

Full Length Research Paper

In vitro antimicrobial activity of selected Ethiopian medicinal plants against some bacteria of veterinary importance

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Accepted 28 May, 2010

Due to rapid development of resistance and high cost of the new generation antibiotics, lots of efforts are being made to discover new antimicrobial agents from different sources. In the current study aqueous and hydro-alcoholic extracts of leaves of *Jasminium abyssinicum*, *Myrsine africana*, *Foeniculum vulgare* and aerial part of *Leonotis ocymifolia* were screened for antibacterial activity using agar well diffusion and broth dilution methods. Species of bacteria that cause various diseases in domestic animals namely, *Escherichia coli*, *Pasteurella gallinarum*, *Manhaemia haemolytica*, *Salmonella gallinarum*, *Salmonella typhimurium*, *Staphylococcus aureus* and *Streptococcus agalactae* were used for investigation of antibacterial activity. Except for aqueous extract of *L. ocymifolia*, all of the plant extracts demonstrated remarkable antibacterial activity on most of the bacterial species tested. The three highest zones of inhibition was exhibited by aqueous extracts of *M. africana* against *S. aureus* (19.5 mm), *J. abyssinicum* against *M. haemolytica* (19 mm) and *F. vulgare* against *P. gallinarum* (19 mm). The minimum inhibitory concentration (MIC) exhibited by the plants against test organisms varied from 10 - 1000 µg /ml. However, no plant extract has shown antibacterial effect against *E. coli* using both agar well diffusion and broth dilution methods at concentrations tested. Further detailed *in vitro* and *in vivo* evaluation of these medicinal plants should be carried out.

Key words: Medicinal plants, bacterial isolates, antibacterial activities, extract types.

INTRODUCTION

The rapid development of multi-drug resistant strains of bacteria increased the occurrence of bacterial infections that cannot be treated with conventional antimicrobial agents (Sieradski et al., 1999). More over the new generation antibiotics are less available and are expensive for resource poor communities (Jones, 1996). Because of the aforementioned reasons, lots of efforts have been made to discover new antimicrobial agents from various sources such as micro-organisms, animals and plants (Tomoko et al., 2002). Plants comprise the largest component of the diverse therapeutic elements of traditional

health care practices both in human and animal. Nearly all cultures and civilizations from ancient times to the present day have used herbal medicines which are antimicrobial sources to cure infections (Erdemeier et al., 1996; Lino and Deogracious, 2006). Plant-based antimicrobials represent a vast untapped source of medicines and are effective in the treatment of infectious diseases while simultaneously mitigating many of the side effects that are often associated with synthetic antimicrobials (Parekh et al., 2005). Herbs are also invaluable source of modern drugs. More than 30% of modern drugs are derived from plants (McCorkle, 1995).

In Ethiopia, ethnoveterinary surveys conducted in different parts of the country show the use of different medicinal plants for treatment of various infectious diseases of livestock by traditional healers (Giday and

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Ameni, 2003; Tollosa, 1997; Sori et al., 2004; Wirtu et al., 1999; Yineger et al., 2007; Lulekal et al., 2008; Wondimu et al., 2007; Weldegerima et al., 2008). A number of medicinal plants with significant antimicrobial activity have also been reported by different workers (Desta, 1993; Ashebir and Ashenafi, 1999; Geyid et al., 2005).

In the current study, crude aqueous and hydro-alcoholic extracts of leaves of *Foeniculum vulgare*, aerial part of *Lyonites ocymifolia*, leaves of *Jasminum abyssinicum* and *Myrsine africana* were screened for possible antibacterial activity against seven pathogenic bacterial species of veterinary importance.

F. vulgare (Mill.) locally called "Insila" is a weed of cultivated or disturbed grounds, sometimes common in grassland areas too. The seeds and dried plant parts are used to flavor local drinks such as "areke" and "tej" in Ethiopia. The boiled or roasted roots are used in the treatment of gonorrhea in human (Getahun, 1976).

J. abyssinicum (Hochst. ex DC.) (Oleaceae) locally called "Tembelet", is climbing shrub, with compound leaves with 5 leaflets and white flower. This plant is claimed for its anthelmintic activity in Africa. A study in Kenya indicated that ground leaves of *J. abyssinicum* induced 69% reduction of *Hemonchus contortus* in naturally infected sheep (Paul et al., 2005). Crude extracts of different parts of the plant has shown antimicrobial and antifungal activities against selected organisms infectious to human (Goji et al., 2006).

