In-vitro Antibacterial Activity of Root and Stem Extracts of Ferula Communis against Staphylococcus aureus and Escherichia coli

Derso Sitotaw, Habtom Kiros Bitsue*, Tsegay Tkue, Niraj Kumar, Berhe Mekonnen Mengistu, Mu-uz Gebru and Girmay Hiluf

College of Veterinary Medicine, Mekelle University, P.O.Box: 2080, Mekelle, Ethiopia
*Corresponding author: E-mail: habtomkb@yahoo.com

ABSTRACT
The use of herbs and medicinal plants as the first medicines is a universal phenomenon. Due to either limited availability or affordability of pharmaceutical medicines, the majority of the rural population in Sub Saharan Africa depends on traditional herbal remedies for primary health care and veterinary use. Some studies in Ethiopia indicated that considerable number of human and livestock population still depends on traditional medicine to fight a number of diseases. This study was conducted with the objective of assessing the antibacterial activity of Ferula communis against two bacterial species namely Staphylococcus aureus (S. aureus) and Escherichia coli (E. coli) using different extractor solvents. The stems and roots of Ferula communis were collected; air dried under the shed, powdered and soaked in 80% methanol and ethanol to harvest the extract of the plant. In vitro antibacterial activity of the extracts was tested at different concentrations (100 mg/ml and 200 mg/ml) by using agar disc diffusion method and measuring the mean zone of inhibition. Accordingly, the results showed that the methanol crude extract of Ferula communis exhibited higher antibacterial activity against the tested organisms rather than ethanol extract of same plant. At 200 mg/ml concentration, the highest antibacterial inhibition against S. aureus (16.83±0.764 mm) and E. coli (15±1.00 mm) was revealed by methanol crude extract of roots of Ferula communis. On the other hand, at 100 mg/ml concentration, lowest inhibition zone (8.00 ±0.00 mm) was obtained from ethanol crude extract of stems of Ferula communis against E. coli. The present result showed that both methanol and ethanol extracts of Ferula communis have antibacterial effect against Staphylococcus aureus and Escherichia coli. However, the pattern of inhibition varied with concentration, bacterial strain, plant part and the solvents used. Hence, further detail study on isolation of the active ingredients of the plant extract should be conducted.

Key words: E. coli, Ferula communis, Mean zone of inhibition, Staphylococcus, S. aureus

INTRODUCTION
Plants have a long therapeutic history over thousands of years and still considered to be a promising source of medicine in the traditional health care system (Hemalatha et al., 2008). Medicinal plants have provided a good source of antimicrobial agent against several infectious pathogens including bacteria (Ahmed et al., 2012). Either due to limited availability or affordability of pharmaceutical medicines, about 80% of the rural population in Sub Saharan Africa depends on traditional herbal remedies for primary health care and veterinary use (WHO, 2002). Pathogenic microorganisms build resistance against antibiotics and also there are many side effects reported by antibiotics, hence, much attention has been paid to extracts and biologically active compounds isolated from plant species used in herbal medicine (Kokoska et
In Ethiopia, about 80% of the human population and 90% of livestock still depend on traditional medicine to fight a number of diseases (Fullas, 2007).

A medicinal plant is any which is one or more of its organ, contains a substance that can be used for the therapeutic purpose or which is a precursor for the synthesis of useful drugs (Edward, 2004). The screening of plant extracts and plant products for antimicrobial activity has shown that higher plants represent a potential source of novel antibiotic prototypes (Afolayan, 2003).

The medicinal plant used in this study was Ferula communis (a common name in Amharic and Tigrigna is “doq”). It is annual or biennial herb up to 3 m high, basal and stem leaves with very conspicuous sheathing. Petals yellow with incurved apex. Propagation is sexual. Fruits vary, strongly dorsally compressed, broadly obviate to broadly elliptic. Ferula communis is distributed in south Europe, West Asia, North Africa, Sudan, Uganda Kenya and Tanzania. In Ethiopia the plant is distributed in Tigray, Gonder, Gojam, Wollo, Shewa, Arsi, Sidamo, Bale and Harerge (Hedberg, 2009). Many reports are available on the traditional medicine uses such as for skin infection, bronchitis, Schistosomiasis, pneumonia and wound infection in Ethiopia (Dawit et al., 2003). Even though, this plant has different medicinal values, there is scarce of scientific data regarding to antibacterial activity especially the stem and root parts of Ferula communis. Therefore, the objective of this study was to screen the anti-bacterial activity of Ferula communis against Staphylococcus aureus and Escherichia coli.

