Acaricidal activities of extracts of *Commiphora swynnertonii* Burtt (Burseraceae), *Melia volkensii* Gürke, *Turraea abyssinica* Hochst., *Turraea floribunda* Hochst and *Turraea cornucopia* Styles & F. White (Meliaceae) against the brown ear tick *Rhipicephalus appendiculatus* Neumann

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Abstract: *Extracts of the stem bark of Commiphora swynnertonii, the root bark of Turraea abyssinica, T. cornucopia, T. floribunda and the ripened fruits of Melia volkensii were tested for acaricidal activities against nymphal and adult stages of Rhipicephalus appendiculatus in the laboratory. At a concentration of 10% (w/v), hexane and ethyl acetate extracts of C. swynertonii induced mean mortalities of 71% and 54% respectively, in two week old nymphs. A 10% (w/v) hexane extract was found to be as effective as the commercial acaricide Triatix against the two-week-old nymphs. Extracts of T. cornucopia, T. abyssinica and M. volkensii induced no mortality in nymphs and adults, but suppressed oviposition capacity during the first day. On the other hand, 500«l of 10% (w/v) methanol extracts of T. cornucopia and T. floribunda delayed attachment by adult females of R. appendiculatus on the rabbit ears, engorgement and hatchability of their eggs.*

Keywords*:* acaricidal, activity, plant extracts, Burseraceae, Meliaceae, *Rhipicephalus appendicullatus*.

INTRODUCTION

Since their introduction into South Africa around 1890, acaricides have been the commonest method of tick control in Africa, leading to many problems such as environmental pollution, development of resistant tick strains and escalating costs (Kaaya *et al.,* 1995). It is therefore necessary to develop other methods of tick control. Such alternative strategies must kill or incapacitate the tick in order to prevent host finding and attachment, especially for multi-host ticks which have more chances of survival than one host ticks (Mwangi *et al*., 1995). In recent years, the use of botanicals has received considerable interest in Africa because many countries wanted to reduce the importation of acaricides and to replace those rendered unusable by tick resistance.

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Studies have shown that plant extracts or isolates caused tick mortality (Dipeolu and Ndung'u 1991; Puyvelde *et al.,* 1987; Chiasson *et al*., 2000; Soon Il. *et al.,* 2004; Lee Hoi-Seon 2003), tick repellence (Dipeolu *et al*., 1992; Malonza *et al*., 1992), tick immobilization (Sutherst *et al.,* 1982; Zimmerman *et al*., 1984; Sutherst and Wilson, 1986; Sutherst *et al.,* 1989).

This paper presents anti-tick activities of *C. swynnertonii,* Burtt., *T. cornucopia,* Styles and F White., *T. abyssinica,,* Hochst., *T. floribunda,* Hochst and *M. volkensii,* Gurke., against *R. appendiculatus,* Neumann under laboratory conditions. These plants are found in East Africa. The exudate of *C. swynnetonii* ("Oldemwai" in Maasai vernacular) is used locally by the Maasai people to control ticks. The other plants have also been reported to have broad biological activities. For example, *T. floribunda* is used as an emetic and purgative (Torto B. *et al,* 1995).

MATERIALS AND METHODS

Plant materials

The leaves and stem bark of *C. swynnertonii* were collected from Kiteto in Central Tanzania. The plant was identified by the late Mr. Samwel Kibua of the National Herbarium of Tanzania (NHT). A voucher specimen No. 2888 is deposited at the NHT. The root barks of *T. cornucopia* and *T. abyssinica* were collected in Kenya from the Ngong Hills Circuit and Naivasha, respectively and ripened fruits of *M. volkensii* from Mtito Andei on the periphery of Tsavo National Park. The voucher number of these specimens, which were deposited at the herbarium of Nairobi University are 93/5, 93/4 and 93/3 respectively. The root bark of *T. floribunda* was collected from Kwale National Park in the Longogadi Forest in Kenya. Its voucher specimen No. 89/401 is deposited at the herbarium of Nairobi University. The four plants were identified by Mr Samwel Mathenge of the Department of Botany, Nairobi University.

