In vitro repellency and contact bioassay of aqueous extracts of Cissus quadrangularis and Gomphocarpus physocarpus plants against Rhipicephalus evertsi evertsi ticks

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In vitro repellency and contact bioassay of aqueous extracts of *Cissus quadrangularis* and *Gomphocarpus physocarpus* plants against *Rhipicephalus evertsi evertsi* ticks

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Abstract

Background: The frequent use of acaricides to control ticks and inadequate flock management has led to the development of tick resistance to many acaricidal drugs. Ethno-veterinary plants with acaricidal activities have a major potential to combat this challenge because they are easily biodegradable, user-friendly, and less toxic to the environment and meat products. The objective of the study was to assess the ethnoveterinary properties of *Cissus quandrangularis. Lin* and *Gomphocarpus physocarpus E. Mey* to control ticks.

Results: The 6 % v/v of *Cissus quadrangularis* for each extract were more effective ($p<0.01$) against *Rhipicephalus evertsi evertsi* ticks. The repellency percentage was highest at 6 % v/v for acetone, methanol and control extracts similar to positive control Amitraz. The acaricidal efficacy of the *Gomphocarpus physocarpus* at 12 % v/v of methanol extracts was as good as that of 6 % v/v, however different to that of 18 % v/v was relatively low. The mortality rate of the control, acetone was similar ($p <0.05$) between 6, 12 and 18 % v/v at 24hrs. The mortality rate of the positive control reached 100 % after 72 hrs ($p < 0.05$) post-treatment, even though it was similar to that of acetone, methanol and control across different concentrations. The use of acetone and methanol extracts resulted in similar tick mortality at 12 and 18 % v/v at 24 hrs post-treatment. The methanol extract of *Gomphocarpus physocarpus* at 6 % v/v reached up to 100 % mortality at 72 hours similar to the positive control.

Conclusions: The bioassays indicated that there was a high efficacy percentage from the lowest concentrations (6 % v/v) of both *Gomphocarpus physocarpus E. Mey* and *Cissus quadrangularis. Lin* plant extracts, which was similar to the positive control (Amitraz) suggesting that suggesting that 6 % v/v could be sufficient for recommendations because less plant material is required.

Keywords: Acaricidal efficacy, bioactive compound, phytochemistry, tick mortality

1. Background

The abundance of ticks in the tropics and subtropics is a major challenge to goat productivity [1]. Ticks are prominent due to low veterinary services and lack of appropriate dipping systems for goats [1]. The increasing temperatures and humidity in most parts of the Southern Africa coupled with the abundance of wildlife that are main reservoirs of ticks create ideal conditions for tick proliferation [2] and survival. Ticks cause anaemia, body condition loss, damage to skin and hides [1]. Damages to teats and testes is also prevalent [3] and they transmit pathogenic viruses, rickettsia and protozoal diseases endemic to most parts of Southern Africa, particularly *ehrlichiosis* [1]. The frequent use of acaricides to control ticks and inadequate flock management has led to the development of tick resistance to many acaricidal drugs.

The use of plant-derived remedies that have repellency and acaricidal activities can be a sustainable alternative to address challenge of acaricide use. The application of plant-derived infusions, concoctions and ointments to livestock has been reported to repel and kill certain mites, mange, tsetse flies and ticks [4]. There is, however, paucity of information on the use of these ethnoveterinary plants to control ticks in goats. *Cissus quadrangularis* is one of the plants where almost all its parts are used in developing medicines. *Gomphocarpus physocarpus* (Apocynaceae) is a small, upright and occasionally-branched shrub usually grows to 0.5 to 2 m tall. It occasionally reaching up to 2.5 m in height. Despite the widespread and value of these plants for medicinal purposes, little information is available about their acaricidal properties. Ethno-veterinary plants with acaricidal activities have a major potential because they are easily biodegradable, user-friendly, and less toxic to the environment and meat products. These plants produce primary and secondary compounds that can be toxic [5] and repellent to ticks.

