World Journal of Advanced Research and Reviews
eISSN: 2581-9615 CODEN (USA): WJARAI
Cross Ref DOI: 10.30574/wjarr
Journal homepage: https://wjarr.com/

(RESEARCH ARTICLE)

Check for updates

Acaricidal activity of Cola *nitida* aqueous and ethanolic stem bark extracts against *Otobuis megnini* (Acari: Argasidae)

Nkechi Catherine Nzekwe *, Uzoma Ozurumba Ferdinand Osuala and Oluchukwu Michael Nwachukwu

Department of biology, Federal University of Technology, Owerri, Nigeria.

World Journal of Advanced Research and Reviews, 2024, 23(02), 1162–1169

Publication history: Received on 28 June 2024; revised on 11 August 2024; accepted on 13 August 2024

Article DOI: https://doi.org/10.30574/wjarr.2024.23.2.2388

Abstract

The present study was designed to evaluate, Acaricidal activity of *Cola nitida* aqueous and ethanolic stem bark extracts against *Otobuis megnini* Stem bark extracts of *C. nitida* were used for In-vitro adult immersion test of soft ticks. Three graded concentrations of the extracts, 15%, 30%, and 45% were tested at different time intervals and tick mortality was recorded for 48 hours. Dieldrin was synthetic acaricide used while distilled water was used as control. Standard procedures were applied to screen the phytochemical constituents of the tested plant part. The phytochemical screening of *C.nitida* stem bark revealed the presence of alkaloids , flavonoids , saponins , steroids , tannins and cardiac –glycoside. The LC50 and LC90 activity of ethanolic and aqueous extracts of stem bark of C. nitida soft ticks at different concentrations were estimated after 48 hours. Dieldrin has the lowest LC50 (0.34mg/ml) and LC90 (1.34mg/ml), followed by 45% ethanolic stem bark extract LC50 (0.71mg/ml) and LC90 (1.30mg/ml), while 15% concentration of ethanolic extracts of leaves has the highest LC50 (4.23mg/ml) and LC90(6.21mg/ml). At 24 hours both 45% ethanolic concentration of stem bark and dieldrin (5%) gave the same mortalities, 60+1.8 and 60+2.4 respectively. Aqueous extracts showed less acaricidal effects than ethanolic extracts. Ticks killing activity of all evaluated plant extracts increases with increasing exposure time and concentration as well.

Keywords: Acaricidal; *C nitida*; Soft ticks; *O megnini*; Stem bark; Dieldrin

1. Introduction

Ticks are considered the second most significant vectors of life-threatening or disabling diseases for both humans and animals, following mosquitoes. [1]. In addition, ticks are known to transmit a wider range of infectious pathogens compared to all other groups of arthropods. Ticks are considered to be the primary ectoparasites of farm cattle in Nigeria due to their extensive infestation, which leads to substantial harm to hides and skin, as well as the transmission of diseases to their host [2]. Ticks and the diseases they spread are widely distributed and commonly seen in regions with warmer weather. The spinose ear tick, *Otobius megnini* is an economically important soft tick as it parasitizes livestock mostly [3]. *O.megnini* is a one host tick with larval and nymphal stages parasitic and adult stage non-parasitic. *O.megnini* can cause waxy exudate and severe inflammation in and around the ear ,and can lead to secondary bacterial infection which can cause irritation [4].Consequences of infestation with *O.megnini* are related to the trauma caused by blood feeding larvae and nymphs within the ear canal and associated discomfort .Local reactions at attachment sites include perivascular to interstitial dermatitis containing abundant eosinophils and neutrophils [4].Large numbers of spinose ear ticks can produce ulceration lesions .Animals may exhibit vigorous head-shaking .Ear scratching and rubbing of the head may lead to excoriation of the ear pinnae.[5].Infection of *O.megnini* with *Coxiella burnetii.*

The detriment of Spinose ear tick infestations seems due to annoyance and direct injury caused by immature instars within the external ear canal [6,4].