Ticks

Adult and two weeks old nymphs of *R. appendiculatus* were obtained from the insectary of the Tropical Pesticides Research Institute, Arusha, Tanzania. The ticks were maintained by feeding on the ears of rabbits according to methods described by FAO (1971).

Commercial acaricide

Triatix 12.5EC (Trade name) or Amitraz (common name) registered by Schering Plough Animal Health was used as a standard material in the study.

Equipment

Chemical composition of the hydrodistrilled oil was carried out using a low resolution mass spectrometer VG Quatro (Fissons, Machester, UK) coupled to a gas chromatograph 5890 series 11 (Hewlett Packard, Boblingen, Germany). The G.C was equipped with methyl silicone column DB-5 MS (J & W Scientific Inc.) 30m, ID = 0.25mm, FD = 0.25 um).

Plant extraction

Freshly collected leaves of *C. swynertonii* Burtt weighing about 0.6 kg were hydro-distilled using a Clevenger apparatus. The distillate collected in hexane was

evaporated in vacuum to yield about 2.1g (0.35%) of oil. The chemical composition of the hydro-distilled oil was determined by using Gas chromatography coupled to a low resolution mass spectrometry. The structures were confirmed by GC co-injection for the components whose authentic samples were present in the laboratory, probability based matching system and fragmentation pattern of each component.

Root barks of *Turraea cornucopia*, *T. abyssinica* and *T. floribunda* and the stem bark of *C. swynertonii*, were air dried and ground to a fine powder. About 1.2 kg of the powder of *C. swynertonii* was allowed to stand sequentially in hexane, ethyl acetate and methanol at room temperature. Each extract was filtered and concentrated to dryness by rotary evaporation to afford approximately hexane (8g), ethyl acetate (240g) and methanol (300g). About 2.2kg of air-dried root bark of *T. cornucopia* and *T. abyssinica* were extracted in methanol. Filtration and concentration of the *T. cornucopia* and *T. abyssinica* extracts in vacuum afforded (100g) and 150g of extract, respectively. About (0.9kg) of fine powder from air dried root bark of *T. floribunda* was allowed to stand in methanol at room temperature. The extract was filtered and evaporated to dryness under vacuum to yield 84g of extract. About 4.5kg of crushed fresh ripened fruits of *M. volkensii* was allowed to stand sequentially in dichloromethane and methanol at room temperature to yield approximately 800g and 2150g respectively of dichloromethane and methanol extracts after filtration followed by evaporation under vacuum conditions.

Acaricidal activity

Bioassays were carried out on the hydro- distilled oil and n-hexane, and ethyl acetate extracts of the stem bark of *C. swynnertonii* for acaricidal properties using the standard method described by Stone and Haydock (1962).

Pieces of filter paper of desired sizes (9cm x 6cm and 6cm x 4 cm) were folded in such a way that a pocket were formed of which the open ends were closed using three dog bull clips. Three such papers were treated with n-hexane, ethyl acetate and methanol extracts at a time. A stock solution of each extract was prepared by dissolving 1mg of sample in 1:2 solutions of olive oil and trichloroethylene (TCE). Serial dilutions were made on each stock solution to give the different doses by drawing 100µl aliquots from each subsequent prepared dose and dissolving it in 100 µl of olive oil: TCE (1:2) solution. The control consisted of olive oil: TCE (1:2). 500 µl of each dose of the test material was applied on the filter papers. TCE was allowed to evaporate leaving behind the test material.

Acaricidal tests were performed on the oil and extract by introducing 25 adult ticks through the open end of a treated piece of paper and closing it with a third clip. Acaricidal tests were also performed on the extracts by introducing 100 two-week old larvae into the test area by using a fine brush. The pockets were closed as previously described for adults. The pockets were laid down flat on a clean bench for 24 h and 48 h for larvae and adults, respectively. The bioassays were carried out at room temperature (24-26ºC). Each pocket was opened after the exposure period and inspected under a 2x lens. Mobile larvae were picked off by a fine brush,

transferred to a wet cotton wool pad and counted. Adult ticks were counted with naked eyes. The dead larvae and adults of *R. appendiculatus* were counted and recorded.