Plant remedies are a potential source of undiscovered compounds with high biologically active compounds against ticks and disease-transmitting vectors. Even though studies have been conducted to determine the efficacy of plant species such as *Aloe forex* and *Acokanthera oppositifolia* (Lam.) Codd [6; 7] in controlling ticks, however, the acaricidal and repellency properties of *Cissus quadrangularis* and *Gomphocarpus physocarpus* need to be determined. It could be cheaper and easier to use than conventional acaricides [4; 8]. Farmers use them but differently, it is important to standardise these so as to disseminate to other farmers without the knowledge. There is need to investigate the effectiveness of plant species in controlling ticks in goats [9; 2]. The objective of the study was to determine the in *vitro* repellency and contact bioassay of aqueous extracts of *Cissus quadrangularis Lin.* and *Gomphocarpus physocarpus E. Mey* plants against ticks. The study hypothesised that the use of plant aqueous extracts has no acaricidal effects against tick infestation in goats.

2. Methods

2.1 Study site

The study was conducted in the Animal and Poultry Science laboratory, University of KwaZulu-Natal. The study complied with the standards required by the Animal Research Ethics Committee of the University of KwaZulu-Natal (Reference Number: AREC/043/017).

2.2 Description of plant material

Description of reference dip

Eraditick 125 (Amitraz 12.5 % w/v) is a registered commercial, non-systematic organophosphate insecticide used by farmers to control external parasites in the study area. It was used as a reference (positive control). It is effective against ticks, lice and mange.

2.3 Plant collection and preparation

Fresh leaves of *Cissus quadrangularis* and *Gomphocarpus physocarpus* used by farmers to control tick infestation were collected in the bushes of Jozini area. Permission of plant collection was granted by the chief authorities and livestock association from Jozini Area. Following personal interviews with the chief authorities and livestock association, plant specimens were identified and collected. During collection of plant parts, leaves were collected as advised by farmers. The specimens were harvested, prepared, packaged and stored according to the herbarium rules and regulations. Plant specimens were authenticated at the Bews Herbarium, Department of Botany, University of Kwa-Zulu-Natal. The voucher specimens were as follows: *Cissus quadrangularis Lin* (NU0068142) and *Gomphocarpus physocarpus E. Mey* (NU0083347). Plants used were the most used plants by farmers in the study site and based on a survey result from Mkwanazi et al. [1]. The plant materials were thoroughly washed using distilled water and shaken to remove debris and tip off extra water droplets. Plant materials were macerated using an electric blender.

The prepared mixture was stored at room temperature overnight for 24 h before use and later strained using a muslin cloth. The concentration percentages of aqueous extract were determined to obtain a 6, 12 and 18 % (v/v) extract. The treatments were calculated to provide a wide range

of dosages that farmers normally use as described by Mkwanazi et al. [1]. Acetone and methanol solvents were used to make these different concentrations. Water was used as a control. The resulting extracts were stored in capped bottles in the refrigerator between 4 and 8 °C until use.

2.4 Phytochemical screening

Phytochemical screening of C. *quadrangularis* and *G. physocarpus* were carried out to assess the qualitative chemical composition of crude extracts. The plants were tested for the presence of tannins, alkaloids, saponin, flavoids and steroids. When testing for tannins 10 mg of each plant extract was dissolved in 45 % of ethanol in test tubes. Test tubes were then boiled for 5 minutes and 1 ml of ferric chloride solution added to each. The appearance of greenish to black colour indicated the presence of tannins in the extract. During the test of alkaloids the plant extract was mixed in 1 % v/v, then warmed and filtered.

