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Evaluation of acaricidal activity of Leaves of *Ficus exasperata* on dog ticks: *Rhipicephalus sanguineus*

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Abstract
The research investigated phytochemical constituents of leaves extract of *Ficus exasperata* (FE) and its acaricidal activity on dog ticks: *Rhipicephalus sanguineus.* Essential oils of FE were extracted using direct steam distillation (DSD) and tested for their relative toxicity as contact acaricides. In an *in-vitro* technique for evaluation of efficacy, percentage mortality of larvae of *Rhipicephalus sanguineus* is 100% at 0.15mg/ml of N-hexane extract and 100% at 0.24mg/ml of methanol extract of FE. Similarly, percentage mortality of the adult *Rhipicephalus sanguineus* is 100% at 0.15mg/ml of N-hexane extract and 98% at 0.18mg/ml of methanol extract. Lethal dose (LD50) of *Ficus exasperata* is 0.12. Chromatographic analysis of *Fiscus exasperata* revealed seven potential essential oil with insecticidal property namely *Neophytadiene*, Citronellyl propionate, Lavandulyl acetate, *Octadecanoic* acid *methyl ester*, *Yadanzioside*, Tridecanoic acid and *Pentanoic* acid, 4-*methyl*- (*Methylvaleric* acid).

Keywords: *Rhipicephalus sanguineus, Ficus exasperata,* methanol, N- hexane

Introduction
Ectoparasitism in animal and man has resulted into great economic loss. They cause direct effect on animal health and also serve as vectors of various diseases ranging from bacteria, viruses and protozoa [1]. Of zoonotic importance is Rickettsioses which has recently been recognized worldwide as emerging and reemerging arthropods-borne infectious diseases [2, 3] *Rhipicephalus sanguineus* being a main dog infesting ticks reported to have biohazards danger and public health importance [4, 5]. *Rhipicephalus sanguineus* have been associated with reemerging tick-borne human and animal infectious diseases such as babesiosis; Q-fever, Lyme disease and life-threatening arboviruses [6]. Dog ownership and high level of environmental parasitism by *R. sanguineus* ticks have been reported severally [7, 8, 9]. In Brazil, occurrences of human parasitism with *R. Sanguineus* especially in dog handler have been on increase [10, 8]. Outbreak of human parasitism by *R. sanguineus* in south-east Nigeria has also been reported [11]. Lack of effective drug often prevents the control of some of these parasitic diseases incremented with tick infestation. The commercially existing drugs have posted much adverse effect that limits their uses. Besides the high cost of treatment, drug-resistance and toxicity to non- target / environmental damage is a problem that remains to be tackled. However, plant-derived compounds are easily degradable and show little or no environmental impact with respect to synthetic drugs.
Plant-based pesticides have been suggested as suitable alternatives for arthropod control, largely due to presence of relatively safe bioactive chemicals with little or no risk to the environment, animal and human health. [12, 13].
Leaves of *Ficus exasperata* have been reported to possess antipyretic, anti-candidal, anti-microbial, insecticidal and pesticidal activities [14-17]. Leaves of *Ficus exasperata* was also reported to be in use to control ectoparasite in some poultry houses [17]. In this study we focused on major acaricidal screening of methanol and N-hexane leave extracts of *Ficus exasperata* whose characteristics comply with Article 23 of the Regulation (EC) No. 1107/2009 [18]. This extract was tested for its effect on mortality and oviposition of the brown dog tick *Rhipicephalus sanguineus*.

Materials and Method
- Extraction and concentration of selected medicinal plant with rotary vacuum evaporator.
- Evaluation and acaricidal assessment of methanol and N- hexane leave extract of *Ficus exasperata* potential against *Rhipicephalus sanguineus* of dog *in a vitro* experiment.
- Suggestive mechanism of action of the acaricidal plant

A. Selection of Indigenous Plant: The leaves of *Ficus exasperata* (Family: *Moraceae*) were collected, identified and authenticated in the Department of Botany with a voucher number UIH- 22635 and kept as herbarium specimen.

B. Extraction from the Plant: The leaves were washed and air dried for two weeks. Dried plant leaves were pulverized using a grinder. The powdered plant material (3 kg) was extracted using 10 litres of methanol and N-hexane cold extraction each for 72 hrs. and concentrated with rotary vacuum evaporator. Finally, the concentrated extract was lyophilized to remove traces of the solvent and collected in the glass tubes to test for acaricidal activity. Seven different dilutions of the extract at the rate of 0.06, 0.09, 0.12, 0.15, 0.18, 0.21 and 0.24 mg/ml were obtained. Asuntol (Organophosphate - ORG) served as a positive control and dimethyl sulfoxide (DMSO) as a negative control. Further fractions were isolated by column and vacuum chromatography using mobile phase (chloroform: ethyl acetate)

Collection of ticks

Fully engorged adult female ticks *Rhipicephalus sanguineus* were collected from the naturally infested adult dogs brought to five different Veterinary Clinics within Ibadan environs. The engorged female ticks were harvested from invested dogs with a history of no recent exposure to any acaricides. The harvested ticks were put in vials and labeled with all details of animals such as age, sex, breed and date of collection. Tick identification was carried out in the Parasitology Laboratory, Faculty of Veterinary Medicine, University of Ibadan, Nigeria. This study followed the guidelines stated by Animal Care and Use Research Ethics Committee, University of Ibadan (UI-ACUREC/App/17/0031).

