

Effectiveness of selected medicinal plants used for management of ticks in cattle in Western Uganda

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ABSTRACT

Tick management among cattle farmers in Western Uganda has become an outstanding problem based on the fact that there is increasing resistance to most of the synthetic acaricides. Several herbs have been mentioned for management of ticks in different parts of the world, and they have been tested for acaricidal properties. This (experimental) study evaluated the in vitro investigation of the efficacy of *Phytolaca dodecandra* L'Her, *Azadirachta indica* A. Juss., *Vernonia amygdalina* Del. and *Tephrosia vogelii* Hook. f. in the management of ticks in Western Uganda. Cold and hot extracts of these herbs were used in comparison with Duodip a synthetic acaricide commonly used by farmers in the management of ticks within the region. Results indicated that there was a significant difference in the effectiveness of the different herbal extracts with cold extracts showing better efficacy ($p < 0.0001$) than the hot extracts ($p = 0.12$) at highest concentration used of 75 mg/ml. The mortality rate of ticks when Duodip was used was 20% which was much lower than that of the medicinal extracts used which gave a mortality rate of 30% and above for the different medicinal plants. The use of medicinal plants therefore could be a better option in the management of ticks if well concentrated. There is need for more in vivo studies to establish the possible dermatological effects on the animals if these herbs were to be used effectively as acaricides.

Keywords: Medicinal plants, phytochemistry, acaricides, cattle, Duodip.

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INTRODUCTION

Tick management among cattle farmers in western Uganda has become an outstanding problem based on the fact that there is increasing resistance to most of the synthetic acaricides (FAO, 2018). Ticks are the most prevalent parasitic arthropods affecting cattle with tick borne pathogens which are responsible for several tick borne diseases in Western Uganda (Muhanguzi et al., 2010). They cause physical damage and other tick borne diseases which are responsible for the ill health and mortalities of cattle. Ticks are considered as the most damaging livestock pests on a global scale and are responsible for a great diversity of livestock health problems (Gakuya et al., 2011). Over 80% of the cattle population in the tropics is at risk of tick infestation and tick-borne diseases (Eyo et al., 2014). Their infestation alone can give rise to severe anaemia, inflammation and swelling at the bite site, irritation and trauma which

results in substantial economic losses from reduced milk yield, skin and hide damage as well as reduced weight gain (Vudriko et al., 2017). As vectors of pathogens, ticks are second only to mosquitoes and are responsible for the transmission of protozoan, bacterial and viral diseases among domestic animals (Rajput et al., 2006). It is estimated that the annual cost of East Coast Fever in the smallholder dairy system in Kenya and Tanzania is at US\$ 54.4 and 4.41 million respectively. In the traditional cattle rearing system in Kenya and Tanzania the annual cost is estimated at US\$ 34.1 and 129.5 million respectively (Minjauw and McLeod, 2003).

In Uganda, tick control costs over 50% of the farmers running costs (FAO, 2018). This has incurred a great deal of economic losses among the farmers arising from mortalities and expenditure on the multiple usage of acaricides (Vudriko et al., 2017). Tick management and

control in this region has been a combined effort for most stakeholders including farmers, extension workers, the government and non-governmental organisations (FAO, 2018). Most of the conventional way of management of these ticks has been through use of synthetic chemicals; however this method has had its short comings especially the ticks' resistance to conventional chemical control methods. Multiple acaricide resistance has been reported in Uganda and is likely to affect future chemical control methods on ticks (Vudriko et al., 2017). In addition to chemical resistance, the chemicals pose a high risk to the environment and may lead to hazardous public health effects on living organisms especially to the users of cattle products. Several herbs have been mentioned for management of ticks in different parts of the world for example *Cymbopogon martini* (Roxb) Wats, *Eucalyptus globulus* Labill, *A. indica* and *Cinnamomum camphora* Nees & Eberm have been tested for acaricidal properties in India (Greeshma et al., 2018). The advantage of using medicinal herbal extracts are that they are biodegradable, less toxic to the environment and to non-targeted species when compared with chemical agents that accumulate and pollute the environment. Other researchers have obtained promising results in controlling ticks by using botanicals (Fouche, 2017).