The filtrate was treated with Mayers reagent (Mercuric chloride + Potassium iodide in water). The presence of alkaloids was shown by the formation of yellow coloured precipitates. During the test of saponin about 10 mg of each plant extract was diluted with 20 ml of distilled water in test tubes. Test tubes were hand-shaken for 15 minutes. Formation of a foam on the top part of a test tube indicated the presence of saponin. When testing for the presence of flavoids 10 mg of each plant extract was added in test tubes, and few drops of NaOH was added on each tube. The appearance of a yellowish colour showed the presence of flavonoids. In addition, when testing for steroids about 10 mg of each extract was added in test tubes, and 1 ml of concentrated H_2SO_4 added by the side wall of the test tube. The appearance of dark-reddish green colour indicated the presence of steroids in the plant extract.

2.5 Statistical analyses

For the analyses of tick repellency and mortality PROC UNIVARIATE (SAS, 2016) was used to check data for normality. The collected data on repellency and contact bioassay were analysed using PROC GLM procedure (SAS, 2016). In each case, ticks were used as an experimental unit. Turkey test was used to compare differences between treatment means. Differences among the least square means were considered significant at $p < 0.05$. The statistical model used was:

$$Y_{ijkl} = \mu + C_k + R_j + (C \times R)_{kj} + \varepsilon_{ijkl}$$

Where; Y_{ijkl} - is the response variable due to treatment (mortality and repellency);

μ - is the overall mean common to all observations;

C_k- is the effect due to concentration;

R_j - is the effect due to aqueous extracts;

$(C \times R)_{ikj}$ - is the interaction between concentration and extract;

ε_{ijkl} – is the residual error;

3. Results

3.1 Qualitative phytochemical screening

Phytochemical screening of both C. *quadrangularis* and *G. physocarpus* are shown in Table 1. Screening of methanol, acetone and water extracts of *Gomphocarpus physocarpus* revealed the presence of saponin, alkaloids, steroids, flavoids and tannins. The presence of these phytochemicals differ with the extraction medium used. Alkaloids, phenolic, tannins and steroids were highly present when extracted with methanol, while saponin became highly present when using water extracts. Extraction using acetone revealed the presence of flavoids, steroids and phenolic and tannins (Table 1). Highly present reflect that the particular colour that is supposed to reveal the phytochemical present in a plant is strong. While moderately present means that the phytochemical are not that strongly present. Low means that the presence of phytochemicals is there but in low amounts as reflected by the presence of light colour. If the colour that is supposed to appear, however is not there it is reflected as absent.

The screening of *Cissus quadrangularis* on the other hand revealed the presence of saponin, alkaloids, steroids, flavoids and tannins. In the methanoic extracts, alkaloids were highly present, while in the acetone the presence of alkaloids were moderately present. The water extract had low alkaloids present. Phenolic and tannins were highly present in all extracts. Flavoids were highly present in the acetone and methanol extract, while in water it was moderately present. In the acetone and water extract the saponin were low while moderately present for methanol. Extraction using acetone and water revealed the presence of low steroids. In the methanol extracts steroids were moderately present.

3.2 In *vitro* repellency bioassay

Tick repelling activity of *C. quadrangularis.Lin* at different concentrations are shown in Table 2. In *vitro* assay showed that both plants have positive acaricidal activity against ticks using different aqueous extracts. Repellency percentage of *Cissus quadrangularis* and different extraction solvents declined with time from 30 min to 5 hrs (Table 2). The 6 % v/v of *Cissus quadrangularis* for each extract were more effective ($p<0.01$) against *Rhipicephalus evertsi evertsi* ticks. The repellency percentage when extracting with acetone, methanol and control were similar at 12 % v/v. The repellency percentage of methanol reached up to 100 % for *Cissus quadrangularis* at 6 % v/v. In the 18 %, v/v concentration the repellency percentage was very low compared to 6 and 12 % v/v.

