In vivo Insecticidal Activity of Formulations Containing Essential Oils of Cymbopogon citratus Stapf and Eucalyptus globulus Labill. against Melophagus ovinus in Sheep

Negero Gemeda*, Hirut Lemma, Ashenif Tadele, Getachew Addis, Mulugeta Guta, Kidist Yirsaw, Frehiwot Teka, Walelegn Mokennen, Zenebech Adela and Asfaw Debella

Ethiopian Public Health Institute, P.O. Box: 1242, Addis Ababa, Ethiopia

*Correspondence: dnegero@gmail.com, Mob: +251911339449

Abstract

Background: National insecticide dipping program has been performed in Ethiopia for the last decade on sheep and goat to control ectoparasites. However, there is still high burden of ectoparasite in the country. On the other hand, the widespread use of chemical insecticides has significant drawbacks including increased cost, handling hazards, threat to human health and environment and insecticide resistance. These facts prompted the search for new alternative insecticides from plants for the control of ectoparasite.

Objective: To determine insecticidal potency of essential oil formulations on sheep naturally infested by Melophagus ovinus (sheep ked).

Method: A randomized block design studies were used on naturally ked infested sheep (n=6) as treatment and control (negative/Tween 80 and positive/diazinon) groups to evaluate insecticidal activity of two essential oils from Cymbopogon citratus and Eucalyptus globulus against Melophagus ovinus ectoparasite.

Result: The essential oils showed promising insecticidal activity against sheep ked. C. citratus essential oil has higher antiparasitic activity than E. globulus essential oils. C. citratus oil showed 94.7%, 87%, and 90.2% parasite reduction of sheep ked parasite at a concentration of 0.6254%, 0.3125% and 0.1563%, respectively. The C. citratus essential oil has no differential (p>0.05) insecticidal activity compared to diazinon at a concentration of 0.6254% and 0.1563%. Moderate antiparasitic activity was recorded in the groups of sheep treated by E. globulus essential oil.

Conclusion: Essential oils from C. citratus and E. globulus are potential plant sources of insecticides that can substitute diazinon for the management of M. ovinus in sheep. It is recommended that essential oils from C. citratus and E. globulus can be potential bio-rational insecticides in eco-friendly control of ectoparasite in sheep.

Key Words: Cymbopogan citratus, essential oil, Eucalyptus globulus, insecticide, Melophagus ovinus

Introduction

Ethiopia has the largest livestock population in Africa with 54.3 million cattle, 25.5 million sheep and 22.78 million goats (CSA 2013). As a result the country has huge leather production potential with 2.4 million hides, 10 million sheepskins and 7.4 million goat skins annually (MoA and ILRI 2013). The sector has been the backbone of the country’s economy by contributing large share to the GDP of the country through the export of finished and semi-finished products to the world, beside, supplying 16-18 million skins and hides for local tanners per annum (MoA and ILRI 2013). However, today, the benefits that this sector provide for the country is deteriorating mainly because of the rejection and down grading due to low quality raw materials (Kebede and Fetene 2012), mainly due to antemortem and postmortem factors, including poor animal husbandry, disease and parasites, bad slaughtering and flaying and bad practicing during curing, collection, transportation, storage and general handling (Abadi 2000). Pre slaughter stage of production was responsible for 65% of these defects of which the majority was due to a disease caused by ectoparasite (Kebede and Fetene 2012; Teshome, 2016). Ectoparasitic skin diseases of domestic ruminants that are caused by lice, sheep ked, ticks and mange mites are among the major problems that result in serious economic loss to smallholder farmers, the tanning industries and the country at large (Teshome 2016).