Experimental Design

A. *In vitro* Screening Method

Larval sensitivity test

The sensitivity test was carried out on *Rhipicephalus sanguineus* larvae at the Veterinary Parasitology Laboratory, University of Ibadan. According to Food and Agriculture Organization [19, 20], double sheets of filter paper (4 cm^2 Whatman) were treated with varying concentration of methanol and N-hexane leave extract of *Ficus exasperata.* 0.05mg/mL of solution containing 3% dimethyl sulfoxide (DMSO) and 0.05mg/ml of 16% Asuntol used to treat Whatman paper as negative and positive control, respectively. Approximately 100 tick larvae were placed on one of the sheets and then covered with the other sheet to form a sandwich. The sandwiched filter papers and larvae were then placed in an envelope of folded non-impregnated filter paper (72.25 cm^2) and sealed with a plastic clothespin. The envelope was placed in an incubator and maintained at 28 °C with relative humidity (RH) 80% for 24 h. Alive and dead larvae were counted [21]. Test for every dilution was done in triplicates.

Adult immersion tests to assess the sensitivity of engorged females

The sensitivity of engorged *R. sanguineus* females was determined by using the immersion test [22]. A group of 10 ticks each were immersed for 5 min in varying concentrations of the leave extract ranging from 0.06 to 0.24 mg/ml. The engorged females were subsequently dried on a paper towel, placed in Petri dishes and maintained in a biochemical oxygen demand (BOD) incubator at 28 °C and RH 80% for 15 days. The lethal dose for 50% and 100% mortality of the larvae and adult females were calculated for test compounds and essential oils using Graph-Pad Prism 5.0 software.

Toxicity test

Acute toxicity test was conducted according to the Organization for Economic Co-operation and Development (OECD) guidelines [23]. Group A received body spray of 0.05 mg/ml of dimethyl sulfoxide (DMSO); group B received 0.05 mg/ml body spray of 16% Asuntol. Groups C, D and E received body spray of 0.06, 0.12, and 0.18 mg/ml of both methanol and N-Hexane leave extract of *Fiscus exasperata* separately. The rats were exposed once daily for three weeks. The animals were observed daily for any signs of toxicity on the skin and general body appearance.

Gas Chromatography Mass Spectrometry (GC-MS)

GC-MS (Agilent Technologies, Palo Alto, CA, USA) 5973 Network selective detector with column DB23 model number J&W 1222362 with internal diameter of 60 m×250 µm×0.25 µm (250^{O}C Max) was used for this analysis. 50 µL of the methanol extract of *Ficus exasperata* was dispensed into 1ml sample vial and diluted to ml with methanol. Total flow of 3.6 mL with column flow of 0.57 mL/min. was used under a pressure of 16.0 kPa at 50 ^{O}C GC temperature. The total runtime was 37 minutes.

Results

Toxicity Test

There was no sign of toxicity (skin bruise, irritation, salivation, incoordination and mortality) within 48 hours of treatment. A significant reduction in kidney and liver body weight ratio were recorded in the positive control group (Organophosphate) compare to the control group and a significant reduction in testes organ weight compare to the extract treated group (*Ficus exasperata*)

(A) Phytochemical analysis

Table 1: Phytochemical Analysis of the Leaves of *Ficus Exasperata*

Biochemical Test *Ficus Exasperata*	
Saponins	++
Tannis	+
Alkaloids	++
Cardiac Glycosides	++
Flavonoids	++
Terpenoids	-
Anthraquinones	-
Steroid	+