When using *Gomphocarpus physocarpus* plant the efficacy decreased with time ($p <0.01$) from 30 min to 5 hrs, respectively (Table 3). The repellency percentage was highest at 6 % v/v for acetone, methanol and control extracts similar to positive control Amitraz. The methanol extracts of *Gomphocarpus physocarpus* at 6 % v/v produced repellency percentage similar to that of 12 % v/v. The repellency percentage of positive control decreased with time from 30 min to 5 hrs. The acaricidal efficacy of the *Gomphocarpus physocarpus* at 12 % v/v of methanol extracts was as good as that of 6 % v/v, however different to that of 18 % v/v was relatively low. The methanoic extracts of *Gomphocarpus physocarpus* were so effective that it's reached up to 100 % repellency at 6 % v/v from 30 min to 1 hr. There was lower repellency activity from acetone and methanol at 18 % v/v. Acaricidal efficacy at 12 % v/v for the control treatment was similar to that of 18 % v/v ($p > 0.05$), respectively. The efficacy of the control was the same ($p <0.01$) for *Gomphocarpus physocarpus* across all the concentrations from 3- 5 hours post-treatment.

3.2 Contact bio-assay

Table 4 shows the in *vitro* mortality of *Rhipicephalus evertsi evertsi* ticks against *Cissus quadrangularis.Lin* 72 hours post-treatment. The acaricidal efficacy of *Cissus quadrangularis* increased with an increase in incubation period (Table 4). The mortality rate of the control, acetone was similar ($p < 0.05$) between 6, 12 and 18 % v/v at 24hrs. The methanoic extracts produced similar efficacy with the control at 4 hrs post-treatment across the different concentrations ($p < 0.05$). The mortality rate of the positive control reached 100 % after 72 hrs ($p < 0.05$) post-treatment, even though it was similar to that of acetone, methanol and control across different concentrations.

Table 5 shows the in *vitro* mortality rate of *Gomphocarpus physocarpus* against ticks. Tick mortality at 6 % v/v for acetone, methanol and control at 24 hrs post-treatment were similar to that of positive control ($p < 0.05$). The use of acetone and methanol extracts resulted in similar tick mortality at 12 and 18 % v/v at 24 hrs post-treatment. There was a similar tick mortality rate across the methanol, control and positive control at different concentrations ($p < 0.05$). The methanol extract of *Gomphocarpus physocarpus* at 6 % v/v reached up to 100 % mortality at 72 hours similar to the positive control.

4. Discussion

Farmers in resource-limited areas use plant extracts to control parasites, including ticks. The use of repellent acaricides for goats protection against ticks, constitute an important prophylactic component of tick management strategy. Same applies to the contact acaricides, which are chemical agents meant to kill ticks and are toxic through contact action. In *vitro* techniques are preferred over in *vivo* methods due to their low cost, simplicity and rapid turnover [10]. Ethno veterinary plants have bioactive substances such as flavonoids, terpenoids, and alkaloids and phenolic that possesses acaricidal properties against ticks than commercial chemicals [11]. The search for alternative methods to control parasites including ticks envisages the importance of determining the efficacy of these plant remedies as it gives the dense understanding of the quality of the plants. Plant remedies used in the current study were identified to be the most used acaricidal agents against ticks.

The aqueous extracts of both *Cissus quadrangularis. Lin* and *Gomphocarpus physocarpus E. Mey* plants were acaricidal against *Rhipicephalus evertsi evertsi* ticks, though the pattern was not anticipated. It was expected that the highest acaricidal efficacy percentage would be from the highest aqueous extracts, however, the lowest concentration (6 % v/v) for both studied plant materials were most effective. Such findings are similar to Madzimure et al. [12] who reported the highest efficacy ratio from the lowest concentration of (5 % w/v). A probable explanation for these findings could be that the extraction process with both methanol and acetone contribute to the efficacy of the plant because they produce a more potent extract, which is similar to the control. Similar repellency percentage at 6 and 12 % v/v for *Cissus quadrangularis. Lin* could probably be influenced by the similar polarity of methanol and acetone [13]. The high presence of alkaloids,

tannins and flavoids in the methanol extract of *Cissus quadrangularis* could have contributed to repellency. Tannins have been reported to bind the glycoprotein of the tick cuticle and lead to mortality [14]. Zenebe et al. [15] reported that the phytochemical screening of *Cissus quadrangularis.Lin* using methanol extract showed the presence of flavonoids and phenols.