The prevalent ectoparasites on ruminants are tick (37.66%), mange (10.38%), lice (29.55%) and sheep ked (23.38%) (Teshome 2016). Melophagus ovinus (sheep ked) which accounts for 32.57% infestation of sheep was the main ectoparasite causing defects followed by Bovicola ovis (22.28%) (Tadesse et al. 2011). Highly potent insecticides are routinely used in Ethiopia for the control of sheep ked infestation (Zewdie 2010). However, indiscriminate use of chemical insecticides has led to insecticide resistance in parasite populations (Wall 2007; Lifschitz et al. 2008; Kumar et al. 2011). Besides their continues application has led to a number of environmental and health problems (Wall 2007). Cognizant of high prevalence of sheep ked among sheep farmers and problems related to synthetic chemical insecticides;
calls for search for alternative bio rational insecticides from natural sources which are safe, effective and environmental friendly. The use of herbal medicines
by traditional healers and the rural community at large for treatment of sheep ked parasite remains the mainstay of health care system for the majority of the population in Ethiopia. A previous study reported promising insecticidal activity of Cymbopogon citratus, and Eucalyptus globulus essential oils against M. ovinus (Gemeda et al. 2014). However, the study was conducted in vitro and no data is available on the insecticidal activity of these essential oils against M. ovinus in sheep (in vivo). This study was therefore conducted to evaluate the insecticidal activity of C.citratus (DC.) Stapf (Poaceae), Eucalyptus globulus Labill (Myrtaceae) essential oils in naturally M. ovinus sheep ked infested sheep.

Material and methods

Plant material collection, identification and extraction: C. citratus and E. globulus essential oils were extracted from aerial and leaf part, respectively in Wondo-Genet Agricultural Research Center, EIAR, Wendo Genet, Ethiopia. Fresh plant materials (250g) were placed in a 5L round-bottom distillation flask and the plant material was wetted with 3L distilled water. The essential oils were obtained by hydro-distillation using Clevenger-type apparatus continuously for 3 hours. The volatile oils were taken from the upper layer. The aqueous layer was further portioned using dichloromethane to extract and enrich the essential oil from the water layer. The organic layer (dichloromethane extract) was filtered and dried with anhydrous sodium sulfate and concentrate using rotary evaporator to give the crude essential oil.

Formulations: The formulations recipe of essential oils were optimized in the Formulation Laboratory of the Traditional and Modern Medicine Directorate, EPHI. The formulation recipes are as follows: Formulation 1: 2.5% E. globulus oil in 2% aqueous Tween 80; Formulation 2: 1.25% E. globulus oil in 2% aqueous Tween 80; Formulation 3: 0.625% E. globulus oil in 2% aqueous Tween 80; Formulation 4: 0.625% C. citratus oil in 2% aqueous Tween 80; Formulation 5: 0.3125% C. citratus oil in 2% aqueous Tween 80 and Formulation 6: 0.156% C. citratus oil in 2% aqueous Tween 80.

In Vivo Insecticidal Assay: Experimental study design recommended by World Association for the Advancement of Veterinary Parasitology was followed in this study (Holdsworth et al. 2006). Sheep of the same weight and age (6-12 months) with adequate natural infestation were bought from the Fiche Local market and housed indoors in individual cages and fed daily. Animals were acclimatized to the facilities and the feeding regime for 14 days prior to the treatment. The animals were identified by uniquely numbered ear tag. A randomized block design was used to randomly allocate the animals (n=6) into treatment and control (negative/Tween 80 and positive/diazinon) groups. The groups were maintained in isolation for the duration of the study to avoid contact with animals in any other group. On day 0, the numbers of live sheep ked found on 40 hair partings (10 cm wide) on four body sites (neck, shoulder, withers, flank and rump) were counted and recorded for each animal. All the treatment groups were treated by the essential oils at day-0 and day-14. Positive controls were treated only once with Diazinon at the start of the experiment (day 0) at a dose rate of 0.001%. The groups were selected by lottery method for each treatment as follows: Group 1: 1.25% E. globulus; Group 2: Diazinon; Group 3: 0.625% C. citratus; Group 4: 0.15625% C. citratus; Group 5: 2% Tween 80; Group 6: 2.5% E. globulus; Group 7: 0.3125% C. citratus; and Group 8: 0.625% E. globulus. Sheep ked parasite counting was done every week for 56 days. At each time point the mean live sheep ked count was taken for each group and the results compared with that of the untreated control group and positive control group. Responses to the treatment were monitored on alternate days at the time of drug application in terms of mean parasite count, and percent parasite reduction. Personnel involved in the collection of efficacy data were masked to the treatment assignment of the animals.

