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Acaricidal Activities of *Hyptis suaveolens* and *Ocimum sanctum* Against African Dog Tick (*Rhipicephalus sanguinneus*)

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Authors' contributions

This work was carried out in collaboration between all authors. Authors EIO and TCNA designed the Study and wrote the protocol. Authors TCNA and SCI wrote the first draft of the manuscript and performed the statistical analysis. Authors EIO and SEB revised the manuscript. All authors managed the literature searches and wrote the first draft of the manuscript. All authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Aim: To determine the acaricidal activities of some solvent extracts (chloroform, methanol and n-hexane) and crude extracts of *Hyptis suaveolens and Ocimum sanctum* against African dog tick (*Rhipicephalus sanguinneus*).
Study Design: The study design involves a 24 h LC_{50} dose-mortality static non-renewal bioassay.
Place and Duration of Study: The study was carried out at Rohi Biotechnology Toxicity Laboratory, Port-Harcourt, Rivers State Nigeria, between August and November 2014.
Methodology: The solvent extracts were assessed against the ticks at varying concentrations in a 2-phased rapid and final screening test.
Results: All extracts showed moderate activities during the bioassay, except the crude extract

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which was not active beyond the rapid screening phase (i.e. LC_{100}>500 ppm). The chloroform, methanol and n-hexane extracts of *H. suaveolens* induced LC_{50} values of 175.00, 81.25 and 225.00 ppm respectively. On the other hand *O. sanctum* induced mortalities of 200.00, 137.50 and 287.50 ppm for chloroform, methanol and n-hexane extracts respectively. Meanwhile, the positive control was lethal at 1ppm, while the tick survived in the negative control.
Conclusion: The result demonstrates that solvent extracts of *H. suaveolens and O. sanctum* can be used as acaricides for the control of dog tick.

Keywords: Acaricide; solvent extracts; tick; Hyptis suaveolens; Ocimum sanctum.

1. INTRODUCTION

In the recent years, the use of synthetic pesticides has attracted global attention due to its toxic impacts on the applied organisms and the environment at large. Notwithstanding, the active sites of the attack by the pesticides depend on the type of pesticides under use. Due to the environmental effects and high cost of synthetic pesticides to indigent disease endemic areas [1], the application of plant-derived pesticides have attracted the attentions of ecotoxicology and parasitology researchers. In Africa, some plants have been reported for their therapeutic applications as pesticides against a variety of animal vectors and parasites. Some plants have found applications as curative agents due to the diverse phytochemicals they produce. Both *H. suaveolens* and *Ocimum sanctum* vital therapeutic components with diverse applications. For instance, *Ocimum* species has been widely reported to possess repellent properties against mosquito [2] and lymphatic filariasis [3]. *H. suaveolens* have also been reported to contain diverse metabolites which have found application as antimicrobial, anti-diarrhoeal, anti-inflammatory, anthelmintic, anti-diabetic, anticancerous, wound-healing and insecticidal agents [4].

In many part many parts of the world, several people domesticate dogs (*Cannis* species) and use them as pets, for food and security. Parasites such as *Rhipicephalus sanguineus* (dog tick), are obligate haematophagous external parasites of some domestic and wild animals; which cause discomfort on infected hosts [5]. Tick infestation often results to loss of appetite by the dog and results to death in cases of poor and inappropriate treatment. Generally, ticks rank second to mosquito amongst parasites of infectious diseases [6,7]. It has been estimated, in literature that 80% of 1.2 million domestic tick-prone animals are at risk of contracting tick-borne diseases, thereby causing a global annual loss of about US$ 7000 million [6]. For instance, literature exist by several authors which reported the incidence of tick-borne diseases for cattle with 7.6% infestation rate, 55.4% for goats and 13.2% for pigs [8-10].

R. sanguineus are controlled by physical and chemical methods and to a lesser extent by biological control. The physical approach involves hand picking of the tick from the skin of the infected animal. Chemical approach involves the use pesticides that contain chemicals in the eradication of the *R. sanguineus*. Some of the common chemical pesticides used for the control of tick in general are delta methrin and amitraz [5,11,12]. The constant use of chemical acaricides could lead to environmental contamination, food toxicity, etc. Hence, the use of plant extracts for the eradication of *R. sanguineus* has gained prominence over physical and chemical methods. Some of the plants species that have been reported to have acaricidal potentials include *Annonas quamosa, Centella asiatica, Gloriosa superba, Mukiamader aspatensis, Pergularia daemia, Phyllanthusem blica*against adult cattle tick (*Haemaphysalis bispinosa*) [6]. Fernandes and Freitas, [13] also reported the acaricidal activity of *Copaifera reticulate* against *R. microplus* larva. Basedon the prospects of using plant-derived extracts for the control of parasitic vectors, the acaricidal potentials of *H. suaveolens* and *O. sanctum* from Nigeria is hereby evaluated.