MATERIALS AND METHODS

Study design

In-vitro experimental study was conducted to investigate and evaluate antibacterial activity of Ferula communis against S. aureus and E. coli.

Medicinal plant

The medicinal plant used in this study was Ferula communis. Ferula is a genus of about 170 species of flowering plants in the family Apiaceae. They are herbaceous perennial plants growing to 3 m tall, with stout, hollow, somewhat succulent stems. The leaves are Tripinnate or even more finely divided, with a stout basal sheath clasping the stem. The flowers are yellow, produced in large umbels (Iranshahi, 2010).

Plant collection and preparation of crude extract

The roots and stems of Ferula communis used in this study were collected during the rainy season from South Gondar zone (Amhara regional state) around Addis-Zemen town, and authenticated at biology department in the University of Gondar, Ethiopia. The test plant parts were washed thoroughly with tap water to remove unnecessary particles, air-dried (dried indoor without exposure to sunlight), chopped, grinded mechanically, sieved and preserved at the College of Veterinary Medicine, Mekelle University until extraction.

The preserved samples were weighed using sensitive digital balance and extracted in 80% ethanol and methanol by maceration method and concentrated according to the procedures described by Debella (2002). For this study, 100 g of powdered plant materials were soaked in
each of 1000 ml of 80% ethanol and methanol in Erlenmeyer flask of two liter capacity. The flask containing dissolved plant materials in 80% ethanol was plugged with cotton wool and kept on a rotary shaker at 100-120 rpm for 72 hours. After 72 hours the supernatant was filtered with Whatman (No.1) filter paper and concentrated via rotary evaporator. Trace solvent was evaporated on water bath of 40°C and under vacuum to dryness. Additional solvent was added to the residue and filtered three more times. Plant extracts were then kept in an incubator at 40°C for 24 hours to help evaporate the remaining solvent. The concentrated plant extracts weighed their initial weight using sensitive balance and labeled, and finally, extracts were stored at +4°C in an airtight container until being used for anti-microbial activity.

Isolation of the test bacteria

Two species of bacteria isolates, a gram positive (S. aureus) and gram negative (E. coli) were isolated from subclinical mastitis milk sample of cow. Catalase test, Methyl red test, indole test, triple iron sugar test and microbial growth medium were used for the identification of these bacterial pathogens using procedures described by Cheesbrough (2002). Isolated colonies were suspended in 0.9% sterilized saline solution and then test suspensions were shacked with vortex mixer so as to get uniform suspension; and turbidity was adjusted by comparing with that of 0.5 McFarland turbidity standards against a white black ground with contrasting black lines. 0.5 McFarland units prepared by mixing 0.5 ml of 1.75% (w/v) barium chloride dihydrate with 99.5 ml 1% (v/v) sulfuric acid as procedures employed by Willey et al. (2008).

Preparation of antimicrobial discs from herb extracts for in-vitro experiment

Discs which were used for this experiment were prepared from sterilized filter paper discs. Discs were impregnated with 10 μl of the test sample, allowed to dry and placed onto inoculated plates. Then, discs which were prepared from a Whatman No. 1 sterile filter paper disc (6 mm diameter), was impregnated with the dissolved extracts at different concentration. 10 μl of the crude extract was loaded in to each prepared discs. For Ferula communis, two groups of discs were prepared; i.e. one group was loaded 2000 μg (10 μl of crude extract solution containing 200 mg/ml), second group was loaded 1000 μg (10 μl of crude extract solution containing 100 mg/ml). Finally, the loaded discs were sterilized under UV for 30 minutes. Procedures used as indicated by Periyasamy and Mahalingam (2010).