* Corresponding author: Nkechi Catherine Nzekwe

Currently, the eradication and control of ticks rely on the use of standard synthetic acaricides, including organochlorides, organophosphates, pyrethroids, amidines, macrocyclic lactones, benzoylphenlureas, and phenylpyrazoles [7]. The application of synthetic acaricides on animals and in the environment is widely employed as a predominant method in many regions across the globe [8]. The frequent utilisation of these compounds frequently leads to the emergence of resistance to acaricides, the buildup of chemical residues in food, and negative impacts on the environment [9]. These concerns have necessitated the investigation of alternative approaches, such as the utilisation of medicinal plants for the management of cattle ticks [10].

One example of a medicinal plant having phytotherapeutic properties is *Cola nitida*, which is classified under the sterculeacea family [11]. *C.nitida* is a prominent tree crop cultivated in Nigeria. Its cultivation is mostly constrained by ecological factors, since it is predominantly planted in the rain forest zones of southern Nigeria and the riverine sections of the savannah region [12]. `*C. nitida* is widely recognised for its multifarious use across the African continent. The substance possesses diverse therapeutic attributes and is implicated in the management of several ailments, including cough, asthma, and malaria [13]. *C. nitida* has been historically utilized for its stimulant effects. [14].

Limited research has been undertaken in Nigeria regarding the acaricidal properties of *C. nitida* extracts. The majority of scholarly investigations pertaining to these plants primarily focus on the examination of the seeds. The scarcity of studies pertaining to other aspects of the *C.nitida* tree necessitates the undertaking of this study endeavour, which is to determine acaricidal activity of *Cola nitida* aqueous and ethanolic stem bark extracts against *O.megnini*

2. Material and methods

2.1. Study area

This research was carried out at Federal University of Technology, Owerri Nigeria.

2.2. Study design

This investigation employed an experimental study design. A cross- sectional study was conducted from April to July,2023 to evaluate the acaricidal activity of *Cola nitida* aqueous and ethanolic *stem* bark extracts against *Otobuis megnini*, using in -vitro immersion method (IIM).

2.3. Plant Sample collection and Processing

Cola nitida stem bark was collected from a tree of *C. nitida* from Imo state Nigeria. The collected plant samples were taken to a taxonomist in the Department of Biology, Federal University of Technology Owerri for proper identification and classification. Then, the samples were chopped into smaller size and washed gently in a tap running water to remove accumulated dust, sand and dirt before air-drying for weeks. The dried samples were pulverized into fine powder using electric blender and sieved using a sieve with an aperture size of 100μm before extraction.

2.4. Collection of Spinose ear tick

Spinose ear tick was collected from two Abattoirs, Somachi Abbatoir at the back of shoprite, Egbu and Gariki abbatoir, obinze, both in Imo state. The soft ticks collected were placed in cardboard containers with perforated lids to allow ventilation and then transported to the laboratory. All collected soft ticks were identified to the species level on the basis of observed anatomical features using the taxonomical keys by [15].

2.5. Soxhlet extraction (Ethanolic Extraction)

This was carried out according to the methodology described by [16] with slight modification. Twenty grams (20g) of the pulverized stem bark sample of *C. nitida* was weighed in an electronic weighing balance and poured into a thimble, 70% ethanol was measured and transferred into a round bottom flask as the extraction solvent. Then, the Soxhlet apparatus was set-up and the reflex worked for 8-10 hours. Upon completion of extraction, the solvent was evaporated using a rotary evaporator to obtain a crude extract. The extracts was transferred into a screw-capped bijou bottle, corked tightly, labeled appropriately, and was kept in the refrigerator at 4^0C for further use.

2.6. Preparation of the concentration

The different extracts from the plant material will be reconstituted in clean water and different concentrations (15%, 30%, 45 %,) will be prepared and store in reagent bottles for immediate use.

2.7. *In-vitro* acaricidal efficacy test

The acaricidal activity of plant extracts against Spinose ear ticks was determined invitro using immersion method (IIM) described by [17]. The design was completely randomized with three groups each containing 20 Spinose ear ticks. Different sets of empty clean grease free 250ml beakers will be used for the set-up. Three different set-ups, each per extracts from stem bark of *C. nitida* will be set. 20ml of the prepared concentrations (15%, 30%, 45%,) of the extracts were measured using measuring cylinder into the empty 250ml beakers and labeled accordingly based on the extract and its concentration, The in-vitro test were started 16 hrs. after collection and identification 20 active Spinose ear ticks and 3ml of each extracts concentration were directly added in each petri- dish of the three replicate, Distilled water were used as control whereas dieldrin 5% concentration was used as synthetic acaricide used , The test solution were removed after two minutes contact time. Each tick in each petri-dish was closely observed for death under magnifying glass at 1hr, 3hrs, 6hrs, and 24hrs, time interval [18].

