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Research Article

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In vitro acaricidal activity of *Azadirachta indica* and *Phytolacca dodecandra* leaves and their combination on *Rhipicephalus* (*boophilus*) *decoloratus*

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ABSTRACT

Rhipicephalus (boophilus) decoloratus (ticks) are causing great economic loss among the cattle rearers from cattle mortalities and costs during the treatments. Synthetic acaricides have been used for a long time in the management of ticks, however chemical resistance from multiple uses of acaricides as well as contamination of dairy products from these acaricides has caused the urgent need for alternative herbal drugs. This study evaluated *in vitro* activity of the combination of *Phytolacca dodecandra L'Her* and *Azardirachta indica* A. Juss against *R.* (*boophilus*) *decoloratus* (ticks) with a view to developing active, less toxic and non-resistant acaricides. *P. dodecandra* and *A. indica* were collected, dried after authentication, pulverised mechanically and extracted with cold water. Ticks were treated with the herbal extracts singly and then in combination with concentrations ranging between 50-100mg/mL of distilled water. *The anti-tick activity of P. dodecandra and A. indica was compared with* Duodip and Ametix which are synthetic acaricides currently being used by farmers in Western Uganda. Results showed that *P. dodecandra* extract gave the highest mortality of ticks (65%) compared with *A. indica (50%)*, Duodip (40%) and Ametix (5%) within a period of 12– 48 hours after exposure to the treatment. A combination of the two medicinal plant extracts (50%) did not show any significant difference in tick mortality compared to *P. dodecandra*. *Phytolacca dodecandra* has potential benefits thus may be used to obtain a natural eco-friendly acaricide for the management of ticks, however, a combination of the two medicinal herbal extracts has little synergistic effect compared to when *P. dodecandra* is used alone.

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INTRODUCTION

Back Ground

Ticks are blood sucking ecto-parasitic arthropods that affect cattle and are the cause of most tick borne diseases (TBDs) which have resulted in huge economic losses. Cattle mortalities and costs associated with the treatment of TBDs (Byaruhanga *et al.*, 2015) in addition to expenditures on synthetic acaricides, to which they have developed resistance have affected farmers' progress in many parts of the country. Synthetic acaricides have been reported to have a serious negative impact on the environment affecting the health of humans and other non-target organisms (de Mattos *et al.*, 2017), human acaricide toxicity was reported in Southwestern Uganda in a survey (Vudriko *et al.*, 2018)

While over 80% of the cattle population in the tropics is at risk of tick infestation and tick-borne diseases (Eyo *et al.*, 2014), in Uganda, tick management costs have gone up to over 50% of the farmers running costs (Ocaido, 2009). This has incurred many economic losses among the farmers' arising from mortalities with TBDs accounting for about 75.4% of (Byaruhanga et al., 2015). Repeated administration of acaricides and use of high doses has been reported resulting from tick resistance to the chemicals conventionally used (*Vudriko et al.*, 2016) though this has been attributed to factors like wrong dilution, application methods and increased acaricide pressure.

Alternative means of managing ticks are being studied including the use of herbal acaricides, vaccination, recommended tick control practices and use of endo symbionts have been suggested (Ghosh *et al.*, 2007; Abbas *et al.*, 2014). Medicinal plants have been studied and found to possess acaricidal properties(Abbas *et al.*, 2014; Abdisa, 2017; Jain *et al.*, 2021). Several medicinal plants like *Vernonia amygdalina* Del., *Calpurnia aurea* (Aiton) Benth, *Schinus molle*, *Ricinus communis* L, *Croton macrostachyus* Hoscht, *Nicotiana tabacum* L, *Phytolacca dodecandra L'Her, Azardirachta indica* A. *Juss.*, *Tephrosia vogelii* Hook and Solanum dasyphyllum Schumac (Van Puyvelde *et al.*, 1985; Kemal *et al.*, 2020; Catherine *et al.*, 2021) have been studied and found to have acaricidal and repellent properties while others like *Tagetes minuta* have been found to possess anti-moulting properties (Nchu *et al.*, 2012) but many could be inhibiting growth (Habeeb, 2010) alongside other effects. *Tagetes minuta*, *Tithonia diversifolia*, *Juniperus procera*, *Solanecio mannii*, *Senna didymobotrya*, *Lantana camara*, *Securidaca longepedunculata*, and *Hoslundia opposita* were cited in Kenya for having repellence effects against *Rhipicephalus appendiculatus* (Wanzala *et al.*, 2014).