Antimicrobial susceptibility test

The antimicrobial susceptibility testing of the isolates was performed by using the disc diffusion method according to the recommendations of the National Committee for Clinical Laboratory Standards (NCCLS, 2002) and Kirby-Bauer (Willey et al., 2008). Test discs were prepared by loading and saturating sterilized filter paper discs with plant extracts. Same sized filter paper discs (6 mm diameter) loaded with the different volume of extract. For negative control discs impregnated by DMSO was used while standard antibiotic discs (chloramphenicol) was used as positive control. The prepared discs were placed on the surface of the media (Muller Hinton agar media) ± and the compound was allowed to diffuse for 5 minutes and the plates were kept for incubation at 37°C for 24 hours. Discs were applied to the cultured bacteria by sterile forceps approximately equidistance to each other. The diameter of zone of inhibition (mean and standard deviation in triplicates) as indicated by clear area which was devoid of growth of bacteria was measured using a digital calliper. Based on the diameter of the zone of inhibition, antibacterial susceptibility was recorded and ranked as described by Periyasamy and Mahalingam (2010).
Data analysis

Data were entered into Microsoft Excel spreadsheet and analyzed using SPSS version 20 software. The zone of inhibition was expressed as mean ± SD. Statistical analysis was also undertaken by analysis of independent t-test (t-test) coupled with Least Significant Difference (LSD) to compare result between plant parts, solvent, test bacteria and concentrations. The result was considered statistically significant at p-value <0.05.

RESULTS

Yield of crude extract

Out of the two solvents used in this study, the maximum yield of crude extract of roots of *Ferula communis* was attained by using methanol solvent which is 25% followed by ethanol with a yield of 14.28%. With stems of *F. communis*, the yield of crude extracts of stems of *Ferula communis* using methanol and ethanol was 20% and 11.42% respectively.

Antibacterial screening

Zone of inhibition of the tested extract against two pathogens is showed significant antibacterial activity as indicated in Table 1. Among the two crude extracts tested, evidenced by a high zone of inhibition methanol crude extract showed the highest degree of inhibition followed by ethanol for all tested bacteria. The methanol crude extract showed maximum may be due to their crude nature, were significant to justify antibacterial activity against *S. aureus* with a diameter of the traditional claim of its use against infections. The zone of inhibition in all the cases increased with an increase in concentration.

DISCUSSION

Ethnobotanical investigations have been found to offer important clues in the identification and development of traditionally used medicinal plants into modern drugs. The present study clearly indicated that both methanol and ethanol extract of *Ferula communis* could able to inhibit both gram positive and gram negative tested bacteria. However, the degree and pattern of inhibition are different.

In this study, the crude extract of *Ferula communis* has shown higher inhibition zone against *S. aureus* but comparatively lower against *E. coli*. The observed difference in the antibacterial activities of the extracts among *E. coli* and *S. aureus* may be attributed to the difference in the outer membrane of the tested bacteria. The plant extracts were more active against the Gram positive microorganisms than Gram negative microorganisms. This is in line with previous reports that plant extracts are more active against Gram positive bacteria than Gram negative bacteria (Parekh and Chanda, 2006). Some previous studies also support this present finding in that Gram positive bacteria are more sensitive to the extracts because of the single layer of their cell wall while the double membrane of Gram negative bacteria make them less sensitive (Arora and Kaur, 2009).

On top of this, Gram negative bacteria may possess high permeability barrier for numerous antibiotic molecules which may be similar for these extracts too. The periplasmic space of the Gram negative also contains enzymes which are capable of breaking down foreign molecules and appears to be less susceptible to plant extracts than gram positive bacteria (Duffy and Power,
2001). Moreover, to justify the reason for the difference in sensitivity between gram positive and gram negative bacteria might be attributed to the differences in morphological constitutions between these microorganisms, gram negative bacteria having an outer lipopolysaccharide membrane.

This makes the cell wall impermeable to antibacterial chemical substances. The gram positive bacteria on the other hand are more susceptible having only an outer peptidoglycan layer which is not an effective permeability barrier. Therefore, the cell wall of gram negative organisms are more complex in lay out than the gram positive ones acting as a diffusion barrier and making them less susceptible to the antibacterial agents than gram positive bacteria (Alonso et al., 2000).

