

THE EFFECTS OF ESSENTIAL OILS EXTRACTED FROM MEDICINAL PLANTS: ALLIUM SATIVUM L, CYMBOPOGON SSP., EUPATORIUM FORTUNE TURCZ, CINNAMOMUM CASSIA BL ON PARASITIC CHIGGERS OF CHICKEN

Nguyen Thi Kim Lan^{*1}, Pham Dieu Thuy¹, Dao Thi Huong² and Dao Van Cuong^{1,3}

¹Faculty of Animal Husbandry and Veterinary Medicine, Thai Nguyen University of Agriculture and Forestry, Thai Nguyen city, Thai Nguyen prefecture, Vietnam.

²PDS Dog Professional Research Center, Faculty of Veterinary Medicine, Vietnam National University of Agriculture, Trau Quy crossing, Gia Lam district, Hanoi, Vietnam.

³Department of Veterinary Pharmacology, Joint Faculty of Veterinary Medicine, Kagoshima University, 1-21-24 Korimoto, Kagoshima 890-0065, Japan.

Corresponding author: Professor Nguyen Thi Kim Lan, DVM, PhD, Faculty of Veterinary Medicine and Animal Sciences, Thai Nguyen University of Agriculture and Forestry, Thai Nguyen, Vietnam.. Tel: (+84) 280-3855-564. Fax: (+84) 280-3852-921.

Abstract

Keywords:

chigger socket, chicken, chigger, medicinal plant, extract, garlic.

Ethnopharmacological relevance: our study evaluated the effects of essential oils extracted from plant materials on chicken infested with chiggers, in order to explain these plants' traditional uses for the treatment of external parasitic diseases. In addition, the demonstration of their *in vivo* effects might be a step forwards to widen their uses in chicken chigger disease.

Materials and methods: plant materials were collected from Vietnam. Extracted essential oils were diluted in ethanol 40% at concentrations of 1%, 2%, 3% and 4% to test with chiggers infested on pastured chicken in Vietnam. Treatment efficacy was evaluated after 5 d of oil application. Our study tested with pastured chicken because the infection of chiggers on this type of domestic chicken is significantly high in Vietnam.

Result and discussion: All of essential oils from medicine plants showed effects on chicken chiggers at different levels. Garlic oil showed the best effect, because the application of this essential oil at concentration of 1% had exterminated chigger infection, while oils of other plants at this concentration still showed no effect. However, at the concentration of 3%, garlic oil exerted the side-effect as the applied skin areas became congested. The application of essential oils from other medicinal plants, such as lemon grass, fortune bogorchid and cinnamon, could not eliminate the chigger infection on chicken. Their highest efficacy observed at concentration of 4% was only 60%.

Conclusion: The present study demonstrated the anti-parasitic effects of garlic, lemon grass, fortune bogorchid and cinnamon on chicken chiggers, and therefore gives pharmacological basis for their therapeutic uses for the treatment of external parasites in traditional medicine. Among our tested materials, garlic showed the best

effect and serve as the most promissory candidate to treat this disease.

INTRODUCTION

The number of farms that raised the pastured chicken has been remarkably increased in these recent years, following the change in consumer taste preferences. However, because of the husbandry characteristics, pastured chicken are more usually affected with parasitic diseases, including the chigger infestation (Nguyen, 2015). Not only induce the significant loss in chicken production efficacy, chiggers might also become the vectors mediating many dangerous diseases that affect both animals and human (Boseret et al., 2013; Chu et al., 2015; Pampiglione et al., 2001). However, researching on the control of this disease has not yet been focused in Vietnam. Researchers have reported the development of resistant strains of parasites to currently available drugs (Clark et al., 1996; Currie et al., 2004; Fernandes and Freitas, 2007; Geerts and Gryseels, 2000; Halley et al., 1993; Kaplan and Nielsen, 2010; Nong et al., 2012; Nong et al., 2013; O'Brien, 1999; Ribeiro et al., 2007; Terada et al., 2010; William et al., 2001). There has been an increasing interest in searching for other control methods to alternate those synthesized drugs application, and among which researchers have been considering the use of medicinal plants as a promissory therapy (Madzimure et al., 2011; Moyo and Masika, 2009). Botanical anti-parasitic plants have been known for many advantages features: they can be degraded in the environment, do not remain in livestock, are not as prone to resistance and are relatively safe for humans, animals and the environment (Nong et al., 2012). In this study, we focused to test the effects of garlic, lemon grass, fortune bogorchid and cinnamon, because these four medicinal plants are well-known for the therapeutic uses with external parasites (Chevallier, 1999; Do, 2005a, b, c, d; Le and Nguyen, 1999 a, b, c). In addition, we chose to test with pastured chicken affected with chiggers, because the infection rate of this disease on this type of domestic chicken is remarkably high in Vietnam (Nguyen, 2015). We also expect that information about the effects of medicine plants on this disease would help the farmers and veterinary managers to select them as the cheap and effective therapy to alternate synthesized drugs in the control of chigger diseases on chicken, and particularly pastured chicken.