A study in Ethiopia indicated use of herbal preparations in the management of ticks. Preparations of *Capsicum* spp, *Euphorbia obovalifolia* A.Rich, *solanum incanum* L and *Ficus brachypoda* Miq have been found to have 30 to 100 % acaricidal properties. Subsequently, *in vivo* treatment trials of these preparations were conducted using indigenous *Bos indicus* cattle naturally infested with ticks. Results obtained indicated that treatments at the rate of once per day for five consecutive days with the latexes of *E. obovalifolia* and *F. brachypoda* could reduce tick burdens by up to 70% on cattle (Regassa, 2000). Leaf extract of *T. vogelii* is used as a low cost acaricide in central Kenya (PACE, 2013) which indicated that the mortality induced by extracts of *T. vogelii* was often very high. There is a mortality rate of 95 and 100% using concentrations of 10 and 20 mg/ml of leaves of the varieties of *T. vogelii* against *Rhipicephalus appendiculatus* (Kalume et al., 2012). The phytochemical screening of the leafy stems of *T. vogelii* revealed the presence of catechol tannins, saponins, reducing compounds, leucoanthocyanins, and sterols-polyterpenes. Tannins showed significant acaricide potential on the cattle tick, *R. Boophilus microplus* (Fernandez et al., 2010).

According to Ogutu et al. (2012), the leaf extract of *P. dodecandra* contains alkaloids, saponins, terpenoids and phenolics. *V. amygdalina* produces a variety of flavonoids and bitter sesquiterpene lactones which contribute to the bioactivities of this plant. In a study done in South Africa, the best acaricidal activity (97.8%) was observed for the whole plant extract of *Monsonia angustifolia* E. Mey which also had the highest mortality of both the ethanol and acetone extracts and then followed by the leaf

extract of *Schkuhria pinnata*. Lam (86.7%), the root, leaf and fruit extracts of *Setaria italica* L and the leaf extract of *Cleome gynandra* L (77.8%) (Fouche, 2017). The use of medicinal plants is a wide spread worldwide alternative eco-friendly way of managing ticks in cattle especially in India (Greeshma et al., 2018). Some herbs have been mentioned to manage ticks in different parts of the world for example *V. amygdalina*, *P. dodecandra* and leaves of *Lepidium sativum* L (Regassa, 2000). Some other aromatic herbs like *Cymbopogon citratus* Stapf and *Lantana camara* L have been mentioned for their repellent properties against ticks (Wanzala et al., 2014). The current research targets to test and identify the phytochemicals in the selected medicinal plants used in control of ticks, the most efficacious medicinal herb and its appropriate concentration in the control of ticks. The results from this study could be a basis on which to develop an alternative eco-friendly and cost effective interventions to control ticks within Uganda.

MATERIALS AND METHODS

Scope of the study

The research project was carried out in MUST pharmaceutical laboratory. Phytochemical screening of the selected medicinal plants was done after which an in-vitro study was done to test for their efficacy in management of ticks. Different concentrations were used for each of the extracts to determine the most effective concentration in the management of ticks.

Plant materials collection and preparation

Fresh leaves of *P. dodecandra*, *A. indica*, *V. amygdalina* and *T. vogelii* were tested for acaricidal properties. The plant materials were collected and air dried under room temperature. The dried samples were then ground into coarse powder. Extracts were obtained by immersing 250 g of each powdered plant material were added to 2 litres of cold distilled water and the mixture for about 48 hours while shaking periodically. 250 g of each powdered plant material was also infused in 2 litres of hot water in a range of 60 to 95°C for 30 min. The extracts were filtered and then air dried. The percentage yield of each of the extracts was calculated from $\frac{W_1}{W_0} \times 100\%$ where W_1 is the weight of the extract after extraction, W_0 is the weight of the sample after drying.

Phytochemical screening

Phytochemistry was done using chemical methods as stated by Sofowora (1993) and Usman *et al.*, (2009). The phytochemicals that were tested in the plant extracts included tannins, phenols, terpenoids, alkaloids, flavonoids and saponins. The procedures of the tests that were carried out are as follows:

Test for alkaloids

To 2 ml of the extract, 1 ml of 0.1 M hydrochloric acid was added, mixture was shaken and then 3-4 drops of Dragendorff's reagent were added. An orange-brown coloration indicated the presence of alkaloids (Sofowora, 1993; Usman et al., 2009).

Test for terpenoids

To 2 ml of the extract, equal volume of trichloromethane was added and then 2 ml of concentrated sulfuric acid, a reddish brown coloration indicated the presence of terpenoids (Sofowora, 1993; Usman et al., 2009).

Test for phenols/tannins

To 2 ml of each extract, 1 ml of 5% ferric chloride was added. A deep blue-black precipitate indicated the presence of phenols and tannins (Sofowora, 1993; Usman et al., 2009).

Test for saponins

To 3 ml of each extract, 3 ml of water was added and the mixture shaken vigorously. A froth that lasted for more than 5 minutes indicated the presence of saponins (Sofowora, 1993; Usman et al., 2009).

Test flavonoids

To 2 ml of each extract, 1 ml of 10% sodium hydroxide was added. A yellow coloration indicated the presence of flavonoids (Sofowora, 1993; Usman et al., 2009).