The observation that the methanol extract of *Gomphocarpus E.May* and *Cissus quandrangularis. Lin* the repellency percentage could reach 100 % at a shorter duration may be attributed to the ability of methanol to attract and also compromise the movement of ticks [13] efficiently. This findings are corroborated by Santhoshkumar et al. [16] who found that the aqueous extract of *C. quadrangularis* (stem) had acaricidal activity against *Rhipicephalus (B.) microplus*. Also, the finding that extracting using 12 % v/v *Cissus quadrangularis. Lin* at 1 hour was as good as that of 6 % v/v using methanol extract could be influenced by the presence of compounds such as β-sitosterol (1), (22E)-3-β-hydroxycycloart-22-en-24-one, uvaol, daucosterol, methyl-3,4-dihydroxybenzoate, emodin, 4-hydroxyphenyl-O-β-D-glucopyranoside, aloin B and rutin isolated from the methanol extract [17]. Luseba et al. [18] and Zenebe et al. [15] reported that the methanol extracts of stems of *Cissus quadrangularis.Lin* has shown to have antimicrobial activity. The moderate levels of alkaloids in the methanol extracts, which has the ability to affects the permeability of the cell membranes of ticks and cause vacuolization and disintegration [14].

The finding that methanol extracts of *Gomphocarpus physocarpus* at 6 % v/v produced repellency percentage similar to that of 12 % v/v could probably be influenced by the increase in polarity as the concentration increases. The observation that methanol extract of *Gomphocarpus physocarpus* was able to extract a wide range of phytochemicals such as alkaloids, phenolic

compounds, flavoids and asteroids could have contribute to the high solubility of methanol. These secondary metabolites are considered as the chemical components responsible for wide acaricidal activities for several ethnoveterinary plants [19; 14].

Delayed repellency activity observed from all extracts including positive control from 4-5 hours post-treatment could mean that as the time of application increases the polarity of the extracts decreases, which might indicate that a lower concentration and shortened duration (6 % v/v) provides the maximum activity for both *Gomphocarpus physocarpus E. Mey* and *Cissus quadrangularis .Lin* plants and is adequate to control ticks. The similar efficacy of the positive control amitraz and 6 % *Cissus quadrangularis. Lin* and *Gomphocarpus physocarpus E. Mey* after 30 min, 1 hour and 2 hours are difficult to explain. These findings, however corroborate that of Benavides et al. [20] in which a 5 % soapy aqueous seed extract of *Azadirachta indica* controlled *Boophilus microplus* tick as effectively as an amitraz-based acaricide. The observed similar repellency for the control treatment at 12 and 18 % v/v for *Gomphocarpus physocarpus* from 3-5 hours post treatment could probably be due to the extraction medium used as different mediums yield different results demonstrating the same strength as the positive control.

It is, however not precisely clear why the repellency efficacy at 18 % v/v from acetone and methanol was lower for *Gomphocarpus physocarpus.* It is possible that at a higher concentration of the extract the acaricidal properties of the plants were washed way such that they become less effective against ticks. These findings are corroborated by Adamu et al. [21] who reported that at higher dilutions the test extracts are moderately effective. The observed acaricidal efficacy of both *Gomphocarpus physocarpus E. Mey* and *Cissus quadrangularis. Lin* aqueous extract at different

concentrations increased with an increase in the incubation period for tick mortality rate are contrary to Zenebe et al [15] who was of the view that mortality increases with an increase in the relative concentration of the product.

The observed dose-dependent response across all extracts and concentrations for *Cissus quadrangularis* is similar to Madzimure et al. [12] who reported a dose-dependent response to acaricidal treatments from 24- 72 hours on tick mortality. The dose-dependent response of *Cissus quadrangularis. Lin* shows that the duration of the treatments can influence the efficacy of the material. The similar mortality rate at 6, 12 and 18 % v/v concentrations when extracted with water and acetone at 24 hrs could suggest that the main factors that influence the efficacy is the duration of exposure to the test material and concentration. Sanhokwe et al. [7] reported that the concentration of 30 and 50 % *Aloe forex* acetone extracts was effective in causing *Rhipicephalus decoloratus* and *Amblyomma hebraeum* tick mortality. Even though it was not expected for acetone to produce such good findings due to its reported low efficiency of solvation [22]. Adults ticks have a protective cuticle layer that is meant to protect the tick from dehydration and other physical and chemical effects.