In the present finding, the zone of inhibition in all the cases increased with an increase in concentration. This may be due to increase in active ingredients as an increase in concentration. The finding of this study is consistent with the previous report, the antibacterial activity of the tested plant showed that the zone of inhibitions increased with an increase in concentration (Parekh and Chanda, 2006).

Previous studies by Alzorkey and Nakahara (2003), showed that influence of the solvents in the extraction of phytochemicals from plant materials. These reports further indicated that both methanol and ethanol are proved to be good solvents in extracting the inhibitory substance from medicinal plants. Those solvents have ability to extract secondary metabolites which have antimicrobial activity and ability to degrade inert part of the plant parts which is inactive.

In this present study, compared to ethanol extract, methanol extract showed slightly higher inhibition zone. This result disagrees with previous reports of, a study of two different solvents evaluated by Amsalu et al. (2001), which indicated ethanol extract showed the most effective solvent that significantly reduced radial growth of the pathogen compared to methanol and similarly in another plant species (Allium sativum) reduced the radial growth of the pathogen by 83%. In another previous report the methanol extracts of another plant Acokanthera schimperi leaves showed strong antibacterial activity against 10 bacterial strains (Biruhalem et al., 2001) which are in agreement with the present finding.

The above difference may be due to methanol has potential to extract saponins which have antimicrobial activity (Ncube et al., 2008). Furthermore, in methanol, there is an enzyme polyphenol oxidase which degrades polyphenol which is inactive part of the plant. Means methanol has potential of cell walls and seed degradation and causes polyphenols to be released from the cells. Antimicrobial property of saponin is due to its ability to cause leakage of proteins and certain enzymes from the cell (Zablotowicz et al., 1996). Moreover, to justify the above difference methanol extracts of selected plants leaves have age and concentration dependent antibacterial activity against some of the tested organisms (Rahid et al., 2014). It should also be advisable to consider the growth stage of plant parts used.

When we compared root and stem part extract, the root extract showed more antibacterial activity than stem which might be due to more active ingredient found in the root than the stem. Furthermore, previous reports justified that the presence of different active ingredients and other chemicals in plants varies by the part of the plant harvested, the maturity of the plant at the time of harvest; the time of year during harvest; geography and soil conditions; soil composition and its contaminants; and year-to-year variations in soil acidity, water, weather conditions and other growth factors reported by (Azaizeh et al., 2005).
The present study revealed that the tested plant has great therapeutic potential. The antibacterial results showed that the tested samples are considered to be a rich source for antibacterial agents and can be used in various pathological conditions due to these different bacteria. This result strongly supports the folkloric use of this plant in various ailments like bronchitis, sinusitis, skin infection and various others (Shinwari and Gilani, 2003). The present antibacterial results are strongly supported by the antibacterial properties of related species like *F. szowitsiana* (Ozek *et al.*, 2008) and *F. hermonis* (Ibraheim *et al.*, 2012).

Al Yahya *et al.* (1998) reported that sesquiterpenes 14 (hydroxycinnamoyloxy) dauc4, 8 diene, ferulenol and ferchromone using petroleum ether from roots of *Ferula communis* have strong antibacterial activity against *Staphylococcus aureus* which agrees with the present finding. Similarly Ibraheim *et al.* (2012) reported that seventeen daucane sesquiterpenoid esters isolated from the root of *Ferula hermonis* which is related species to *Ferula communis* showed broad spectrum activity against *S. aureus* and *E.coli*. However, *Ferula hermonis* in the previous report showed more strong antibacterial effect than *Ferula communis*. This variation might occur due to species differences among the plants. It is not surprising that there are differences in the antimicrobial effects of plant groups, due to phytochemical properties and differences among species. The investigated plants did not show strong antibacterial activity as compared to *Ferula hermonis*; however, lower results do not mean the absence of bioactive constituents nor is that the plant inactive. The active compound(s) may be present in insufficient quantities in the crude extracts to show activity with the dose levels employed (Taylor *et al.*, 2001). Lack of activity can thus only be proven by using large doses (Farnsworth, 1993). Alternatively, if the active principle is present in high enough quantities, there could be other constituents exerting antagonistic effects of the bioactive agents (Jager *et al.*, 1996).