2. MATERIALS AND METHODS

2.1 Collection of Plant Materials

The leaf of *H. Suaveolens* and *O. Sanctum* were collected from, Igarra, Edo State, Nigeria in August 2014. The taxonomic identification of both plants was carried out identification keys as described by Ogunkunle [14].

2.2 Plant Extraction/ Phytochemical Analysis

The leaves were shade-dried for 7 days at ambient environmental temperatures (31±2ºC).

The dried leaves were powdered using domestic electrical blender and the powdered leaves (400 g) were macerated for 72 h in n-hexane (700 ml, Fisher Scientific international Company), chloroform (700 ml, BHD Chemical Ltd. Poole England) and methanol (700 ml, BHD Chemical Ltd. Poole England); meanwhile for the crude extraction, the juice of the fresh leaves of both plants were used. The active ingredients in the filtrates were respectively extracted in a rotary evaporator (60ºC), leaving no trace of solvent. The residue obtained was stored at 4ºC until it was ready for use. Phytochemical screenings of the plants were carried out following standard protocols [15].

2.3 Parasite Collection

The attached adults of *R. sanguinneus* were collected from the ears and foot of the dog. The parasites were identified using identification keys as described by Dantas-Torres [16].

2.4 Experimental Setup

The applied experimental setup of this investigation to verify the acaricidal activity of different solvent plant extracts against adult of *R. sanguinneus* was developed following standard protocol [17,18], with slight modifications incorporating rapid and final screening as described by several authors [19-21]. The bioassay was set up with different concentrations of the extracts impregnated with series of prepared filter paper envelopes (Whatman filter paper No. 1), inoculated with a minimum of ten ticks.

The treated envelopes inoculated with adult ticks was tagged and sealed with metallic clip in order to prevent escape. The envelopes were incubated at laboratory conditions (28-30ºC and 80–90% relative Humidity for 24 h), as described by Begavan et al. [6]. The envelopes were opened sequel to the incubation period (24 h), and the mortality rates (%), were recorded. The positive control was impregnated with Zimitraz 12.5% (Zampharm Limited, London-England), while the negative control was set up with distill water.

2.5 Rapid Screening and Final Screening

The rapid screening was set up with their respective replicates at concentrations of 1000 and 500 ppm of the extracts. Furthermore, only extracts active at 500 ppm (i.e. extracts with an LC_{100} of 500 ppm within 24 h), were sanctioned for the final screening.

2.6 Statistical Analysis

The mean mortality and standard deviation of data from the bioassay were calculated, after which they were further subjected to concentration-mortality curve using statistical analysis (Microsoft Excel, 2013 version, with 5% error), to estimate the median lethal concentration.

3. RESULTS

The phytochemical screening and general acaricidal activities of different solvent extracts (chloroform, methanol and n-hexane), of *H. suaveolens and O. sanctum* is presented in Tables 1-3 and Fig. 1. The result of the phytochemical analysis indicated higher amount of phenol in all extracts of *H. suaveolens* compared to *O. sanctum*. On the other hand, higher amount of flavonoid was found in extracts of *O. sanctum* compared to *H. suaveolens*. Tannins and steroids were absent in all extracts of *O. sanctum*. Solvents extracts of *H. suaveolens* witnessed higher amount of steroids compared to tannins. On the other hand, tannins and steroids were absent in the extracts of *O. sanctum*, as well as alkaloids and saponins in all extracts of the plant.