Long term effects of plant extracts on adult ticks

Plant material extracts (10% w/v) including dichloromethane and methanol extracts of ripening fruits of *M. volkensii* and methanol extracts of root bark of *T. cornucopia, T. abyssinica* and *T. floribunda* were screened against ticks for long-term residual effects.

The tests were performed on the extracts by introducing 20 adult females of *R. appendiculatus* into the filter paper (as described above) which had previously been treated with 500µl of 10% w/v solutions of extracts. Each pocket was opened after 48 h and the treated ticks were used to infest the ears of rabbits. Each test was replicated three times. After the introduction of the adult ticks, ear tags were used to cover them on the ears of rabbits in order to prevent escaping. Rabbits which were reared in the animal house at the Tropical Pesticides Research Institute, Arusha, Tanzania were used in the efficacy tests. Three rabbits were used for each of the test materials namely methanol extracts of *M. volkensii, T. cornucopia*, *T. abyssinica* and *T. floribunda* and dichloromethane extract of *M. volkensii.*

The time used by ticks to attach on the ears of rabbits was recorded. The time taken for each tick to engorge and oviposit was also recorded. Other data recorded included total number of eggs laid by each tick and percentage hatchability of the eggs laid.

Method of counting eggs

Eggs laid by the ticks which were previously treated by the different extracts were separated from the engorged ticks, kept in stoppered glass tubes, labeled and stored at room temperature. A portion of eggs from each tube was taken and the number of eggs counted and their weights taken. Each portion of eggs was put back to the original tube and weight of all the eggs from each tube taken. The total number of eggs was then calculated.

Statistical analysis

The mean and Standard Error (±SE) of tick counts were estimated for the samples, including dead ticks after being exposed to *C. swynnertonii* extracts. Also, ±SE of tick counts were estimated for the number of ticks attached to ears of rabbits, number of engorged ticks, weight of treated ticks, number of ticks oviposited, eggs laid and percentage hatchability after being exposed to *Turraea cornucopia, T. abyssinica , T. floribunda* and *M. volkensii* extracts. The means were then subjected to One Way Analysis of Variance to compare performance of different extracts against the ticks. The ANOVA was then followed by a Post Hoc Multiple Comparisons, using LSD test, to identify the differences among the sample means. Significance was considered when the probability was below 0.05.

RESULTS AND DISCUSSION

In this study we report acaricidal activity of the hydro-distilled oil and hexane and ethyl acetate extracts of the stem bark of *C. swynnertonii* against *R. appendiculatus*.

The study also reports long term residual effects of methanol extracts of the root bark of four plants belonging to family meliaceae. These plants were *T. cornucopia*, *T. abyssinica* and *T. floribunda* and the ripening fruits of *M. volkensii*. Dichloromethane extract of *M. volkensii* was also tested.

At a concentration of 10% (w/v), hexane and ethyl acetate extracts of *C. swynnertonii* induced mean mortalities of 71% and 54% respectively in two weeks old nymphs. A 10% (w/v) hexane extract was found to be as effective as the commercial acaricide, Triatix, against the two-week-old nymphs. Extracts of *T. cornucopia, T. abyssinica T. floribunda* and *M. volkensii* induced no mortality in nymphs and adults of *R. appendiculatus.*

Hexane and ethyl acetate extracts of the stem bark of *C. Swynnertonii* and the oil obtained by hydro-distillation of the leaves of *C. swynnertonii* killed larvae of *R. appendiculatus* (Table 1). It has been found out that for those analyses which resulted into zero Standard Error (SE), the figures obtained from the tests were incidentally the same for the three replicates. This resulted into zero SE, indicating that there was no variability in the data obtained from the tests.