Preparation of the concentrations

Samples of each medicinal plant were tested for acaricidal properties using different concentrations. Three different concentrations for the hot extract were used; 25, 50 and 75 mg/ml of water. Similar concentrations of cold water extracts were also used. This test was used to determine the most effective concentration as per each extract.

Herbal extract administration

The ticks were collected from infested areas, either from the cows directly or by passing a white cloth in farms where there was a high likelihood of tick infestation. Ticks were picked from four farms; two from Kashari Mbarara District and two from Nyakambu Buhweju District, where tick resistance had been reported by farmers. They were transported in big buckets with grass containing moisture which was covered with a muslin cloth to allow air saturation. Ten (10) cattle ticks (*R. appendiculatus*) were used for each of the experiments. The ticks were dipped in the prepared extracts, for 30 seconds and then removed and then placed on a porcelain dish. The mortality rate of ticks was observed in a period of 12 to 36 hours for each of the herbal extracts. A control experiment was done in which case the ticks were dipped in *Duo dip* a synthetic acaricide at a recommended concentration of 1 ml of Duodip per 1 litre of water, which is commonly used by farmers in Uganda. Close observation of the body parts using a magnifying lens was used to confirm whether the tick is dead or not. The numbers of ticks dead per each experiment were recorded.

Ethical considerations

Ethical approval was sought from Mbarara University of Science & Technology research Ethics Committee (MUST-REC). Farm owners were contacted and requested for permission to obtain tick specimen from their farms.

Statistical analysis

Analysis of variance (ANOVA) was used to test for differences in the efficacy of the herbal extracts between the treatment groups, different concentrations and the survival time after exposure to the extracts. Level of significance was set at 5% and $p \leq 0.05$ for statistically significant results.

RESULTS

Percentage yield composition

The percentage yield of each of the extracts was obtained, and results are shown in Table 1. Cold extracts of *P. dodecandra*, *A. indica* and *V. amygdalina* had the highest percentage yield compared to their hot water extracts. Only *T. vogelii* had its percentage yield of the hot extract higher than that of the cold one.

Phytochemistry

Phytochemical screening done indicated presence of phenols, tannins and alkaloids in all the plant extracts of *Azadirachta indica*, *Tephrosia vogelii*, *Vernonia amygdalina* and *Phytolacca dodecandra* (Table 2). Saponins were lacking in *Tephrosia vogelii* but present in the other extracts. Flavonoids and terpenoids were not found in *Vernonia amygdalina* and *Phytolacca dodecandra*.

Acaricidal properties

Mortality rates in Table 3 showed that the cold extracts were more effective in killing ticks more than the hot extracts. With hot extracts, there was no significant difference between the different medicinal plants used ($p = 0.13$) and different concentrations ($p = 0.2$). However, duration after exposure to the extract had an effect on the mortality of ticks though evidence was not strong ($p = 0.027$).

With the cold extracts, there was strong evidence that the longer the duration after exposure to the extract, the higher were the mortality rates ($p < 0.0001$). Mortality was observed up to 36 hours but by 24 hours most mortalities had occurred. However beyond the 36 hours no more deaths were recorded. The type of herb influenced mortality of ticks, which means that there was a significant difference between the different herbal extracts ($p < 0.0001$) and the different concentrations of each of the herbs ($p < 0.0001$). *P. dodecandra* showed the greatest efficacy with the highest mortality rate at 60% at its highest concentration of 7.5 g/ml of the cold extract. *A. indica* was the second in the effectiveness with 50% mortality rate, then *V. amygdalina* with 40% mortality rate and lastly *T. vogelii* at 30% mortality rate, all at their highest recorded concentrations of 7.5 g/ml of the cold

Table 1. Percentage yields of the different plant extracts used.

Medicinal plant extract	Percentage yield (%)	
	Cold extracts	Hot extracts
1. <i>Phytolacca dodecandra</i>	32	25
2. <i>Azadirachta indica</i>	30	23
3. <i>Vernonia amygdalina</i>	39	33
4. <i>Tephrosia vogelii</i>	25	28

Table 2. Phytochemicals present in the tested plant extracts.

Plant extract	Saponins	Phenols/Tannins	Alkaloids	Terpenoids	Flavanoids
<i>Azadirachta indica</i>	+++	+++	++	+++	++
<i>Tephrosia vogelii</i>	-	+++	+++	+	+
<i>Vernonia amygdalina</i>	+++	++	+++	-	-
<i>Phytolacca dodecandra</i>	+++	+++	+++	-	-

Key: (+++) indicates high abundance of the phytochemical, (++) moderate amounts of the phytochemical and (+) traces of the phytochemical. (-) indicates absence of the phytochemical.