Table 1. Phytochemical analysis of various leaf solvent extracts of the plants

Plants	Extracting medium	Phytochemicals					
		Phenol	Alkaloid	Tannin	Steroid	Saponin	Flavonoid
H. Suaveolens	chloroform	++	-	+	++	-	+
	methanol	++	-	+	++	-	+
	n-hexane	++	-	+	++	-	+
	crude extract	++	-	+	+	-	+
O. Sanctum	chloroform	+	-	-	-	-	++
	methanol	+	-	-	-	-	++
	n-hexane	+	-	-	-	-	++
	crude extract	+	-	-	-	-	++

++: present in abundance; +: present; -: absent

Table 2. Rapid screening results

Plants	Extracting medium	% Mortality ± SD	
		1000 ppm	500 ppm
H. Suaveolens	chloroform	100±0.000	100±0.000
	methanol	100±0.000	100±0.000
	n-hexane	100±0.000	100±0.000
	Crude extract	100±0.000	83±1.314
O. Sanctum	chloroform	100±0.000	100±0.000
	Methanol	100±0.000	100±0.000
	n-hexane	100±0.000	100±0.000
	crude extract	100±0.000	67±1.0403

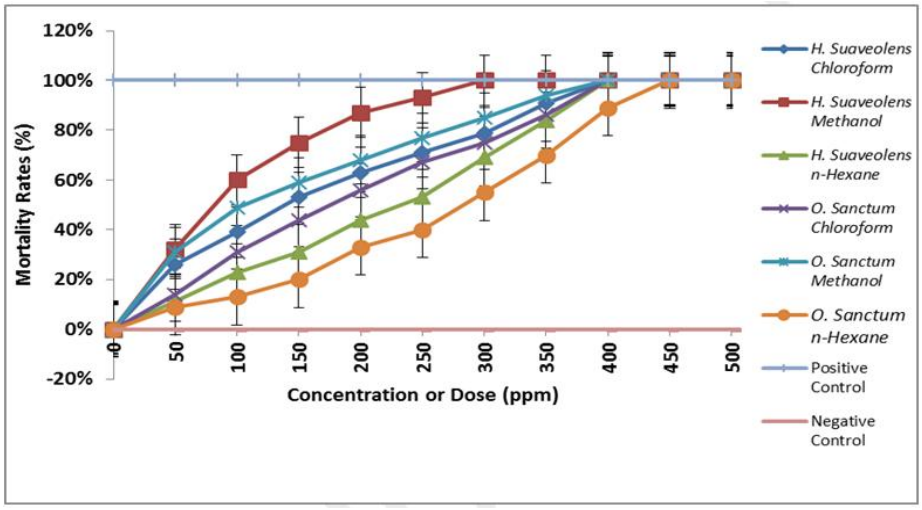

Fig. 1. Dose-mortality graph (5% error ± SD)

Compared to other extracts, results of the rapid screening (Table 2), shows that the crude extracts (for both plants), had total mortality rates (i.e. 100% mortality), at a higher concentration (above 500 ppm). Hence the crude extracts of both plants were not regarded for the final screening.

Results of the final screening (Table 3), shows that the solvent extracts of *H. suaveolens* had minimal total mortality rates (*MTMrt*), at concentrations of 400, 300 and 450 ppm for chloroform, methanol and hexane extracts respectively. While the *O. sanctum* extracts were toxic to the parasites with *MTMrt* at 400 ppm for both chloroform and methanolic extracts compared to the hexane extract with *MTMrt* at 450 ppm.

The median lethal dose (LC_{50}) was statistically determined. Results (Fig. 1) show that, the chloroform, methanol and n-hexane extracts of *H. suaveolens* induced LC_{50} values of 175.00, 81.25 and 225.00 ppm respectively. On the other hand *O. sanctum* induced mortalities of 200.00, 137.50 and 287.50 ppm for chloroform, methanol and n-hexane extracts respectively. Meanwhile, the positive control was lethal at 1 ppm, while the tick survived in the negative control.

4. DISCUSSION

Compared to the crude extracts (LC_{100}>500 ppm), the solvent extracts of both plants (*H. suaveolens and O. sanctum*), were effective against African dog tick, *R. sanguinneus*. The higher rates of acaricidal activities observed amongst the solvent extracts (The *H. suaveolens*-chloroform extract =175.00 ppm, *H. suaveolens*-methanol extract =81.25 and *H. suaveolens*-Hexane extract = 225.00 ppm; *O. sanctum* extracts 200.00, 137.50 and 287.50 ppm for Chloroform, methanol and n-hexane extracts respectively) had been demonstrated from the foregoing. There are several literature demonstrating the acaricidal activities of plant solvent extracts by other researchers.