L. ocymifolia (Burm.f.) (*Lamiaceae*) locally called "Ras kimir" is an erect and stiff woody herb or shrub up to 3 m tall. It grows around houses, along road sides, and abundant at forest edges and mountain bush land between 1500 and 3400 m (Abebe and Ayehu, 1993). The leaves of this plant are used for treatment of hookworm infection, flowers and roots for treatment of gout, leishmaniasis and tumor "mishiro/Nekersa" (Abebe and Ayehu, 1993). Aqueous and hydro-alcoholic extracts of aerial part of *L. ocymifolia* has shown *in vitro* ovicidal and larvicidal effect on eggs of *H. contortus* (Tadesse, 2008).

M. africana, (L.) (*Myrsinaceae*), locally called "Kechemo" is an erect, densely branched, ever green shrub, usually 1 – 3 m high, with grey-brown twigs; leaves are alternate, elliptic or abrogated, usually leathery. It is used as aphrodisiacs and as hair dye (Abebe and Ayehu, 1993). The fruit is edible and has anthelmintic property and is particularly effective for expulsion of the tape worms. Decoctions of the leaves are used as blood purifier (Chauhun, 2009).

MATERIALS AND METHODS

Plant material collection and extraction

Aerial parts of *L. ocymifolia*, leaves of *F. vulgare*, *J. abyssinicum* and *M. africana* were collected from different parts of the country. *L. ocymifolia* was collected from 120Km along Addis Ababa Debrebrhan road, *F. vulgare* and *M. africana* from 25 Km south west of Addis Ababa along Addis Ababa-Butajira road while leaves

of *J. abyssinicum* was collected around Portugise bridge about 100 Km North of Addis Ababa along Addis Ababa Fiche road. Voucher specimens were identified by Dr Mirutse Giday of Aklilu Lemma Institute of Pathobiology and voucher samples (Herbarium No. TE/33/07, TE/41/07, TE/39/07 and TE/52/07) were deposited for *J. abyssinicum*, *M. africana*, *F. vulgare* and *L. ocymifolia*; respectively, at the herbarium of the Addis Ababa University, Biology Department. The garbled plants were dried in air at room temperature, powdered using pestle and mortar and kept in amber colored bottle for further use.

Aqueous extraction was performed by soaking 100 g of the dry powder of plant materials in distilled water (500 ml) and shaken for 3 h by electric shaker. The suspension was filtered through muslin gauze and the filtrate kept in deep freezer for 24 h, which was then lyophilized. The lyophilized dry powder was then collected in stoppered sample vial, weighed and kept in desiccators to avoid absorption of water until used. Hydro-alcoholic extraction was conducted by percolating 200 – 300 g of the dried and powdered plant materials using 80% methanol for 5 days, which was then filtered through whatman filter paper No.1. The solvent was evaporated using a Rota vapor and the extract was kept in a stoppered sample vial at 4°C until used.

Preparation of bacteria for the experiment

Bacterial species used in the current study were obtained from Bacteriology Laboratory, National Animal Health Diagnostic and Investigation Center, Sebeta, Ethiopia. The specimens were originally isolated from animals during disease investigation and kept lyophilized. These were *Escherichia coli*, *Pasteurella gallinarum*, *Pasteurella hemolyticum*, *Salmonella gallinarum*, *Salmonella typhimurium*, *Staphylococcus aureus* and *Streptococcus agalactiae*. The isolates were homogenized with 3 ml of nutrient broth and a loopful of broth containing the bacteria was inoculated on blood agar. It was then incubated at 37°C for 24 h. Purity and viability of the organisms was checked by plating, gram staining, by conducting primary and secondary biochemical tests (Acheampong et al., 1988).

The test bacteria were suspended into sterile universal bottles containing nutrient broth separately and incubated at 37°C for 18 h. Normal saline was added gradually to adjust the culture turbidity to that of McFarland turbidity standard, which corresponds to approximately (10^7 CFU/ml).

Determination of antibacterial activity

The antibacterial activity was tested using agar well diffusion and broth dilution methods according to (Lino and Deogracious, 2006; Sahm and Washington 1990), respectively.

Agar well diffusion

Briefly, 1 ml of the test culture (10^7 CFU/ml) was inoculated into a sterile plate with 20 ml Muller Hinton molten agar and the plate was shaken for even spread and proper mixing of the organisms and agar. It was then allowed to solidify. Six wells of approximately 8 mm in diameter and 7 mm depth were made on the surface of the agar plates using a sterile borer. The plates were then turned up side down and the wells were labeled with a marker. Stock solution of each plant extract was prepared at concentration of 250 mg/ml in distilled water. Each four wells of the six wells were filled with 0.35 ml of the plant extracts. The fifth and sixth well were filled with 0.35 ml of distilled water and 13.13 µg equivalent of gentamycin in 0.35 ml of distilled water and served as a negative and a positive control respectively. The plates were then incubated at 37°C for 24 h and

Table 1. Percentage yield of plant extracts using aqueous and hydro-alcoholic extraction methods.