In general, the diameter of the conventional antibiotic disc and crude extracts impregnated discs are almost comparable. The diameter of inhibition zone of roots of *Ferula communis* at 200 mg/ml/ disc concentration was found almost comparable with noble antibiotics discs chloramphenicol. The dimethyl sulfoxide impregnated discs did not show any inhibition against the test organism which implies that the inhibition observed was exclusively by crude extracts. Zone sizes of 6 mm (equivalent to the size of the discs) indicated no activity while zone sizes equal to or greater than 7 mm indicated antibacterial activity of the crude extract. The controls were the solvents used for the extraction and they showed no inhibitions, hence any inhibitions observed in the plant extracts were not due to the solvents. In conclusion, this *in vitro* study showed that the plant extracts inhibited bacterial growth but the pattern of inhibition varied with concentration, bacterial species, plant part and the solvents used. Thus, *Ferula communis* can be employed as a source of natural antimicrobials and there is a possibility to isolate potential antibacterial drugs for the treatment of bacterial disease.

**ACKNOWLEDGEMENTS**

The authors would like to acknowledge Mekelle University, College Veterinary Medicine for financial support and provision laboratory facilities.
REFERENCES


NCCLS, 2002. National Committee for Clinical Laboratory Standards. Performance standards for antimicrobial disc and dilution susceptibility tests for bacteria isolated from animals; approved standard 2nd Ed.


Table 1: Mean zone of inhibition of *Ferula communis* against *S. aureus* and *E.coli* under different settings of plant parts and concentration

<table>
<thead>
<tr>
<th>Plant part</th>
<th>Solvent</th>
<th>Concentration (mg/ml)</th>
<th>Zone of inhibition <em>S. aureus</em> (mm)</th>
<th>p-value</th>
<th>Zone of inhibition <em>E.coli</em> (mm)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Root</td>
<td>Methanol</td>
<td>200</td>
<td>16.83 ± 0.764</td>
<td>0.004</td>
<td>15.00 ± 1.000</td>
<td>0.003</td>
</tr>
<tr>
<td>Stem</td>
<td>Methanol</td>
<td>200</td>
<td>12.00 ± 1.000</td>
<td></td>
<td>10.67 ± 0.577</td>
<td></td>
</tr>
<tr>
<td>Root</td>
<td>Methanol</td>
<td>100</td>
<td>11.67 ± 0.577</td>
<td>0.007</td>
<td>10.33 ± 0.577</td>
<td>0.013</td>
</tr>
<tr>
<td>Stem</td>
<td>Methanol</td>
<td>100</td>
<td>10.00 ± 0.000</td>
<td></td>
<td>8.3 ± 0.577</td>
<td></td>
</tr>
<tr>
<td>Root</td>
<td>Ethanol</td>
<td>200</td>
<td>15.00 ± 1.000</td>
<td>0.002</td>
<td>12.00 ± 1.000</td>
<td>0.026</td>
</tr>
<tr>
<td>Stem</td>
<td>Ethanol</td>
<td>200</td>
<td>10.33 ± 0.577</td>
<td></td>
<td>10.00 ± 0.000</td>
<td></td>
</tr>
<tr>
<td>Root</td>
<td>Ethanol</td>
<td>100</td>
<td>10.33 ± 0.577</td>
<td>0.016</td>
<td>9.33 ± 0.577</td>
<td>0.016</td>
</tr>
<tr>
<td>Stem</td>
<td>Ethanol</td>
<td>100</td>
<td>9.00 ± 0.000</td>
<td></td>
<td>8.00 ± 0.000</td>
<td></td>
</tr>
<tr>
<td>Root</td>
<td>Methanol</td>
<td>200</td>
<td>16.83 ± 0.764</td>
<td>0.006</td>
<td>14.9600 ± 1.000</td>
<td>0.003</td>
</tr>
<tr>
<td>Root</td>
<td>Methanol</td>
<td>100</td>
<td>11.57 ± 0.404</td>
<td></td>
<td>10.20 ± 0.724</td>
<td></td>
</tr>
<tr>
<td>Root</td>
<td>Ethanol</td>
<td>200</td>
<td>15.00 ± 1.000</td>
<td>0.005</td>
<td>11.9433 ± 0.91632</td>
<td>0.008</td>
</tr>
<tr>
<td>Root</td>
<td>Ethanol</td>
<td>100</td>
<td>10.40 ± 0.529</td>
<td></td>
<td>9.0500 ± 0.42720</td>
<td>0.026</td>
</tr>
<tr>
<td>Stem</td>
<td>Methanol</td>
<td>200</td>
<td>12.0900 ± 0.86850</td>
<td>0.010</td>
<td>10.7800 ± 0.29866</td>
<td>0.002</td>
</tr>
<tr>
<td>Stem</td>
<td>Methanol</td>
<td>100</td>
<td>9.6667 ± 0.28868</td>
<td></td>
<td>8.5000 ± 0.50000</td>
<td></td>
</tr>
<tr>
<td>Stem</td>
<td>Ethanol</td>
<td>200</td>
<td>10.567 ± 0.4041</td>
<td>0.002</td>
<td>9.700 ± 0.2646</td>
<td>0.006</td>
</tr>
<tr>
<td>Stem</td>
<td>Ethanol</td>
<td>100</td>
<td>8.783 ± 0.2021</td>
<td></td>
<td>8.00 ± 0.005</td>
<td></td>
</tr>
<tr>
<td>Control (+)</td>
<td>Chloramphenicol</td>
<td>30 µg</td>
<td>17.00 ± 1.000</td>
<td></td>
<td>16.67 ± 1.155</td>
<td></td>
</tr>
<tr>
<td>Control (-)</td>
<td>DMSO</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>