2.8. Statistical Analysis

The data generated were stored in Microsoft Excel database system used for data management. SAS (Statistical Analysis System) package version 20.1 was used and statistical significance was determined by one way analysis of variance (ANOVA), with multiple comparison tests. Results of the study were expressed in means, percentage ± standard error.

The LC_{50} and LC_{90} values of the extracts were determined applying the probit transformation data analysis for mortality at P values < 0.05 were regarded as significant.

3. Results

Table 1. Shows the screening, of *C.nitida* extract and, it revealed the presence of various phytochemical components. The stem bark of *C. nitida* extract were found positive for, alkaloids, flavonoids, saponins, steroids, tannins, and cardiac –glycosides. *C. nitida* stem bark contains large quantity of alkaloids, saponins, steroids. The presence of all these phytochemical constituents reveals that the plant exhibits medicinal as well as pharmacological activities.

Table 1 Phytochemical qualitative analysis of *C.nitida*

S/N	PARAMETER	*C.nitida* STEM BARK
1	Alkaloids	++
2	Flavonoids	++
3	Tannins	++
4	Saponins	++
5	Steroids	++
6	Cardiac-glycosids	+

Note: + = Present; ++= Present in large quantity.

Table 2 shows phytochemical quantitative analysis of *C.nitida* Stem bark. Data are shown in Table 2. The amount of alkaloids, saponins, steroids, tannins, and cardiac –glycosides,are 59.13,53.06.30.50,1.72,0.62,93.06,respectively.

Table 2 Phytochemical quantitative analysis, of *C.nitida*

S/N	PARAMETER	*C.nitida* STEM BARK
1	Alkaloids	59.3
2	Flavonoids	30.50
3	Tannins	0.62
4	Saponins	53.06
5	Steroids	1.72
6	Cardiac-glycosids	93.06

Table 3 shows LC_{50} and LC_{90} activity of Ethanolic stem bark extract of *C. nitida* on *O.megnini*.The LC_{50} and LC_{90} values (with 95% fiducial limits), of ethanolic stem bark extract of *C.nitida* were estimated as shown in Table 2. The lowest LC_{50} and LC_{90} were estimated at 45 % concentration of stem bark extract

Table 3 LC_{50} and LC_{90} activity of ethanolic stem bark extract of *C. nitida* on spinose ear tick

Concentration of Ethanolic extracts	**Activities (ppm)95% LC50 (LCL-UCL)**	**fiducial limit LC90 (LCL-UCL)**
Stem bark extract		
15%	2.42(1.96-2.71)	4.03(3.27-4.41)
30%	1.51(1.37-1.67)	2.14(1.97-2.38)
45%	0.39(0.29-0.75)	1.09(1.01-1.22)
Dieldrin	0.34(0.26-0.68)	1.34(0.96-1.48)
Distilled water	0.0(0.0-0.0)	0.0(0.0-0.0)

LC = Lethal concentration, LCL = Lower control limit, ULC = Upper control limit

Table 4 shows LC_{50} and LC_{90} aqueous extract of *C. nitida* Stem bark against *O.megnini* **.**

After 48 hrs., the LC_{50} and LC $_{90}$ values of the stem bark extracts of *C.nitida* against *O.megnini* were estimated. The LC_{50} and LC_{90} at 15 % concentration of stem bark aqueous extract, are 4.23 mg/ml and 6.21 mg/ml respectively as shown in Table 4. The LC_{50} aqueous extract of stem bark of *C. nitida* at 45 % were 1.07 mg/ml . Dieldrin has the lowest LC_{50} (0.34mg/ml) and LC_{90} (1.34mg/ml), followed by 45% ethanolic stem bark extracts, LC_{50} (0.71mg/ml) and LC_{90} (1.30mg/ml).