Table 1: Acaricidal activity of hexane and ethyl acetate extracts of *C. swynertonii*, oil and triatix against two week larvae of *T. appendiculatus*

Dose % (w/v)	Hexane	Ethyl acetate	Oil	Triatix
10	71 ± 4.1^{a}	54.0 ± 4.04^{b}	84.0 ± 3.05^{a}	70.3 ± 3.18^{a}
1	53.3 ± 2.6^{a}	41.3 ± 12.87^{b}	56.0 ± 6.92^{a}	59.3 ± 2.5^{a}
0.1	40.3 ± 2.7^{b}	31.7 ± 4.4^{c}	46.7 ± 6.7^{b}	55.0 ± 2.9^{a}
0.01	24.3 ± 1.8^{c}	24.0 ± 1.2^{c}	31.7 ± 6.0^{b}	43.0 ± 2.9^{a}

Mean values with the same letters within the same dose are not significantly different at 5% level.

The methanol extracts of *T. cornucopia* and *T. floribunda* delayed the attachment of ticks on the ears of rabbits on day three (Table 2). Also, these extracts delayed engorgement of the ticks during the first three days after the rabbits were infested with adult ticks (Table 3).

Table 2: Mean number ($\pm$ S.E) of ticks attached to the ears of rabbits

Extract	Time (days)		
	Day 1	Day 2	Day 3
Control	2.91 ± 0.09^{a}	1.77 ± 0.09^{a}	0.71 ± 0^{b}
M. volkensii (dichloromethane)	1.95 ± 0.08^{a}	2.41 ± 0.06^{a}	1.34 ± 0.12^{ab}
*M. volkensii (*methanol extract)	2.79 ± 0.15^{a}	1.73 ± 0.26^{a}	0.71 ± 0^{b}
*T.abyssinica (*methanol extract)	2.52 ± 0.49^{a}	1.71 ± 0.53^{a}	0.99 ± 0.29^{ab}
*T.cornucopia(*methanol extract)	2.24 ± 0.25^{a}	1.93 ± 0.22^{a}	1.55 ± 0.19^{a}
*T.floribunda (*methanol extract)	2.54 ± 0.11^{a}	1.77 ± 0.09^{a}	1.46 ± 0.12^{a}

Mean values with the same letter within the same day are not significantly different at 5% level.

Table 3: Mean number (+ S.E) of engorged ticks on a daily basis

Extract	Time (days)						
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Control	2.91 ± 0^{a}	1.68 ± 0.96^{a}	1.65 ± 0.21^{a}	0.71 ± 0^{c}	0.71 ± 0^{b}	0.71 ± 0^{c}	0.71 ± 0^{b}
M. volkensii (methanol extract)	1.94 ± 0.18^{b}	0.88 ± 0.17^{bc}	1.68 ± 0.09^{ab}	1.72 ± 0.33^{a}	1.05 ± 0.17^{b}	0.88 ± 0.17^{bc}	0.88 ± 0.17^{b}
T.abyssinica (methanol extract)	1.77 ± 0.09^{b}	1.86 ± 0.16^{a}	1.56 ± 0.19^{ab}	1.34 ± 0.12^{ab}	1.05 ± 0.17^{b}	0.71 ± 0^{c}	0.88 ± 0.17^{b}
M.volkensii (dichloromethane extract)	1.22 ± 0^{c}	1.05 ± 0.17^{bc}	1.68 ± 0.09^{ab}	0.88 ± 0.17^{bc}	1.05 ± 0.17^{b}	1.77 ± 0.09^{a}	1.65 ± 0.21^{a}
T.cornucopia (methanol extract)	$1.05 + 0.17^{c}$	0.71 ± 0^{c}	1.34 ± 0.2^{b}	1.68 ± 0.09^{a}	1.95 ± 0.08^{a}	1.50 ± 0.17^{bc}	0.71 ± 0^{b}
T.floribunda (methanol extract)	$0.87 + 0^{c}$	0.71 ± 0.14^{c}	1.34 ± 0.14^{b}	1.78 ± 0.11^{a}	2.19 ± 0.21^{a}	2.20 ± 0.17^{a}	2.25 ± 0^{a}

Mean values with the same letters within the same day are not significantly different at 5% level.