Table 3. Final screening results

Plant–extracts		% MORTALITY RATES ± SD						
		450 ppm	**400 ppm**	**350 ppm**	**300 ppm**	**250 ppm**	**200 ppm**	**150 ppm**
H. suaveolens	Chloroform	100±0.00%	100±0.00%	91±1.31%	79±1.14%	68±2.134%	57±0.41%	44±0.11%
	Methanol	100±0.00%	100±0.00%	100±0.00%	100±0.00%	93±1.099%	87±3.03%	75±1.40%
	n-hexane	100±0.00%	97±0.44%	84±1.33%	69±1.13%	53±1.34%	44±2.30%	31±0.31%
O. Sanctum	Chloroform	100±0.00%	100±0.00%	82±1.44%	71±2.03%	67±1.33%	50±0.43%	39±2.20%
	methanol	100±0.00%	100±0.00%	89±1.11%	80±2.02%	77±0.42%	61±1.10%	51±1.13%
	n-hexane	100±0.00%	89±1.13%	70±0.73%	55±3.04%	40±1.10%	33±1.11%	20±0.42%
CONTROLS								
Positive control		100±0.00%	100±0.00%	100±0.00%	100±0.00%	100±0.00%	100±0.00%	100±0.00%
Negative control		0±0.00%	0±0.00%	0±0.00%	0±0.00%	0±0.00%	0±0.00%	0±0.00%

Bagavan et al. [6] investigated the acaricidal activities of several plants against cattle tick (*Haemaphysalis bispinosa*) and results demonstrates that the leaf from the hexane extract of *Annona squamosal* induced LC_{50} value of 145.39 ppm, while the acetone and methanolic extracts of *Gloriosa superb* leaf against had LC_{50} value of 419.83 ppm and 225.57 ppm respectively. The authors further reported that methanolic and ethyl acetate leaf extracts of *Pergularia daemia* and *Phyllanthus emblica* had LC_{50} values of 294.46 and 256.08 ppm respectively. Zaman et al. [22] reported the synergicidal efficacy of the aqueous extract of several plants (*Azadirachta indica* leaves, *Nicotiana tabacum* leaves, *Calotropis procera* flowers and *Trachyspermum ammi* seeds) against cattle tick and reported fecundity suppression index of 0.371404, reduced hatching of 22.35% as well as mortality and reduced tick intensity of 50 mg/ml and detachability of 45%. A recent research with the oil of *Camellia sasanqua* seed against the *Rhipicephalus microplus* (cattle tick) and the dog tick (*R. sanguineus*), showed LD_{50} and LD_{100} values of 4.61% and 9.18% for *R. microplus* and 5.43% and 9.50% with *R. sanguineus* [23]. Politi et al. [24] showed that 70% ethanolic extract of *T. patula* was toxic to adult female dog tick with an immersion time of 5 minutes at a concentration of 50.0 mg/ml. The authors also reported a decrease infecundal (21.5%) and mortality (99.78%) rates of larvae. Fernandes et al. [25], reported the acaricidal activity of crude ethanol extract of *Magonia pubescens* stem bark against *Rhipicephalus sanguineus* with an LC_{50} value of 1503 ppm.

Some phytochemicals indicated in our study have also been reported by several authors [4, 26,27]. For instance, Cook and Samman, [26] reported that the antimicrobial activities of *O. sanctum* was attributed to phytochemical like phenol, flavonoid and other carotenoid compounds. Sikkema et al. [27] reported the activity of *H. suaveolens* was due to the presence of monoterpene constituents which could exert membrane disruption. Cox et al. [28] also reported that *H. suaveolens* could stimulate cellular leakage of potassium ions, and further results to mortality. Mandal et al. [29], observed higher microbial activity in steam distillation and petroleum ether extracts of *H. suaveolens* than that of ethanolic extract. Our finding validates previous observations, documented in literature.

Furthermore, earlier studies have shown that extracts belonging to the genus, *Ocimum,* induced various degrees of activities against ticks [30,31] and even malaria [2] and elephantiasis [3] vectors. As such, this line of research should be encouraged, including phytochemical fractionation, purification as well as mode of action (chemistry), of the plant .Also, the evaluation of the actual bioactive components which was lethal to the ticks should be assayed. Notwithstanding, much attention has been given to ongoing multifaceted and integrated approach exploring eco-friendly alternatives in the control of this tick and other pathogenic arthropods.