Table 4 LC_{50} and LC_{90} aqueous extract of *C. nitida* Stem bark against *O.megni*

Concentration of Ethanolic extracts	**Activities (ppm)95% LC50 (LCL-UCL)**	**fiducial limit LC90 (LCL-UCL)**
Stem bark extract		
15%	3.25(2.83-3.42)	5.01(4.73-5.40)
30%	2.01(1.78-2.19)	4.35(3.89-4.65)
45%	0.71(0.53-0.97)	1.30(1.00-1.50)
Dieldrin	0.34(0.26-0.68)	1.34(0.96-1.48)
Distilled water	0.0(0.0-0.0)	0.0(0.0-0.0)

LC= Lethal concentration, LCL= Lower control limit,ULC=Upper control limit.

Table 5 shows the In vitro acaricidal activity of Ethanolic stem bark extract of *C. nitida* on *O.megnini*

Mortality of spinose ear ticks treated with different concentration of ethanolic stem barks extracts of *C. nitida,* are shown in Table 5. All the concentrations of *C.nitida* failed to show any significance on spinose ear ticks after 30 minutes post exposure. There was significance difference between the concentrations and the control. Also, with the exception of 45% concentration and dieldrin (5%), the lower concentrations of the extract failed to show acaricidal activity at 1 hr.

Table 5 *In-vitro* acaricidal activity of Ethanolic leaf and bark extracts of *Cola nitida* on cattle soft ticks

Time	Number of ticks introducing	Concentrations and mortality				
		Ethanolic bark extracts		Dieldrin Distilled water		
		15%	30%	45%	5%	0%
30 Min	60	0 ± 0.0^{c}	0 ± 0.0^{c}	0 ± 0.0^{c}	4 ± 0.16^{a}	0 ± 0.0^{c}
1hour	60	0 ± 0.0^{c}	0 ± 0.0^{c}	2 ± 0.03^{b}	8 ± 1.1^{a}	0 ± 0.0^{i}
3 hours	60	4 ± 0.16^{e}	2 ± 0.01^{f}	8 ± 1.06^{cd}	14 ± 3.6^{a}	0 ± 0.0^{f}
6 hours	60	14 ± 2.6^{c}	8± 1.1^{e}	12 ± 1.32^{d}	20± 3.92^{a}	0 ± 0.0^{i}
12 hours	60	30 ± 5.3^{c}	18 ± 3.4^{f}	22 ± 3..76^{e}	21 ± 3.97^{b}	0 ± 0.0^{f}
24 hours	60	50 ± 4.75^{c}	54 ± 3.38^{b}	60 ± 1.0^{a}	60 ± 1.0^{a}	0 ± 0.0^{f}
48 hours	60	58 ± 1.80^{a}	60 ± 1.0^{a}	60± 1.0^{a}	60 ± 1.0^{a}	0 ± 0.0^{b}

Mean having different superscripts of alphabets along the row differ significantly according to Duncan Multiple Range Test at P< 0.05 level

Table 6 shows the In vitro acaricidal activity of aqueous stembark bark extracts of *C. nitida* on Spinose ear tick. All the concentrations of aqueous stem bark extracts of *C. nitida* showed no acaricidal activity at 30 minutes interval. At 6 hrs. the lowest concentrations (15 %) showed no significance, (P< 0.05). When compared to the Untreated control (distilled water), all the concentrations of the extracts showed significant (P<0.05), difference in mortality of ticks.

Table 6 *In- vitro* acaricidal activity of aqueous stem bark extracts of *Cola nitida* on *O.megnini*

Time	Number of ticks introducing	Concentrations and mortality				
		Aqueous stem bark extract			Dieldrin Distilled water	
		15%	30%	45%	5%	0%
30 Min	60	0 ± 0.0	0 ± 0.0	0 ± 0.0	1 ± 0.01	0 ± 0.0
1hour	60	0 ± 0.0^{c}	0 ± 0.0^{c}	6± 0.2^{b}	9 ± 1.2^{a}	0 ± 0.0^{c}
3 hours	60	0 ± 0.0^{d}	2 ± 0.03^{c}	6 ± 0.2^{b}	18 ± 3.4^{a}	0 ± 0.0^{e}
6 hous	60	4 ± 0.3^{d}	8 ± 1.1^{c}	10 ± 1.40^{c}	32± 5.45^{a}	0 ± 0.0^{e}
12 hours	60	12 ± 1.32^{e}	18 ± 3.40^{d}	22 ± 3.76^{c}	43 ± 7.04^{a}	0 ± 0.0^{f}
24 hours	60	24 ±4.03^{d}	34 ± 6.34^{c}	41± 6.33^{b}	57 ± 2.42^{a}	0 ± 0.0^{e}
48 hours	60	45 ±7.75^{c}	60 ± 3.36^{b}	60± 1.0^{a}	60 ± 1.0^{a}	0 ± 0.0^{d}