Data analysis: The in vivo sheep ked counts (every week for 56 days after treatment application) were recorded. Repeated measure analysis of variance was used to test for the effects of time and the time-treatment interaction. One-way analysis of variance (using Minitab 16.0 software) was used to test for treatment effects on each day and treatment groups were compared to the controls using Tukey test with a family error rate of 0.05. A probability of p<0.05 was considered to be significant.

Results

Table 1 shows M. ovinus reduction effect of C. citratus oil at different concentration on sheep on various days’ post treatment. As can be seen in Table1, differences between mean percent reductions as identified by Tukey’s Tests are displayed. Different concentration of C. citratus essential oils has comparable effect of reducing M. ovinus parasite with Diazinon after 7, 14, 28 and 49 days post treatment of sheep. Compared to standard drug used in the study, C. citratus essential oil at a concentration of 0.3125% has statistically lower (p<0.05) toxic effects on M. ovinus parasite in sheep on 7, 21, 35, 42 and 56 days of post treatment. On the
other hand, at all study points in post treatment day, all essential oil concentrations have statistically
no differential parasite reduction potency. The mean percentage reduction of parasite in sheep treated with C. citratus oils ranged between 73.8% – 94.7%, 38.2%–87.0%, 48.2%–90.2% at a concentration of 0.625%, 0.3125% and 0.1563 respectively.

Table 1: In vivo insecticidal activity of C. citratus essential oil formulation on naturally M. ovinus infested Sheep, Fiche, Oromia region, Ethiopia

<table>
<thead>
<tr>
<th>Sheep Ked percent reduction</th>
<th>Treatment dose (v/v)</th>
<th>2%T80</th>
<th>0.1000% D</th>
<th>0.625% C</th>
<th>0.3125% C</th>
<th>0.1563% C</th>
</tr>
</thead>
<tbody>
<tr>
<td>7 (PT)</td>
<td>39.5±26.4c</td>
<td>100.0±0.0a</td>
<td>75.5±15.6ab</td>
<td>38.2±21.2a</td>
<td>48.2±24.6c</td>
<td></td>
</tr>
<tr>
<td>14(PT)</td>
<td>36.1±21.9c</td>
<td>100.0±0.0a</td>
<td>73.8±14.9ab</td>
<td>87.0±12.5ab</td>
<td>61.6±22.1bc</td>
<td></td>
</tr>
<tr>
<td>21(PT)</td>
<td>25.2±20.3c</td>
<td>100.0±0.0a</td>
<td>91.5±9.0ab</td>
<td>75.5±7.4b</td>
<td>81.4±8.5ab</td>
<td></td>
</tr>
<tr>
<td>28(PT)</td>
<td>43.3±20.6c</td>
<td>100.0±0.0a</td>
<td>86.0±10.5ab</td>
<td>72.9±13.6ab</td>
<td>80.4±13.2b</td>
<td></td>
</tr>
<tr>
<td>35(PT)</td>
<td>36.1±16.8c</td>
<td>100.0±0.0a</td>
<td>93.2±5.5a</td>
<td>61.5±6.8b</td>
<td>84.4±10.1a</td>
<td></td>
</tr>
<tr>
<td>42(PT)</td>
<td>32.6±21.7c</td>
<td>100.0±0.0a</td>
<td>84.7±11.8ab</td>
<td>66.5±10.6b</td>
<td>65.6±23.2b</td>
<td></td>
</tr>
<tr>
<td>49(PT)</td>
<td>33.3±18.6c</td>
<td>100.0±0.0a</td>
<td>90.0±6.2ab</td>
<td>70.9±15.9b</td>
<td>86.6±13.7ab</td>
<td></td>
</tr>
<tr>
<td>56(PT)</td>
<td>38.0±16.3c</td>
<td>100.0±0.0a</td>
<td>94.7±4.6a</td>
<td>71.9±7.7b</td>
<td>90.2±11.9a</td>
<td></td>
</tr>
</tbody>
</table>