5. CONCLUSION

Ticks are important parasite and vectors of human and animal diseases due to their devastating morbidity rate. The control of tick commonly involves the use of synthetic acaricides, which constitute various degrees of environmental toxicity. This article investigated the acaricidal efficacy of *H. suaveolens* and *O. sanctum* against dog tick. Solvent extracts of both plants induced moderate mortality rates against the tick. This study justifies the indigenous application of *H. suaveolens* and *O. sanctum* which are known for their insecticidal activities. We also recommend the *ex-situ* trial of these plant in order to actualize their eco-tolerant dose against non-targeted species.

CONSENT

It is not applicable

ETHICAL APPROVAL

It is not applicable

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Angaye TCN, Ohimain EI, Zige DV, Didi B, Biobelemoye N. Biocidal activities of Solvent extracts of *Azadirachta indica*

*a*gainst Some Endemic Tropical Vector-borne Diseases. International Journal of Tropical Disease & Health. 2014;4(11):1198-1208.
2. Egunyomi A, Gbadamosi IT, Osiname KO. Comparative effectiveness of ethnobotanical mosquito repellents used in Ibadan, Nigeria. Journal of Applied Biosciences. 2010;36:2383-2388.
3. Malebo HM, Imeda C, Kitufe NA, Katani SJ, Sunguruma R, Magogo F, et al. Repellence effectiveness of essential oils from some Tanzanian *Ocimum* and *Hyptis* plant species against afro-tropical vectors of malaria and lymphatic filariasis. Journal of Medicinal Plants Research. 2013;7(11):653–660.
4. Ngozi LU, Ugochukwu N, Ifeoma PU, Charity EA, Chinyelu IE. The Efficacy of Hyptis Suaveolens: A Review of Its Nutritional and Medicinal Applications. European Journal of Medicinal Plants. 2014;4(6):661-674.
5. Adarsh KTP, Ajeesh KTP, Chithra ND, Deepa PE, Darsana U, Sreelekha KP, et al. Acaricidal Activity of Petroleum Ether Extract of Leaves of Tetrastigmal eucostaphylum (Dennst.) Alston against *Rhipicephalus* (Boophilus) annulatus. The Scientific World Journal. 2014;1–6.
6. Begavan A, Kamaraj C, Elango G, AbduzZhar A, Abdul Rahuman A, Adulticidal. Larvicidal efficacy of some medicinal plant extracts against tick, fluke and mosquitoes. Veterinary Parasitology. 2011;166(3-4):286-92.
7. Sonenshine DE, Lane RS, Nicholson WL. Ticks (Ixodida), in: Mullen G, Durden L, (Eds.). Medical and Veterinary Entomology, Academic Press, San Diego. 2002:517–558.
8. Van den Broek AH, Huntley JF, Halliwell RE, Machell J, Taylor M, Miller HR. Cutaneous hypersensitivity reactions to Psoroptes ovis and Der p 1 in sheep previously infested with P. ovis—the sheep scab mite. Vet. Immunol. Immunopathol. 2003;91:105–117.
9. Wall R. Ectoparasites: Future challenges in a changing world. Vet. Parasitol. 2007;1481:62–74.
10. Ghosh S, Bansal GC, Gupta SC, Ray D, Khan MQ, Irshad H, et al. Status of tick distribution in Bangladesh, India and Pakistan. Parasitol. Res. 2007;101(2):207–216.
11. Davey RB, Miller RJ, George JE. Efficacy of amitraz applied as a dip against an amitraz-resistant strain of *Rhipicephalus* (*Boophilus*) microplus (Acari: *Ixodidae*) infested on cattle. Veterinary Parasitology. 2008;(1-2):127–135.
12. Barre N, Li AY, Miller RJ, Gaia H, Delathiere JM, Davey RB, et al. *In vitro* and *in vivo* evaluation of deltamethrin and amitraz mixtures for the control of *Rhipicephalus* (*Boophilus*) microplus (Acari: *Ixodidae*) in New Caledonia. Veterinary Parasitology. 2008;(1-2):110–119.
13. Fernandes FF, Freitas EPS. Acaricidal activity of an oleoresinous extract from Copaifera reticulate Leguminosae: Caesalpinoideae against larvae of the southern cattle tick, *Rhipicephalus microplus* Acari: *Ixodidae.* Veterinary Parasitology. 2008;147(1-2):150-154.
14. Ogunkunle ATJ. Properties, users' assessment and applicability of nine types of taxonomic keys in diagnosing some Nigerian species of *Ocimum* L. *Hyptis* Jacq. And *Ficus* L. African Journal of Plant Science. 2014;8(1):6-24.
15. Association of Official Analytical Chemists, AOAC—Official methods of analysis. 16th edition. Arlington, V. A. USA; 1995.
16. Danta-Torres F. Biology and Ecology of the Brown dog tick, *Rhipcephalus sanguineus*. Parasites & Vectors. 2010;3:26-37.
17. World Health Organisation (WHO). Report of the WHO Information Consultation on the evaluation on the testing of insecticides CTD/WHO PES/IC96. 1996;1:96.
18. FAO (Food and Agriculture Organization of the United Nations). Ticks: Acaricide resistance: Diagnosis management and prevention. In: Guidelines Resistance Management and Integrated Parasite Control in Ruminants, FAO Animal Production and Health Division, Rome. 2004;Module 1.
19. Agboola IO, Ajayi GO, Adesegun SA, Adesanya SA, Comparative Molluscicidal activity of fruit pericarp, leaves, seed and stem Bark of *Blighiauni jugata* Baker. Pharmacology Journal. 2011;3:63-66.
20. Bassey SE, Ohimain EI, Angaye TC. The Molluscicidal Activities of Methanolic and Aqueous Extracts of *Jatropha curcas* leaves against *Bulinus globosus* and *Bulinus rholfsi*, Vectors of Urinary