Several studies have been done on medicinal plants used in the management of ticks for example in Ethiopia a study was done on the effect of essential oils of *Tagetes minuta* L., it was noted that about 60 % of the nymphs delayed moulting up to 25 days compared to the control (Nchu *et al.*, 2012). In another *in vitro* study, *P. dodecandra* L'Her, *A. indica* A. *Juss.*, *V. amygdalina* Del. and *T. vogelii* Hook were studied for their acaricidal properties and they all showed anti- tick properties and (Catherine *et al.*, 2021).

However, several of these *in vitro* studies have shown that medicinal herbs have greater efficacy than the conventional acaricides in the market for example *in vitro* studies indicated higher efficacy for selected medicinal plants against *R. appendiculatus* than Duodip which is a synthetic acaricide commonly used (Catherine *et al.*, 2021).

Carvacrol and thymol were some of the bioactive compounds that were found toxic against organo-phosphate resistant strains of R. microplus larvae (Costa-Júnior *et al.*, 2016). *Argemone mexicana* whole plant was found effective showing more than 50 % mortality of treated ticks; alkaloids, terpenoids, flavonoids and phenolics were analysed in the plant sample used (Ghosh *et al.*, 2015). *Azadirachta indica* and *Phytolaca dodecandra* among the many medicinal plants, have been studied for their acaricidal properties singly (Siyoum *et al.*, 2014; Catherine *et al.*, 2021) and have shown some percentage of efficacy in the management of ticks but the effect of their combination has not been studied.

There is a need to further study the possibility of increasing the efficacy of *P.dodecandra* while combining it with other potential medicinal plants with acaricidal properties. Therefore this study investigated the effect of combining *P. dodecandra and A. indica* at different concentrations while testing their invitro efficacy against ticks. The results from this study may enhance knowledge to researchers which will be a basis for the development of an eco-friendly herbal product for the management of ticks.

METHODS AND MATERIALS

Scope of the Study

The research project was carried out in Pharmaceutical Laboratory, Mbarara University of Science and Technology (MUST), Mbarara where live ticks were exposed to the plant extracts of different concentrations and later with the combination of the two medicinal plants. The observation was done in a period of 12 – 48 hours after exposing the live ticks to the herbal extracts.

Plant Materials Collection and Preparation

Fresh leaves of *P. dodecandra* and A. *indica*, were collected in the mid-morning hours. A sample of each fresh herbal material was taken to a botanist for proper identification and authentication before any processing proceeded. The leaves air dried under room temperature on wooden racks above the ground. The dried samples were ground into coarse powder. Extracts were obtained by separately macerating 110 g of each powdered plant material in 1000 mL of distilled water for 24 hours. Also, combination of *P. dodecandra* and A. *indica* was made by measuring 55g of each herbal powder which was combined to make 110 g of a mixture. The combination was then macerated in 1000 mL of cold distilled water for 24 hours. The combination were left for 24 hours with periodic shaking. The extracts were then filtered, concentrated *in vacuo* using a rotary evaporator at 50°C and lyophilised using freeze dryer.

Herbal Extract Administration

Samples of each medicinal plant and their combination were evaluated for acaricidal properties using different concentrations. Two different concentrations of 75 and 100 mg/mL of aqueous extract was used. The ticks were collected from infested farms directly from the cattle into a tin covered with a muslin cloth to ensure air saturation. Twenty (20) ticks were used for each of the experiments. The ticks were dipped into the prepared extracts for 1 minute and then removed. They were then placed on a petri dish. The mortality rate of ticks was observed in a period of 12 to 48 hours for each of the extracts. A positive control experiment was done in which *Ametix* and Duodip synthetic acaricides of 0.1 mL/100mL each was used based on standard and general use while distilled water was used as negative control. The number of ticks mortality per each experiment was recorded at different time intervals after exposing the ticks to the different treatments.

Mortality rates of the ticks were calculated and a comparison was made between the treatment groups and the control group.

RESULTS AND DISCUSSION

The result showed that from all the tested herbal extracts; *Phytolacca dodencandra* used against *Rhipicephalus (boophilus) decoloratus*, gave the highest activity with 65% mortality which was followed by the combination of *P. dodecandra* and A. *indica*

(50%) with A. *indica* alone being the least active (20%) after 48 hours. Duodip and Ametix which were used as positive controls gave 40% and 5% mortalities respectively. Distilled water that was used gave 0 % mortality as shown in figure 1.