Table 3. Mortality rates for different herbal extracts at different hours post exposure time.

Treatment	Concentration of herbal extracts (%)	Tick mortality rate (%) at different hours after exposure					
		Hot extracts			Cold extracts		
		12 hours	24 hours	36 hours	12 hours	24 hours	36 hours
1 <i>Phytolacca dodecandra</i>	2.5	0	10	10	30	30	30
	5.0	10	10	0	40	50	60
	7.5	10	10	30	50	60	60
2 <i>Azadirachta indica</i>	2.5	0	10	30	0	0	0
	5.0	0	10	10	0	30	30
	7.5	10	20	30	30	30	50
3 <i>Tephrosia vogelii</i>	2.5	0	0	0	0	0	0
	5.0	10	10	10	0	10	10
	7.5	0	0	10	0	30	30
4 <i>Vernonia amygdalina</i>	2.5	0	0	20	0	10	10
	5.0	10	10	10	0	0	0
	7.5	10	10	10	30	40	40

extracts. Duodip was used as a positive control and only indicated 20% mortality rate after the sixth hour through the time of observation. Distilled water which was used as a negative control indicated 0% mortality rate of the ticks.

DISCUSSION

The in vitro experiment of medicinal plants in the management of ticks produced positive results with all

the tested herbs causing a higher mortality rate on ticks than the conventionally used Duodip acaricide which is commonly used in Western Uganda. The high acaricidal activity observed in the aqueous extract of *Phytolacca dodecandra* is in agreement with previous studies on several species of genus *Phytolacca*, which have shown that aqueous extract of leaves of these plants carry bio-active compounds with acaricidal properties (Armstrong, 2009). Tannins which were reported to have acaricidal properties (Matos et al., 2017) were found present in all the plant extracts though their abundance

was quite different within the different plant extracts. Comparison of acaricidal properties of these medicinal plants grown in different geographical regions would give satisfactory results and determine the best way to conserve these plants appropriately for better efficacy.

There were some variations in the phytochemistry of the medicinal plants in this experiment with those of some studies previously done. For example in this experiment *Phytolacca dodecandra* lacked terpenoids and flavonoids which disagrees with Kosgei et al (2017) where terpenoids and tannins were present. Absence of flavonoids in *Vernonia amygdalina* disagrees with a study by Erasto et al (2006). These variations in the phytochemistry could be due to many reasons like the geographical location and the type of soils in which they were growing. These variations could be the reason for the differences in the efficacy of acaricidal activity of these medicinal herbs with other studies done on these plants. For example *Tephrosia vogelli* which reported 100% efficacy in Ethiopia (Abdisa, 2017) is in disagreement with this study.

Vernonia amygdalina showed moderate mortality rate at the highest concentration used of 75mg/mL compared to another study (Jelalu et al., 2020) where moderate activity was realised at a higher concentration of 100 mg/ml of the extract yet found no mortality at all at lower concentrations which agrees with this study. This study indicated that mortality increased significantly with increasing concentrations of the herbal extracts and increasing post exposure time to the extracts. This agrees with a study by Jelalu et al. (2020) in which case most mortalities occurred at post exposure time of 24 hours, though the most effective concentration used in this study was 100 mg/ml which was far above the concentration used in this research. However, the mortality rates were much lower which could be attributed the less time of exposure (30 seconds) compared to the 2 minutes which were used by Jelalu et al. (2020).

Generally the cold extracts had higher percentage yields than the hot extracts, and this is of advantage due to a minimised cost of obtaining the extract and convenience of using cold water. The difference in the effectiveness between the hot and cold extracts could be an indicator that the phytochemicals that possess acaricidal properties are sensitive to heat thus are easily denatured by high temperatures. In some studies done on effect of heat on phytochemicals, saponins were found to degrade when steamed at higher temperatures of 105°C (Le et al., 2015). The higher yields and better efficacy of the cold extracts over the hot extracts gives an advantage for convenience and cost effectiveness for the users in the management of ticks.

CONCLUSION AND RECOMMENDATIONS

The *in vitro* experiment on these medicinal plants have shown that they possess acaricidal properties thus can

be a source of raw materials for developing an eco-friendly acaricide given that they were able to show higher efficacy than one of the conventionally used acaricides. A study on their synergy would be of great importance in determining possibilities of improved efficacy in causing mortalities of ticks in cattle. Repeated *in vivo* studies on these herbs are required to test for their efficacy and thus their dermatological effects on the animals.

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Competing interests

The authors declared that there are neither competing nor conflict of interests that exist.

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