Mean having different superscripts of alphabets along the row differ significantly according to Duncan Multiple Range Test at P< 0.05 level

4. Discussion

In the past decades, the control of spinose ear tick faced major issues, such as the rapid development of resistance in targeted vectors and non-target effect on human health and the environment, due to the employ of synthetic acaricides and repellent. Plant products are a rich source of bioactive organic chemicals and offer an advantage over synthetic pesticides as these are less toxic, less prone to development of resistance and easily biodegradable [19]. Botanical pesticides are characterized by bioactive mixtures/extracts/compounds from plant materials, which serve as insecticides and repellent but also as bactericides, fungicides, herbicides, and nematicides [18].

In vitro testing is important during the early stages of research on new acaricidal products to determine whether they constitute therapeutic options for the control of spinose ear tick. Several plant species have shown in –vitro acaricidal activity against soft ticks [20]. The plant species evaluated in this study were selected using ethno pharmacological criteria. Until now, the acaricidal activity of *C. nitida* against spinose ear tick has not been tested and reported. This study evaluated the acaricidal activity of *Cola nitida* aqueous and ethanolic stem bark extracts against *Otobuis megnini.* In the phytochemical screening test stem bark of *C. nitida* was found positive for alkaloids, saponins, steroids, tannins,

flavonoids, cardiac-glycosides. Alkaloids, saponins, tannins, and steroids are presents in large quantities. This finding is in consistency with [21]. The extent to which a compound injures or kill the target parasite defines its toxicity level as an acaricide [22]. Data collected showed that extracts of *C. nitida* stem barks have a toxic effect on spinose ear tick, causing hemorrhagic swelling, darkening of the cuticle and skin lesions, as well as rapid (Two days post treatment), and increase in the rate of mortality. Various extracts from other plant species have been shown to exhibit acaricidal activity against spinose ear tick within two days. [23,24]. Moreover, the mortality rate of soft ticks was dose – dependent when treated with different concentrations of aqueous and ethanolic stembark extracts of *C. nitida.* This is in line with [25], who reported that difference between medicinal and a toxic effect of plant is often a matter of dosage.

The current study revealed that the mean mortality of spinose ear tick was increased significantly with increased dosage (concentrations) exposure time after in vitro treatment for the tested botanicals. The mortality in the treated tick group started from 1 hour after invitro immersion test, with peak mortality at 48 hours. All the *C. nitida* extracts at tested concentrations, induced significant acaricidal effect against Spinose ear tick compared with the control. The ethanolic stem bark extract of *C. nitida* presented the highest yield, among the extracts. The lowest was observed at 15% concentration of aqueous extracts of *C. nitida* leaves. Tick mortality was significantly increased starting from 1hr and 30 minutes post exposure of 45% concentration of stem bark extract and dieldrin respectively. From this study the extraction method with the best efficacy of control of *O.megnini* is the ethanolic extracts of *C. nitida.* This is because the ethanolic extract was able to extract most of the phytochemical constituents. It has been reported previously that many natural products have been low water solubility and need to be dissolved in organic solvents or surfactant agents before being used in experimental systems [26]. As with other arthropod, the body of soft ticks is caused by the cuticle which protects the body organs against mechanical pressure, desiccation, pathogens and offers attachment sites for the muscles. [27]. Passage of water and other molecules through the cuticle is restricted by a thin layer of wax (lipid), on the outer surface of the cuticle. Hence, the more non- polar a chemical compound is the greater will be its ability to penetrate the cuticle. Majority of the commonly used synthetic acaricides such as Pyrethroids, Fipronil, Ivermectin and Dieldrin are insoluble in water (Lipid – soluble). As such water is a cheap universal solvent, there may be need to use organic solvents to fully optimize the extraction process, since water has its polarity limitations. [20]. However different constituents of the plants screened may be responsible for the toxic effect of the extracts that caused mortality of cattle ticks. Due to the efficacy of this plant extracts, and availability in the rural area. The stem bark of this plant could be an excellent acaricidal option.