Schistosomiasis. Journal of Parasitology. 2013;103:115-122.
21. Angaye TCN, Ohimain EI, Siasia EP, Asaigbe PI, Finomo OA. Larvicidal activities of the leaves of Niger Delta mangrove plants against *Anopheles gambiae*. Sky Journal of Microbiology Research. 2014;2(7):032-036.
22. Zamana MA, Iqbalb Z, Abbasb RZ, Khanb MN, Muhammad G, Younusa M, et al. *In vitro* and *in vivo* acaricidal activity of herbal extract. Veterinary Parasitology. 2012;186(3-4):431-6.
23. Hai NT, Atsushi M. Evaluation acaricidal efficacy of *Camellia sasanqua* thumb seed oil against the cattle tick *Rhipicephalus (Boophilus) microplus* and the dog tick *Rhipicephalus sanguineus*. International Journal of Medicinal Plant Research. 2014;3(3):284-289.
24. Politi FAS, Figueira GM, Araújo AM, Sampieri BR, Mathias MIC, et al. Acaricidal activity of ethanolic extract from aerial parts of Tagetes patula L. (Asteraceae) against larvae and engorged adult females of *Rhipicephalus sanguineus* (Latreille, 1806). Parasites & Vectors. 2012;5:295.
25. Fernandes FF, D'alessandro WB, Freitas EPS. Toxicity of Extract of Magonia pubescens (Sapindales: Sapindaceae) St. Hil. To Control the Brown Dog Tick, Rhipicephalus sanguineus (*Latreille*) (Acari: *Ixodidae*). Neotropical Entomology. 2008;37(2):205-208.
26. Cook NC, Samman S. Flavonoids-chemistry, metabolism, cardio protective effect and dietary sources. J Nutr Biochem. 1996;7(2):66-76.
27. Sikkema J, De Bont JAM, Poolman B. Interactions of cyclic hydrocarbons with biological membranes. J. Biol. Chem. 1994;269:8022-8028.
28. Cox SD, Gustafson JE, Mann CM, Markham JL, Liew YC, Hartland RP, et al. Tea tree oil causes K+ leakage and inhibits respiration in *Escherichia coli.* Lett. Appl. Microbiol. 2008;26(5):355-358.
29. Mandal SM, Mondal KC, Dey S, Pati BR. Antimicrobial activity of the leaf extracts of *Hyptis suaveolens* (L.) Poit. Indian J Pharm Sci. 2007;69:568-569.
30. Del Fabbro S, Nazzi F. Repellent effect of sweet basil compounds on Ixode sricinus ticks, Exp. Appl. Acarol. 2008;45:219–228.
31. Mwangi EN, Hassanali A, Essuman S, Myandat E, Moreka L, Kimondo M. Repellent and acaricidal properties of *Ocimum* suave against *Rhipicephalus appendiculatus* ticks, Exp. Appl. Acarol. 1995;19:11–18.

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