C: C. citratus; D: Diazinon; PT: post treatment; T: Tween; Values are expressed as percent reduction mean ± SD; mean percent reductions that do not share letter in the same rows are significantly different (P<0.05)

As indicated in Table 2, E. globulus essential oil has moderate insecticidal activity on M. ovinus on sheep. Analysis of parasite on sheep on 14th and 28th days after spray of different concentrations of E. globulus essential oil formulations in 2% Tween 80 on sheep have no differential (p<0.05) sheep ked reduction activity compared to Diazinon. The percent reduction of M. ovinus parasite in sheep at lowest concentration of E. globulus (0.625%) was statistically lower compared to Diazinon. The highest percentage of parasite reduction (82%) by this oil was observed at a concentration of 2.5% on 28th day post treatment of sheep, while the lowest was observed at 1.25% concentration of E. globulus oil on 7th day of post-treatment.

Table 2: In vivo insecticidal activity of E. globulus essential oil formulation on naturally M. ovinus infested sheep, Fiche, Oromia region, Ethiopia

<table>
<thead>
<tr>
<th>Sheep Ked percent reduction</th>
<th>Treatment dose (v/v)</th>
<th>2%T80</th>
<th>0.1000% D</th>
<th>2.5000% E</th>
<th>1.2500% E</th>
<th>0.6250% E</th>
</tr>
</thead>
<tbody>
<tr>
<td>7 (PT)</td>
<td>39.5±26.4c</td>
<td>100.0±0.0a</td>
<td>43.9±7.6c</td>
<td>38.9±29.3c</td>
<td>53.9±29.7bc</td>
<td></td>
</tr>
<tr>
<td>14(PT)</td>
<td>36.1±21.9c</td>
<td>100.0±0.0a</td>
<td>78.1±13.2ab</td>
<td>61.5±17.0b</td>
<td>54.2±21.9bc</td>
<td></td>
</tr>
<tr>
<td>21(PT)</td>
<td>25.2±20.3c</td>
<td>100.0±0.0a</td>
<td>62.5±17.8b</td>
<td>61.5±17.0b</td>
<td>54.2±21.9bc</td>
<td></td>
</tr>
<tr>
<td>28(PT)</td>
<td>43.3±20.6c</td>
<td>100.0±0.0a</td>
<td>82.3±14.5ab</td>
<td>54.4±14.4cd</td>
<td>43.2±20.6bc</td>
<td></td>
</tr>
<tr>
<td>35(PT)</td>
<td>36.1±16.8c</td>
<td>100.0±0.0a</td>
<td>68.6±14.0b</td>
<td>48.4±25.3bc</td>
<td>51.1±15.4bc</td>
<td></td>
</tr>
<tr>
<td>42(PT)</td>
<td>32.6±21.7c</td>
<td>100.0±0.0a</td>
<td>69.5±21.8b</td>
<td>60.4±14.3b</td>
<td>56.0±13.4c</td>
<td></td>
</tr>
<tr>
<td>49(PT)</td>
<td>33.3±18.6c</td>
<td>100.0±0.0a</td>
<td>71.2±17.5b</td>
<td>67.9±9.3b</td>
<td>54.0±18.4bc</td>
<td></td>
</tr>
<tr>
<td>56(PT)</td>
<td>38.0±16.3c</td>
<td>100.0±0.0a</td>
<td>78.9±16.6bc</td>
<td>58.8±11.6bc</td>
<td>38.0±16.3c</td>
<td></td>
</tr>
</tbody>
</table>

E: E. globulus; D: Diazinon; PT: post treatment; T: Tween. Values are expressed as percent reduction mean ± SD; mean percent reductions that do not share letter in the same rows are significantly different (P<0.05)