Plant species	Parts used	Extraction type	% yield (W/W)
<i>F. vulgare</i>	Leaves	Aqueous	22.17
		Hydro-alcoholic	14.72
<i>L. ocymifolia</i>	Aireal	Aqueous	4.97
		Hydro-alcoholic	17.31
<i>J. abyssinicum</i>	Leaves	Aqueous	14.28
		hydro-alcoholic	10.94
<i>M. africana</i>	Leaves	Aqueous	8.83
		Hydro-alcoholic	26.57

Table 2. Antibacterial activity of aqueous and hydro-alcoholic extracts of medicinal plants using agar well diffusion method.

Microorganism	Mean zones of inhibition(mm)								Gentamicin	Distilled water
	<i>F. vulgare</i>		<i>L. ocymifolia</i>		<i>J. abysynicum</i>		<i>M. africana</i>			
	Aq	Ha	Aq	Ha	Aq	Ha	Aq	Ha		
<i>E. coli</i>	-	-	-	-	-	-	-	-	13	-
<i>P. gallinarum</i>	19	-	-	8	12	-	13.5	12	22	-
<i>M. hemolyticum</i>	11.5	-	-	15.5	19	11	17.5	15	36	-
<i>S. gallinarium</i>	12	-	-	14	16.5	-	-	-	17	-
<i>S. typhimurium</i>	-	-	-	12	18	-	16	-	14	-
<i>S. aureus</i>	-	11.5	-	-	-	11	19.5	17	20	-
<i>S. agalactae</i>	-	11	-	-	17	16.5	-	11.5	18	-

-No zone of inhibition detected, Aq- aqueous extract, Ha- hydro-alcoholic extract.

zone of inhibition was measured with a pair of calipers and ruler millimeter and results were tabulated.

Broth dilution method

One ml of 24 h culture of test organisms (10^7 CFU/ml) adjusted to McFarland turbidity standard were incubated in serial dilution of 10, 100 and 1000 µg/ml of plant extracts in physiological saline at 37 °C for 24 h. The concentration at which the lowest dilution with no detectable bacterial growth was considered as minimum inhibitory concentration (MIC). Both types of tests were conducted in duplicate and the mean was taken. Absence of growth was confirmed by absence of turbidity and by inoculating in to agar. Both types of tests were conducted in duplicate and the mean was taken.

RESULTS

The species of plants have shown variation in percentage yield in both aqueous and hydro-alcoholic extraction process. The highest yield was recorded for hydro-alcoholic extract of *M. africana* (26.57%) and the lowest yield was observed for aqueous extract of *L. ocymifolia* which was 4.97% (Table 1). Zone of inhibition of bacterial growth by different plant extracts are shown in Table 2. *E.*

coli was not inhibited by any of the plant extracts examined at, the concentrations tested. Of all plant extracts tested aqueous extract of *J. abyssinicum* and *M. africana* showed antimicrobial activity against most of the bacterial species investigated indicating its wide spectrum of activity while aqueous extract of *L. ocymifolia* did not induce inhibition of growth of any bacterial species. The maximum zone of inhibition of 19.5 mm was recorded for aqueous extract of *M. africana* against *S. aureus*. Both extracts of *M. africana* and *J. abyssinicum* showed antibacterial activity against most bacterial species for both extract types while others show affect either in aqueous or hydro-alcoholic extract alone.

The MIC obtained using broth dilution method for different plant extracts are shown in Table 3. No inhibition was recorded for *E. coli* for all plant extracts tested at all concentrations. Aqueous extract of *F. vulgare* is highly effective against *P. gallinarum* and *S. agalactae* (MIC = 10 µg/ml), while *S. gallinarum*, *S. typhimurium* and *S. aureus* were not inhibited at all concentrations tested. Hydro-alcoholic extract also induced inhibition of most of the bacterial species tested but at relatively higher concentration. Aqueous extract of *L. ocymifolia* inhibited

Table 3. Minimum inhibitory concentration (MIC) of plant extracts against different bacterial isolates (µg/ml).