5. Conclusion

The present study has demonstrated the acaricidal activity of *Cola nitida* aqueous and ethanolic stem bark extracts against *O. megnini* (Acari: Argasidae).

at different concentrations and time intervals. The results of *C. nitida* stem bark extracts against spinose ear tick showed good acaricidal activity in killing the ticks 48 hours after treatment. Efficacy of the extracts increases with increasing concentrations and time intervals. This provides evidence that this bioacaricides can be used to control *O.megnini*.

Interestingly, ethanolic stem bark extracts of *C. nitida*, possess strong acaricidal activity compared to aqueous extracts of *C.nitida*. Thus, the plant extracts are a potential material for the development of new therapeutic for the control of *O.megnini*.

Compliance with ethical standards

Disclosure of conflict of interest

The authors declare no conflict of interest financial or otherwise.

Statement of ethical approval

The research was approved by research ethic committee of the School of Biological Sciences, Federal University of Technology Owerri. Cattle breeders head was informed and a verbal consent was given to the Cattle rears, explaining the benefit of the study, and to obtain tick specimen from their farms.

Statement of informed consent

Informed consent was obtained from all participant in this study before commencement.

References

[1] Breedlove, B. Up close with ticks. Journal of emerging infectious diseases. 2023, 29: 229.

[2] Adehan. S. B., Biguezoton. A., Adakal. H., Assogba. M. N., Gbaguidi. A. M, & Tonouheura. A. Acaricide resistance of Rhicephalus microplus ticks in Benin. African Journal of Agricultural resources. 2016,11:119.

[3] Ariyarathne S., Apanaskevich D. A., Amarasinghe P. H., Rajakaruna R. S., Diversity and distribution of tick species (Acari:Ixodidae) associated with human otacariasis and socio-ecological risk factors of tick infestations in Sri Lanka. Experimental and Applied Acarology .2016; 70: 99-123.

[4] Njaa, B.L., The ear (Chapter 20) J. F. Zachary (Edition) Pathologic Basis of veterinary Diseases (6th edition), Elsevier, St. Louis, Missouri (2017) pp. 1223.

[5] Saari, S. Canine Parasites and Parasitic Diseases; Arzchnida. Academic Press, London, UK, 2019 . pp. 187.

[6] Sonenshine, D. E., Roe, M. Chapter 1 - Overview: ticks, people and animals (2nd edition) Biology of ticks, Vol. 1, Oxford University Press, New York, 2013 pp. 122-167.

[7] Adenubi, O. T., Mcgaw, L. T., Eloff, J. N, & Naido. U. L, In vitro bioassays used in evaluating plant extracts for tick repellent and acaricidal properties; A critical review. Veterinary parasitology. 2018, 254: 160-171.

[8] Baran, A. L., Jahanghiri, F., Hajipour, N., Sparango, O. A., Narouzi, R., & Moharramnejed, S, Invitro acaricidal activity of essential oil and alcoholic extract of Trachyspermum ammi against Dermanyssus gallinae. Veterinary parasitology. 2020,278:109030.

[9] Ahmed M., Peiwen Q., Gu Z, Liu Y, Sikander A, & Hussain D.Insecticidal activity and biochemical composition of Citrullus colocyn, cannabis indica and Artemisia extracts against cabbage aphid (Brevicoryne brassicae). Veterinary parasitology. 2020. 10:522.

[10] Erukainure, O., Sanni, O., Ijomone, O., Ibeji. C. U., Chukwuma, C. I., & Islam, M. S.,The anti-diabetic properties of the hot water extract of kolanut (Cola nitida). In type two diabetic rats. Journal of ethnopharmacology. 2019.242: 112033

[11] Adebayo, L. D., Olumide, O. A., Awobajo, F. O., Adebayo, B.A., Adebisi, J.A., Bankole, T. J, & Opeyemi, A. G. Methanol extract of Cola nitida ameliorates inflammation and nociception in experimental animals. Neurobiology of pain. 2012,5:100027.