This cuticle is important in the reproduction process as a site of pheromone production [23]. Therefore, acetone and methanol extract being exceptionally good solvents to active components of plants, they can dissolve the cuticle layer of ticks thus causing mortality. This could in part explain the high mortality rate observed, which was similar to the positive control (100 %), when extracted with acetone, methanol and control across different concentrations after 72 hours post treatment. Even though it was expected that the control will yield positive results because it is

328 regarded as a safe universal solvent for preparing traditional remedies. It is also worth noting that
329 several authors have raised concerns pertaining to the usage of water as a solvent because of high
330 polarity. The observation that at 6 % v/v the mortality rate of *Gomphocarpus physocarpus* due to
331 acetone, methanol and control at 24 hours was similar to the positive control could be the
332 influenced by high active compounds extracted. Even though the high mortality rate at 6 % v/v of
333 methanol extract is contrary to Sanhokwe et al. [7] who reported the highest mortality rate of 89
334 % using 50 % methanol extract of *A. oppositifolia*.
335
336 The unexpected finding that acetone and methanoic extract of *Gomphocarpus*
337 *physocarpus* produced similar tick mortality rate at 12 and 18 % v/v at 24 hrs post treatment is
338 difficult to explain. A low larvae mortality after 24 hours in the methanol extracts [23]. A plausible
339 explanation for these finding could be due to the number of active compounds extracted that
340 depend on the solvent and method of extraction used. The similar mortality rate of *Gomphocarpus*
341 *physocarpus* at 6 % v/v with positive control at 72 hours could be influenced by the presence of
342 hydroxyl (-OH) on the methanol formula (CH3OH), which contains a greater negative charge than
343 the methane structure, thus making it every effective solvent. It is imperative to note that although
344 organic solvents are known for their superiority for extraction of bioactive ingredients, nonetheless
345 in the search of least cost animal health products for resource-limited farmers, the use of organic
346 solvents is limited by affordability. Henceforth, it could of interest to explore the use of different
347 alcohols that are most frequently used as liquor in rural retail outlets.
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5. Conclusions

The study revealed that the plant materials the farmers use as acaricides vary in terms of their efficacy to control ticks. The efficacy of the different concentrations is influenced by the duration of the application and the type of extracts used. Even though at higher concentration (18 % v/v) the acaricidal plant treatments reduced low tick populations than expected, but the 6 % v/v treatments for both repellency and mortality of ticks were as good as a commercial acaricide. Thus the 6 % concentration is sufficient for recommendations to farmers because less plant material is required. While the current study corroborates that both *Gomphocarpus physocarpus E. Mey* and *Cissus quadrangularis. Lin* are effective in controlling tick populations, however, it would be of interest to further laboratory experiments to determine whether the plant extracts can reduce tick feeding, moulting and fecundity.

ABBREVIATIONS

Not applicable

DECLARATIONS

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The University of KwaZulu-Natal granted ethical clearance (Reference number: HSS/0852/017). The participants granted consent.

CONSENT TO PUBLISH

Not applicable.

AVAILABILITY OF DATA AND MATERIAL

The datasets generated and/or analysed during the current study are not publicly available due to cooperating producer privacy and confidentiality, but are available from the corresponding author on reasonable request.

COMPETING INTEREST

The authors declare that there is no conflict of interest.

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AUTHORS CONTRIBUTIONS

MVM, SZN and MC designed the study, MVM, SZN collected the data, MVM interpreted the data, and wrote the manuscript. All authors read and approved the final manuscript.

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