In this study, *P. dodecandra* was more effective in the management of ticks than the other treatments that were used. The combination of *P. dodecandra and* A. *indica* were effective in causing 50% mortality of the ticks, but less effective than *P. dodecandra (65%)*. This could be affected by intrinsic drug interactions which reduced the activity of *P. dodecandra.* Combining the two extracts did not result into better results compared to when each is used singly. There is need for more studies about the type of interaction that may have occurred and the bioactive compounds involved that could have resulted into inhibition of the action of *P. dodecandra* by A. *indica*. *Phytolaca dodecandra* has been reported for *in-vitro* acaricidal properties, this study has further established the previous outcomes as promising acaricidal drug. The study is still on going to improve the activity through the combination with other plants in order to reduce the chance of resistance that may occur from mono-therapeutic approach of this plant. Mortality of ticks increased as the post exposure time increased from 12 hours to 48 hours (Table 1). Therefore, the extract applied should not be removed from the animal up to about 36-48 hours post exposure time. Means of retaining the extract on the skin of the animal need to be studied to increase on the exposure time for the extract onto the ticks.

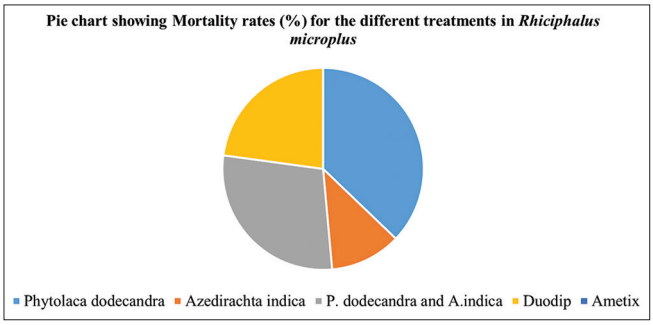

Figure 1: Showing mortalities of ticks after 48 hours post exposure time

Table 1: Tick mortalities between 12 to 48 hours after exposure to different treatments

Treatment	Concentration (mg/mL)	Mortality (%) of ticks recorded between 12 to 48 hours* post exposure time.				
		12*	18*	24 *	36 *	48*
P dodecandra	75	0	10	20	35	55
	100	10	10	40	50	65
A. indica	75	5	5	10	10	20
	100	0	0	10	10	20
P. dodecandra : *A. indica* (1:1)	75	0	0	5	20	20
	100	15	15	20	20	50
Duodip *(synthetic chemical)*	0.1mL/100mL	15	15	35	35	40
Ametix *(synthetic chemical)*	0.1mL/100mL	0	0	5	5	5
Distilled water	100ml	0	0	0	0	0

P. dodecandra has been found to possess acaricidal properties; several studies have been done and have consistently showed that it contains acaricidal and insecticidal properties (Onyango, 2016; Kumar, 2019). In this study, *P. dodecandra* was compared with A. *indica* and then the combination of the two medicinal plants in an *in vitro* study, *P. doecandra* showed higher mortalities of ticks. In some other studies, fruits of *P. dodecandra* in comparison with neem (A. *indica*) oil, where neem oil showed better efficacy than the *P. dodecandra* (Siyoum *et al.*, 2014). The difference is in the plant materials which were used in the experiment could be the result for the difference in the efficacy of the two medicinal plants. It was noted that the highest concentration that was used was 100mg/ml at which increased mortalities were noted for each of the herbal extracts used. This shows that *P. dodecandra* has potential benefits as an acaricide. The cytotoxicity of *P. dodecandra* using vero cells has been done (Kosgei, 2014) and thus may be used on the skin of cattle without causing any dermatological effects, though this remains an area of further investigation using *in vivo* studies.

In a study done by Mahran *et al.* (2020), A. *indica* showed mortality up to 100% contrary to this study where it caused a mortality of only 50%, but this could have been brought about by the difference in the immersion time which was up to 30 minutes compared to this study that used immersion period of only 1 minute during the *in vitro* test. The 1 min exposure time used in this study was likened to the dipping time used by farmers in the management of ticks using synthetic acaricides.

It is important to have *in vivo* report on *P. dodecandra* leaf in order to establish whether it would still cause mortality as the tick continues feeding while attaching to the animals, or if an anti-feedant activity could be introduced in the formulation against ticks.

CONCLUSION AND RECOMMENDATIONS

The combination of *P. dodecandra* and A. *indica* did not increase the effectiveness of *P. dodecandra*, thus combining the two medicinal plants may not be recommended as acaricidal drug. Therefore, *P. dodecandra* could be studied deeply in isolation for acaricidal activity so that an eco-friendly product against ticks is developed in future. Its anti-feedant properties need to be studied *in vivo* and other effects associated with its application.

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CONFLICTS OF INTEREST

Authors have declared no conflicts of interest.

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