Organisms	<i>F. vulgare</i>		<i>L. ocymifolia</i>		<i>J. abyssinicum</i>		<i>M. africana</i>	
	Aq	Ha	Aq	Ha	Aq	Ha	Aq	Ha
<i>E. coli</i>	-	-	-	-	-	-	-	-
<i>P. gallinarum</i>	10	1000	-	100	100	100	100	100
<i>M. haemolyticum</i>	100	1000	-	1000	100	1000	100	1000
<i>S. gallinarum</i>	-	100	1000	-	100	-	-	-
<i>S. typhimurium</i>	-	-	-	1000	10	100	-	-
<i>S. aureus</i>	-	100	-	-	-	-	10	10
<i>S. agalactae</i>	10	-	-	-	100	-	-	100

Aq- aqueous extract, Ha- hydro-alcoholic extract.

only growth of *S. gallinarum* at the maximum concentration tested (1000 µg/ml) while the hydro-alcoholic extract of the same plant inhibited growth of *P. gallinarum*, *P. haemolyticum* and *S. typhimurium* at 100, 1000 and 1000 µg/ml respectively. Both extracts of *J. abyssinicum* and *Myrsine africana* inhibited growth of most bacterial species. Both extracts of *M. africana* were effective against *S. aureus* at concentration of 10 µg/ml. In general, *P. gallinarum* and *M. haemolyticum* were susceptible to most of the plant extracts.

DISCUSSION

The current study showed that most of the plant extracts have antibacterial activity against some of the common microorganisms of veterinary importance. This work could justify their traditional use in treatment of different diseases in human and animals. The fact that both aqueous and hydro-alcoholic extracts of some plants are showing similar efficacy against some species of bacteria could be due to extraction ability of active ingredients responsible for antibacterial activity by the two extraction systems. On the other hand, some plants have shown variable activity by the two extraction methods presumably because of difference in extracting ability of specific active ingredient by the two extraction methods (Lino and Deogracious, 2006). Parekh et al. (2005) also reported difference in antibacterial activity when two extraction methods were used.

Groups of phytochemical compounds commonly implicated for antimicrobial activity in medicinal plants are flavonoids, alkaloids, tannins, and triterpenoids and different essential oils (Lutterodt et al., 1999; Cowan, 1999). Presence of these bioactive components in the crude extracts could be linked to their activities against microorganisms. Previous works have shown that *F. vulgare* contains alkaloids, flavonoids, tannins, saponins and cardiac glycosides (Kaur and Arora, 2009). *J. abyssinicum* contains polyphenols and triterpens, oligomeric secoiridoid glucosides (Geyid et al., 2005; Delle et al., 2006.); Sixty-eight essential oil components were identified from aerial part of *L. ocymifolia* (Nibret and

Wink, 2010) *M. africana* contains flavonol glycosides (Arot et al., 1996).

The finding of the current study is in agreement with the previous study (Kaur and Arora, 2009) with regard to *F. vulgare* in which the aqueous and organic extracts were active against different bacterial species. The previous study also revealed that extracts from *J. abyssinicum* showed activity against *S. aureus*, *P. aeruginosa* and *S. pyogenes* isolated from human patients (Goji et al., 2006).

Previous study has also shown that oils from *L. ocymifolia* exhibited strong antibacterial activity against gram-negative bacteria and oral pathogens (Vagionas et al., 2007). The lack of any measurable antimicrobial activity for all plant extracts against *E. coli* is in agreement with the earlier findings reported for extracts of *J. abyssinicum*, *Annona senegalensis*, *Solanecio gigas*, *Lagenaria siceraria* (Goji et al., 2006; Lino and Deogracious, 2006) and less sensitivity to other plants (Ajaiyeoba, 2002; Mahmood et al., 2008). This could be due to the fact that *E. coli* is inherently resistant to many antibiotics and due to the permeability barriers afforded by its outer membrane (Lino and Deogracious, 2006).

Aqueous extract of *M. africana* has shown the highest zone of inhibition against *S. aureus* which was supplemented by the MIC value of 10 µg/ml against the same organism. Among these four plants; aqueous extract of *J. abyssinicum* exhibited antibacterial activity against most organisms tested, followed by *M. Africana*. The *in vitro* finding is not always dependable, plants which are effective *in vitro* might not work when used *in vivo* and some plants which showed little or no effect *in vitro* study might also be effective when evaluated in animals due to various factors that affect or favor the release of active ingredients in animal bodies. Therefore, further detailed *in vitro* and *in vivo* evaluation of these medicinal plants should be carried out.

ACKNOWLEDGEMENTS

National Animal Health Diagnostic and Investigation Center is highly appreciated for provision of materials and

facilities required to conduct the experiment. We are grateful for Mr. Melaku Sombo and Mrs. Letebirhan Yigezu for the technical assistance in screening of plant materials. Ethiopian Health and Nutrition Research institute is also acknowledged for their help in extraction of the plant materials. We are also grateful to Dr Mirutse Giday of Akililu Lemma Institute of Pathobiology, Addis Ababa University for identification of medicinal plants.

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