Key – Absent
+ Present
++ Highly present

(B1) Effect of percentage mortality on larvae of *Rhipicephalus sanguineus*

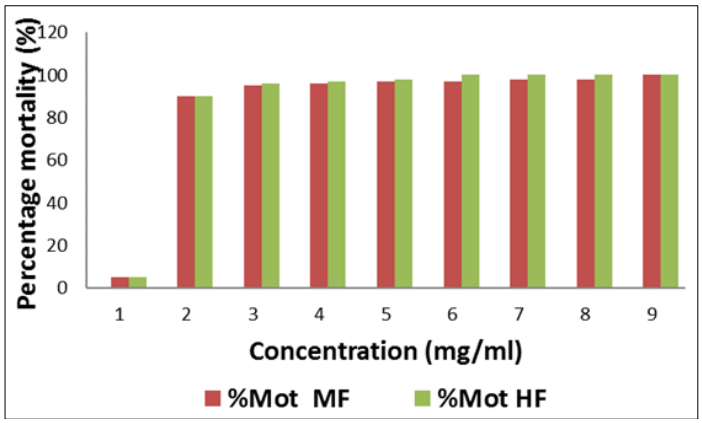

Fig 1: Effects of different concentrations of methanol and N-hexane extract of *Ficus exasperata* on larvae of *Rhipicephalus sanguineus*

(B2) Effect of percentage mortality on adult female *Rhipicephalus sanguineus*

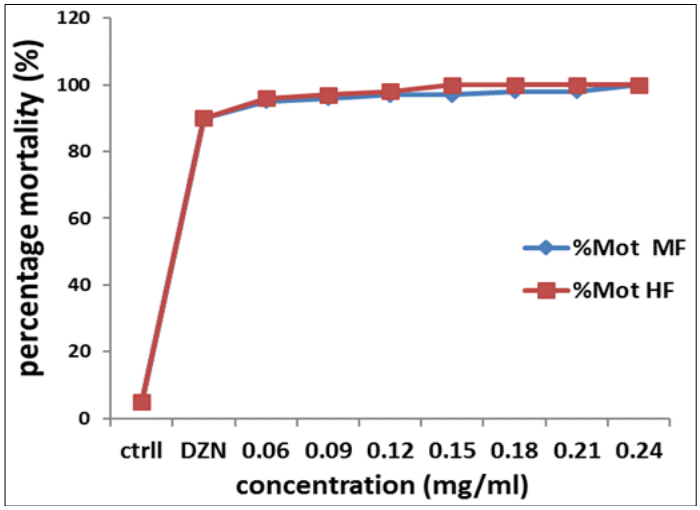

Fig 2: Effects of different concentrations of methanol and N-hexane extract of *Ficus exasperata* on adult female *Rhipicephalus sanguineus*

(C) Organ body weight

Table 2: The organ weight (grams)

Experiments	Liver	Kidney	Testes	Brain
CTRL	5.71±0.87	1.08±0.07	2.06±0.13	1.06±0.25
ORG	4.62±0.30[a]	0.93±0.08 [a]	1.85±0.07	1.50±0.01
FE1	5.05±0.47	0.92±0.12 [a]	2.10±0.23	1.54±0.10
FE2	5.18±0.25	0.96±0.03	2.16±0.14	1.54±0.09
FE3	4.27±0.57 [a]	0.99±0.06	2.27±0.27 [b]	1.58±0.13

(D1) Gas Chromatography Mass Spectrometry (GC-MS)
Detected compounds at different retention time

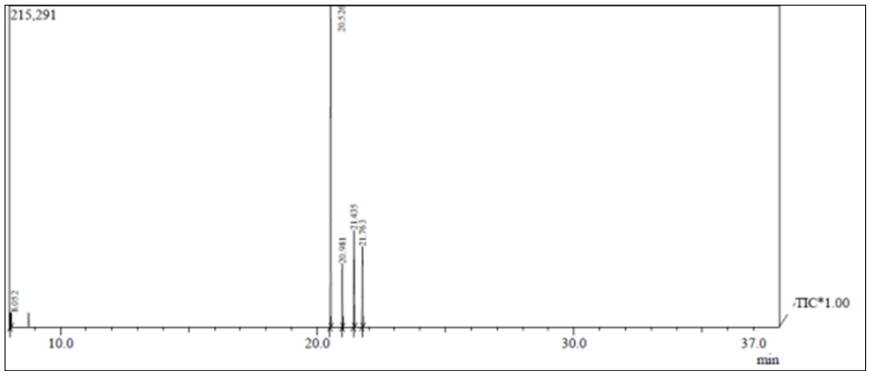

Fig 3: Chromatograph of methanol leaf extracts of *Ficus exasperata*

Table 2: GC-MS analysis of methanol leaf extracts of *Ficus exasperate*

S/N	Retention Time	Compound name	Formula	Molecular weight
1	20.525	Neophytadiene	$C_{20}H_{38}$	278
2	20.525	Citronellyl propionate	$C_{13}H_{24}O_2$	212
3	20.525	Lavandulyl acetate	$C_{12}H_{20}O_2$	196
4	20.525	2-Endo,3-Endo-Dimethybornane (Bicyclol)	• $C_{12}H_{22}$	166
5	20.983	2-Nonylfuran	$C_{13}H_{22}O$	194
6	20.983	Imidazole-4-acetic acid	$C_5H_6N_2O_2$	126
7	21.433	Octadecanoic acid, methyl ester (methyl stearate)	$C_{19}H_{38}0_2$	298
8	21.767	Yadanzioside	$C_{23}H_{28}O_{11}$	480
9	21.767	Tridecanoic acid	$C_{13}H_{26}O_2$	214
10	21.767	Pentanoic acid, 4-Methyl- (Methylvaleric acid)	$C_6H_{12}O_2$	116