The two aromatic plant essential oils tested in this study showed parasite reduction activity in sheep against M. ovinus (Fig 1). The figure demonstrates that E. globulus oil has considerable lower sheep ked reduction activity compared to C. citratus, though the concentration used for E. globulus (0.625%) is fourfold higher than that of C. citratus (0.1563%). A significantly high sheep ked parasite reduction was observed on day 35 and 49 by C. citratus essential oil compared to E. globulus oil. Analysis of the data revealed that C. citratus essential oil has comparable reduction effect to Diazinon while E. globulus essential oil showed significantly lower (p<0.05) percent reduction.
Discussions
In the present study, six essential oil formulations from two medicinal plants were examined on sheep naturally infested by sheep ked. *C. citratus* essential oil has statistically comparable insecticidal activity in vivo with synthetic insecticide Diazinon. Synergy between the complex chemical constituents in the essential oil may be responsible for this insecticidal activity or may be due to potency of major compounds against *M. ovinus*. This finding showed *C. citratus* essential oil have similar activity with that of synthetic insecticide and can be the best candidate to alternate synthetic insecticides which corroborates the previous study in which showed *C. citratus* oil has comparable results with a synthetic insecticide Ivermectin (Gemeda et al. 2014). This finding also corroborates with the previous study of various researchers. Freitas et al. (2010), Vera et al. (2014) and Kabera et al (2011) reported insecticidal activity of *C. citratus* essential oils and its major constituents against adult and larvae of insects. The insecticidal activity of *C.* attributed to its major constituents citral, or its isomers geranial and neral (Freitas et al. 2010). The essential oil and its constituents have recognized wide variety of biological activity against house fly (Sinthusiri and Soonwer 2013), tick (Singh et al. 2014), mite (Hanifah et al. 2011),pest (Ayyaz et al. 2010, Olorunniola et al 2014, Sonker et al. 2014), antimicrobial (Sonker et al. 2014), mosquito (Soonwera and Phasomkusolsi 2014) and leishmanias (Machado et al. 2012).

Insecticidal activity was observed against *M. ovinus* sheep ked parasite by *E. globulus* essential oil in in-vivo model. Moderate parasite reduction by the essential oil from the eucalyptus may be due to phytochemicals including the major constituents 1, 8-cineole that could affect the nerve of the sheep ked leading to a disruption of vital system (Slimane et al. 2014). This finding was in line with previous study of Gemeda et al. (2014) that reported insecticidal activity of *E. globulus* oil against sheep ked. Song et al. (2016) also reported the effect of eucalyptus oils against stored mite and grain insects where they found fifty percent toxicity. The plant has insecticidal activity against egg, larvae and adult stage of insects (Maciel et al. 2009; Kumar et al. 2012). Moreover, other biological activities were reported including acaricidal activity (Pirali-Kheirabadi et al. 2009).

Superior insecticidal activity by *C. citratus* essential oils may be attributed to major constituents citral and other active compounds including myrecene, citronellal and neral found in the oils. *C. citratus* oil has better insecticidal activity against sheep ked in the present study than *E. globulus* essential oil. The same conclusion has been reported in the study conducted by a previous study (Gemeda et al. 2014), where they reported high insecticidal activity of *C. citratus* essential oils against *M. ovinus*. In a similar study, Bossou et al. (2013), *C. citratus* has the best insecticidal activity against *Anopheles gambiae* compared with other tested essential oils.

Conclusion
This study that was conducted to evaluate in vivo insecticidal activity of two aromatic medicinal plants at three different concentrations against sheep ked showed promising results. The essential oil from *C. citratus* showed the highest insecticidal activity against *M. ovinus* at lowest concentration tested. Moreover, moderate insecticidal activity was observed by *E. globulus* essential oil against *M. ovinus*. These aromatic plants could be potential sources of biorational insecticides against ectoparasites.
Further study is needed to evaluate the stability of the formulations and dosage validation using community based study.

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