastrum showed good activity suggesting that they could be having bioactive compounds.

5. Conclusions and recommendations

From the in vitro screening of a total of 152 extracts from 20 medicinal plants selected by Maasai, Kuria and Luo ethnic groups in Kenya, those of five plants, including: S. aculeastrum (berries), A. coriaria (stem bark), E. capensis (stem bark), P. thonningii (stem bark) and E. divinorum (stem bark) were active against all the Mmc, Mcc and Mmm strains tested in this study. The outcome of the present study should be shared with the smallholder farmer especially those who undertook the interview for effective management of livestock mycoplasmal infections. Further, investigations of the active extracts should be carried out to characterize the phytochemistry that would be responsible for their potencies.
Table 7
Minimal inhibitory concentrations (MICs) of the most active crude extracts.

| Plant name     | Parts used          | Solvent of extraction | Mean of MIC against Mm (mg/ml ± SEM) | Mean of MIC against Mm (mg/ml ± SEM) | MIC against Mc (mg/ml ± SEM) |
|----------------|---------------------|-----------------------|-------------------------------------|-------------------------------------|-----------------------------|
| A. coriaria    | Stem bark           | CH$_2$Cl$_2$/MeOH (1:1)| 0.32 ± 0.110                        | 0.13 ± 0.002                       | 0.05                        |
| A. coriaria    | Stem bark           | MeOH                  | 0.13 ± 0.092                        | 0.41 ± 0.090                       | 0.005                       |
| A. coriaria    | Stem bark           | EtOH                  | 0.22 ± 0.114                        | 0.23 ± 0.110                       | 0.05                        |
| S. aculeastrum | Berries             | CH$_2$Cl$_2$/MeOH (1:1)| 0.023 ± 0.110                       | 0.23 ± 0.110                       | 0.05                        |
| S. aculeastrum | Berries             | MeOH                  | 0.23 ± 0.031                        | 0.14 ± 0.020                       | 0.005                       |
| S. aculeastrum | Berries             | EtOH                  | 0.13 ± 0.299                        | 0.23 ± 0.110 –                    | –                           |
| S. aculeastrum | Berries             | Water                 | 0.22 ± 0.114                        | 0.51 ± 0.150                       | 0.5                          |
| S. aculeastrum | Stem bark           | Water                 | 0.02 ± 0.011                        | 0.60 ± 0.100                       | –                           |
| P. thomningii  | Stem bark           | CH$_2$Cl$_2$/MeOH (1:1)| 0.5 ± 0.000                         | 0.41 ± 0.090                       | 0.05                        |
| P. thomningii  | Stem bark           | MeOH                  | 0.5 ± 0.000                         | 0.23 ± 0.110                       | 0.05                        |
| E. capensis    | Stem bark           | CH$_2$Cl$_2$/MeOH (1:1)| 0.13 ± 0.092                        | 0.40 ± 0.099                       | 0.5                          |
| E. capensis    | Stem bark           | MeOH                  | 0.40 ± 0.186                        | 0.23 ± 0.110                       | 0.5                          |
| E. divinorum   | Stem bark           | EtOH                  | 0.5 ± 0.000                         | 0.41 ± 0.090                       | 0.5                          |
| Controls       | Tetracycline        | N/A                   | 0.005                               | 0.005                              | 0.005                       |

Keys: CH$_2$Cl$_2$ (Dichloromethane); MeOH (Methanol); EtOH (Ethanol); P < 0.05; SEM (Standard error of mean); N/A (Not Applicable); – (Not active).

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.jep.2016.09.034.