Discussion

The plant *Ficus exasperata* extract was not toxic to the skin unlike the positive control group B that gave a sharp body irritation on contact signifying a level of toxicity of Organophosphate to the animal. This was also reflected in the significant decrease in organ body weigh resulted from organophosphate treated group [24]. A total clearance of both larvae and adult *Rhipicephalus sanguineous* observed at 0.15 mg/ml of N-hexane extract of *Ficus exasperata* demonstrated its higher efficacy over methanol extract. Organic solvents may work better in acaricidal bioassays as a result of externally wax made of tick cuticle [25]. The more non-polar a chemical compound is, the greater its ability to penetrate the cuticle [26]. Higher efficiency from N- hexane extracts of commercial acaricide formulations have been reported [27]. Potency of ethyl acetate extract of *Senna italica subsp. arachoides* acaricidal activity against adults of *Hyalomma marginatum rufipes* in comparism with other extracts (chloroform, dichloromethane, ethyl acetate and methanol) tested was also reported [28]. There was an excellent result of ethanol extracts of *Cleome gynandra* (leaves) with mortality of 97.8% almost as potent as cypermethrin (100%) similar to the result of this study [29]. High quantity of phytochemicals such as Alkaloids, Saponins, Cardiac glycosides and Flavonoids observed in this study predict the acaricidal efficacy of the plant *Ficus exasperata*. The presence of alkaloids, phenol, tannins and flavonoids in *Cissus quadrangularis* to its acaricidal property [30]. There was confirmation of the presence of 1, 5-*dihydroxy*-3-*methyl anthraquinone* and *anthraquinone* from the aerial parts of *Cassia italica sub sp. arachoides* to be responsible for its acaricidal activity [31]. The presence of phenols, flavonoids, cardiac glycosides, steroids, saponins and alkaloids found in the acetone and ethanol extracts of *Cleome gynandra* (leaves) have been attributed to its acaricidal property [32]. Presence of Tanins in this plant extract though in lower quantity is also suggestive of its additional acaricidal property. Tannin rich plant extracts have been reported to have acaricidal effects against larvae of *R.* (*B.*) *microplus* [33].

Bio-organic compounds observed in the leave extract of *Ficus exasperata* especially Citronellyl propionate, Lavandulyl acetate, Octadecanoic acid and Yadanzioside are indicative of its acaricidal property. β-citronellol and their derivatives are most promising acaricide of *Pelargonium graveolens* oil against *Dermatophagoides farinae* and *Dermatophagoides pteronyssinus* [34]. *Citronellyl propionate* as direct sprays is efficacious against insects of urban and medical importance [32]. Both direct contact and inhalation of essential oil of *Lavandula angustifolia* against *Psoroptes cuniculi* have been reported [35]. Presence of Octadecanoic acid in natural anti-tick tropical legumes of *Stylosanthes humilis* and *Stylosante shamata* was also reported [36]. Insecticidal property of Yadanzioside in *Pestalotiopsis microspore* has also been documented [37].

Conclusion

Following the promising result obtained from this study, N-hexane and methanol leave extract of *Ficus exasperata* suggested a potential acaricide of natural origin against *Rhipicephalus sanguineus* tick of dog. However, there is need to conduct pharmacokinetic investigations to ensure that extract of *Ficus exasperata* is standardized. Most importantly, toxicological studies to identify risks to human and animal health cannot be neglected. A number of studies focusing on the acaricidal efficacy of essential oil, some of these plants are used in the food industry (4). For instance, the leaves of *Ficus exasperata* is used locally for ripen certain fruits. It is thus expected to be safe both to the animal and environment. An alternative botanical acaricide can also be effective in tick management instead of chemical acaricides provided the safety can be established in animal trials. This alternative method for controlling dog ticks would not only be useful to safeguard livestock production but could also be an alternative means to combat resistant strain.

List of Abbreviations

Word or Phrase	Abbreviations
Biochemical oxygen demand	BOD
Control	Ctrl
Dimethyl sulfoxide	DMSO
Direct steam distillation	DSD
Food and Agricultural Organization	FAO
Ficus exasperata	FE
Gas Chromatography Mass Spectrometry	GC-MS
Hexane fraction	HF
Lethal dose	LD
Methanol fraction	MF
Mortality	Mot
Organization for Economic Co-operation and Development	OECD
Organophosphate	ORG
Rhipicephalus (Boophilus)	R (B)
University of Ibadan	UI

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