The test materials including dichloromethane extract of *M. volkensii* and methanol extracts of *M. volkensii*, *T. cornucopia*, *T. abyssinica* and *T. floribunda* showed no negative effects on the weight of engorged ticks (Table 4). Methanol extracts of *T. floribunda, T. abyssinica T. cornucopia,* and dichloromethane extract of *M. volkensii* were found to reduce oviposition of ticks, that is, they are significantly different from the control (Table 5).

Table 4: Mean weight (± S. E) of ticks

Extract	Mean weight of ticks (g)
Control	1.0 ± 0.1^{a}
T. cornucopia (methanol extract)	0.98 ± 1.8^{a}
M. volkensii (methanol extract)	0.98 ± 0^{a}
*M. volkensii (*dichloromethane extract)	0.97 ± 0^{a}
T. abyssinica (methanol extract)	0.97 ± 0.02^{a}
T. floribunda (methanol extract)	0.97 ± 0.01^{a}

Mean values with the same letters are not significantly different at 5% level.

Table 5: Mean number (+ S. E) of ticks which have oviposited

Extract	Time (days)				
	Day 1	Day 2	Day 3	Day 4	Day 5
Control	$2..92 \pm 0^{a}$	0.86 ± 0.33^{b}	1.46 ± 0.33^{a}	0.71 ± 0^{a}	0.71 ± 0^{b}
M. volkensii (methanol extract)	1.86 ± 0.58^{ab}	1.17 ± 0.58^{ab}	2.0 ± 1.20^{a}	1.57 ± 1.20^{ab}	0.17 ± 0^{b}
T. abyssinica (methanol extract)	1.77 ± 0.33^{c}	1.34 ± 0.33^{ab}	1.27 ± 0.88^{a}	1.05 ± 0.33^{b}	0.71 ± 0^{b}
M. volkensii (dichloromethane extract)	1.22 ± 0^{c}	1.86 ± 0.57^{a}	1.64 ± 0.88^{a}	1.17 ± 0.57^{b}	1.77 ± 0.33^{a}
T. cornucopia (methanol extract)	1.17 ± 0.58^{c}	2.08 ± 1.15^{a}	1.47 ± 1.16^{a}	1.34 ± 0.33^{b}	1.71 ± 0^{b}
T. floribunda (methanol extract)	1.05 ± 0.33^{c}	1.56 ± 0.33^{ab}	1.85 ± 0.57^{a}	2.19 ± 0.33^{b}	0.71 ± 0^{b}

Mean values with the same letters within the same day are not significantly different at 5% level.

The methanol extract of *T. abyssinica* reduced the number of eggs laid more significantly as compared to the other extracts (Table 6). On the other hand, *T. floribunda* and *T. cornucopia* reduced hatchability of the ticks (Table 7).

Table 6: Number (mean number (+ S. E) of eggs laid

Extract	Mean (± S. E) number of eggs
Control	6692.6 ± 243.77^{a}
M. volkensii (dichloromethane extract)	6169.4 ± 673.06^{a}
T. cornucopia (methanol extract)	6018.2 ± 162.38^{a}
M. volkensii (methanol extract)	5933.4 ± 478.94^{a}
T. floribunda (methanol extract)	5221.4 ± 88.82^{a}
T. abyssinica (methanol extract)	3459.4 ± 764.35^{b}

Mean values with the same letter are not significantly different at 5% level.

Table 7: Mean (+ S. E) percentage hatchability

Extract	Mean (± S. E) % hatchability
Control	73.0 ± 4.64^{a}
M. volkensii (methanol extract)	69.0 ± 4.0^{ab}
T. abyssinica (methanol extract)	59.0 ± 10.04^{abc}
M. volkensii (dichloromethane extract)	45.0 ± 11.62^{bcd}
T. cornucopia (methanol extract)	34.0 ± 4.85^{cd}
T. floribunda (methanol extract)	29.0 ± 3.32^{d}

Mean values with the same letter are not significantly different at 5% level.