[12] DahNouvlessounon, P., Adjanohoun, A., Sina, H., Noumavo, P. A., & Diarrasouba, N. (). Nutritional and Anti nutrient, composition of three kola nuts (Cola nitida, Cola acuminata, Garcinia kola), produces in benin. Food and nutrition sciences. 2015, 6:1395-1396.

[13] Amadi, N. C, & Nwachukwu, I. W. The effect of oral administration of Cola nitida on the pharmacokinetic profile of metoclopramide in rabbits. BMC pharmacology and toxicology. 2020, 21 (4) 1-2.

[14] Walker, A. R., Bouattour, A., Camicas, J. L., Estrada. P. A., Horak, L. G., Latif, A. A., Pegram, R. G., & Preston, P. M,. Ticks of domestic animals in Africa; a guide to identification of species . UK Bioscience Reports. Edinburgh scot land U.K. 2014 pp,227.

[15] Mbembo M. B., Inkoto C. l., Jean-jacques O. A., Colette M. A., Jonas M. S., & Puis. T. Mini-review on the phytochemistry, pharmacology and toxicology of Cola nitida: A medically interesting bio resource of multiple purposes in Africa. Natural resources for human health.2022, 2(3). 335-342.

[16] Shyma, K. P., Kumar, S. & Sangwan, A., Acaricide resistance status of Rhicephalus (Boophilus) microplus and Hyalomma anatolicum. Collected from Haryana. India. Journal of Animal sciences. 2016, 83(6).591-592.

[17] Nwachukwu, M. O., Azorji, J. N., Ubah, V. C. S., Nwachukwu, P. C., Anochie, C. C. . Effects of Bioinsecticides made from plant leaves and pepper seeds on the growth of Spinach. FNAS Journal of scientific innovations. 2024, 5(3) 40- 45.

[18] Castro – Janer, E., Martins, J. R., Mendes, M. C., Namindome, A. M., Klafke G. M., &Schumaker, T. T. Diagnosis of fipronil resistance in Brazilian cattle ticks. (Rhicephalus microplus), using in vitro larval bioassay. Veterinary Parasitology. 2018.173:5.

[19] Adenubi, O. T., Mcgaw, L. T., Eloff, J. N, & Naido. U. L,. In vitro bioassays used in evaluating plant extracts for tick repellent and acaricidal properties; A critical review. Veterinary parasitology. 2018, 254: 160-171.

[20] Shehu S. A., M. L., Sonfada, A, Danmaigoro, A. A., Umar S. A., & Hena, I. M,. Kolanut (Cola acuminata) extracts as a substitute to histological tissue stain eosin. Scientific Journal of veterinary advances. 2012, 1(2). 33-37.

[21] Ribeiro, V. L. S., Dos Santos, J. C. Bordigon, S.A., Apel, M. A. Henrique, A. T., & Von Poser, G. L.. Acaricidal properties of the essential oil from Hezperozygus ringens (Lamiaceaa) on the cattle tick Rhicephalus microplus. Bioresource technology 2010.101.2506-2509.

[22] Fernandez-Sales. A., Alonso–Diaz, M. A., Acosta Rodriguez. R., Torres–Acosta, J. F. J., Sandoval-Costa, C. A., & Rodriquez –Vivas R. I., In vitro acaricidal effect of tannin–rich plants against the cattle tick, Rhicephalus microplus, (Acari: Ixodidae). Veterinary parasitology. 2011, 175: 113-118.

[23] Muhammed, K. O., Nabilla. I., Abdulaziz, A., Alam, Z., Habajara da Silva, V., Tetsuya, T., Abid. A. Acaricide Resistance in ticks: Selection diagnosis mechanisms and mitiation. 2022. 12:94-95.

[24] Mbembo M. B., Inkoto C. l., Jean-jacques O. A., Colette M. A., Jonas M. S., & Puis. T.Mini-review on the phytochemistry, pharmacology and toxicology of Cola nitida: A medically interesting bio resource of multiple purposes in Africa. Natural resources for human health. 2022. 2(3). 335-342.

[25] Akintola. P. O., Lateef. A., Asafa. T. B., Beukers. L. S., Hakeem. A. S., & Irsad. H. M, Multifunctional titanumdioxide nanoparticles biofabricated via phytosynthetic route using extracts of Cola.nitida antimicrobial dye degradation antioxidant andanticoagulant activities. Heliyon. 2020. 6: 4610