Table 2: Mean zone of inhibition under different bacterial strain with the same plant part, concentration and solvent

<table>
<thead>
<tr>
<th>Plant part</th>
<th>Solvent</th>
<th>Concentration (mg/ml)</th>
<th>Zone of inhibition <em>E.coli</em> (mm)</th>
<th>p-value</th>
<th>Zone of inhibition <em>E.coli</em> (mm)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Root</td>
<td>Methanol</td>
<td>200</td>
<td>16.83 ± 0.764</td>
<td>0.002</td>
<td>14.96 ± 1.000</td>
<td>0.002</td>
</tr>
<tr>
<td>Root</td>
<td>Methanol</td>
<td>100</td>
<td>11.57 ± 0.404</td>
<td>0.001</td>
<td>10.20 ± 0.724</td>
<td>0.001</td>
</tr>
<tr>
<td>Root</td>
<td>Ethanol</td>
<td>200</td>
<td>15.00 ± 1.000</td>
<td>0.007</td>
<td>11.94 ± 0.91632</td>
<td>0.007</td>
</tr>
<tr>
<td>Root</td>
<td>Ethanol</td>
<td>100</td>
<td>10.40 ± 0.529</td>
<td>0.026</td>
<td>9.0500 ± 0.42720</td>
<td>0.026</td>
</tr>
<tr>
<td>Stem</td>
<td>Methanol</td>
<td>200</td>
<td>12.09 ± 0.869</td>
<td>0.039</td>
<td>10.7800 ± 0.29866</td>
<td>0.039</td>
</tr>
<tr>
<td>Stem</td>
<td>Methanol</td>
<td>100</td>
<td>9.67 ± 0.289</td>
<td>0.028</td>
<td>8.50 ± 0.500</td>
<td>0.028</td>
</tr>
<tr>
<td>Stem</td>
<td>Ethanol</td>
<td>200</td>
<td>10.57 ± 0.404</td>
<td>0.036</td>
<td>9.70 ± 0.265</td>
<td>0.036</td>
</tr>
<tr>
<td>Stem</td>
<td>Ethanol</td>
<td>100</td>
<td>8.78 ± 0.202</td>
<td>0.046</td>
<td>8.00 ± 0.500</td>
<td>0.046</td>
</tr>
<tr>
<td>Control (+)</td>
<td>Chloramphenicol</td>
<td>30 µg</td>
<td>17.00 ± 1.000</td>
<td></td>
<td>16.67 ± 1.155</td>
<td></td>
</tr>
<tr>
<td>Control (-)</td>
<td>DMSO</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>