In this study, it was found that methanol extracts of *T. floribunda* and *T. cornucopia* had more adverse effects on *R. appendiculatus* as compared to the other plant extracts tested. This is probably due to different chemical composition of these plants. On the other hand, the hydro-distilled oil of *C. swynnertonii* was as effective as the commercial acaricide, triatix. This is probably due to synergistic effects of the components of the oil. Analysis of the hydro-distilled oil by Gas Chromatography-Mass Spectrometry revealed the presence of five sesquiterpenoids and four sesquiterpenoid derivatives. The main compound was the enolate isomer of 9-(3-furanyl) – 2,6-dimethyl – 3,5 – nonadien – 4 – ol (52.23%) followed by 9-(3-furanyl – 2,6 – dimethyl – 2,5 – nonadien – 4 – one (13.84%) (Kaoneka *et al*., 2007).

It has been shown in different studies that several plant extracts have exhibited residual effects on ticks. For instance, Williams, L. (1991) found out that topical application of crude ethanol extracts of five marine algae; namely *Laurencia obtuse* (Hudson) Lamouroux, *Padina vickerisiae, Liagora farinose* Lamouroux, *Liagora elongate* Zanardini and *Stypopodium lobatum* (C. Agath) Kutzing affected the survival of engorged adult female *B. microplus* Canastrini and inhibited oviposition and embryogenesis in the ticks.

Dipeolu and Ndungu (1991) reported that “Kupetaba”, a ground mixture of dried tobacco leaves and a mineral called ‘ magadi soda’ was acaricidal to *R. appendiculatus* and caused a marked (65%) inhibition of oviposition at a concentration of 100%.

The *Margaritaria discoidea* oil extract was not only acaricidal to adults of both *R. apendiculatus* and *Amblyomma variegatum* engorging on rabbits, but had a high residual activity lasting over 4 days during which 100% of adult *R. appendiculatus* and *A. variegatum* were unable to attach on treated rabbit ears (Kaaya et al., 1996). A 100% concentrated "Kupetaba" when applied at the time of infestation on the ears of rabbits and calves was reported to prevent tick attachment for up to 72 hr in rabbits and 120 hr in calves (Dipeolu and Ndung'u 1991).

It was reported by Carroll *et al*., (1998) that the hexane extract of the gum of *Commiphora erythraea* Engler was active against *Amblyomma americanum* L. and *Dermacentor variabilis* Say larvae. Maradufu (1982) reported that a hexane soluble viscous oil extract from the gum of the tree *C. myrr* Neels was repellant to adult *R. appendiculatus* and house flies *Musca dometica* L. Three furanosesquiterpenoids isolated from *C. erythraea* were found to be toxic to larvae of *R. appendiculatus* (Maradufu 1982).

The hexane extract of the gum of *C.* erythraea engler has also been reported to possess larvicidal and repellant activities against *A. americanum, D. variabilis* and repellence activity against adults *of ixodes dammini*, *D.* variabilis and *A. americanum* (Carroll *et al*., 1989). The essential oils of the leaves of *C. swynnertonii* Burtt was found to repel adults of *R. appendiculatus* at doses of 0.1 and 10% (v/v) in tick climbing repellency bioassays. (Kaoneka *et al*., 2007).

The exudate of *C. swynnertonii* is now on the market in Northern Tanzania in the fight against ticks. It is mainly used by resource poor farmers. The plant grown vegetatively can be propagated in some areas in Northern Tanzania and hence make it easily available to users. The use of the exudates in the control of ticks is promising and it can be incorporated in integrated tick management approaches. Also its suitability is due to low toxicity making it useful in traditional medicine.

Plants in the family Meliaceae are characterized by the presence of limonoids (tetranortriterpenoids) that exhibit a wide range of anti-insect effects (Ndug'u *et al*., 2004). In East Africa, a number of Meliaceae plants are used in traditional medicine. This means that the use of *M. volkensii*, *T. abyssinica*, *T. floribunda* and *T. cornucopia* in the control of ticks may not pose danger to both the animals and people who eat the meat.

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