MATERIALS AND METHODS

Collect and extract plant materials

Plants were collected in Vietnam. The plants in scientific, English and local names, along with their collected parts are shown in Table 1. Their identity was confirmed by Professor Nguyen Thi Kim Lan, PhD, DVM, Faculty of Animal husbandry and Veterinary Medicine, Thai Nguyen University of Agriculture and Forestry, Vietnam. The fresh plant materials were washed, preliminarily dried in the shadow and then pulverized into powder. The essential oil extraction was performed employing the facilities of Gaia's Gift Natural Compound Company (Thuong Tin district, Hanoi). In brief, each 30 kg of plant materials were mixed with 70 liters of distilled water and extracted for

2 h in 95°C and at 780 mmHg atmospheric condition. Obtained essential oils were then dissolved in ethanol 40% to produce the oils at concentrations of 1%, 2%, 3% and 4% to test with chicken.

Table 1. List of plant materials

No	English name	Local name	Latin name	Collected part
1	Lemon grass	Cay sa	<i>Cymbopogon ssp.</i>	Leaves and bulbs
2	Garlic	Cu toi	<i>Allium sativum</i> L	Bulbs
3	Fortune Bogorochid	Cay man tuoi	<i>Eupatorium fortune turcz</i>	Aerial parts
4	Cinnamon	Vò que	<i>Cinnamomum cassia</i> Bl	Bark

TEST EFFECTS OF ESSENTIAL OILS ON CHICKEN AFFECTED WITH CHIGGERS

Chicken affected with chiggers at same level were divided into groups of 5 individuals, and the number of chigger sockets in all infested chicken were recorded. The identification of chiggers and chigger sockets on experimental chicken was performed under the supervision of Professor Nguyen Thi Kim Lan, PhD, DVM, Faculty of Animal husbandry and Veterinary Medicine, Thai Nguyen University of Agriculture and Forestry, Vietnam. Chicken infested with chiggers were then treated with different oil extracts at different concentrations by spraying the oils directly on the infected places once a day for continuous 5 d. After 5 d, chicken were examined again and the numbers of sockets were recorded, in order to compare with that numbers before the treatment and evaluate the oil effects.

RESULTS

The treatment effects of essential oils of tested medicinal plants on parasitic chiggers infested on chicken

The effects of different concentrations of essential oils extracted from garlic, lemon grass, fortune bogorochid and cinnamon on parasitic chiggers infested on chicken are shown in Table 2. We observed that garlic showed the outstanding effect, because only at the concentration of 1%, it were able to eliminate the infested chiggers in all tested chicken and exerted 100% of treatment efficacy. On the other hand, essential oils of lemon grass, fortune bogorochid and cinnamon, at all tested concentrations, were not able to terminate this disease. Their highest treatment efficacy were only 60%, and observed when they were applied at the highest concentration (4%). However, at concentration of 3%, garlic oil exerted the side-effect, which shown by the congestion appeared on chicken treated skins. For this reason, we did not further tested garlic oil at concentrations that higher than 3%.

Table 2. Treatment effects of the essential oils extracted from garlic, lemon grass, fortune bogorchid and cinnamon on parasitic chiggers infested on chicken

Concentration	Medicine plants	Before treatment		After 5 d of treatment			Treatment efficacy		Record of any abnormal signs
		Infected chicken (No)	Infectious degree (socket/ chick)	Infected chicken (No)	Infectious degree (socket/ chick)	Chigger-free chicken (No)	Efficacy (%)		
		1%	Garlic	5	8-11	0	0	5	
	Lemon Grass	5	8-11	5	5-8	0	0	No	
	Fortune Bogorchid	5	8-10	5	4-7	0	0	No	
	Cinnamon	5	9-11	5	5-9	0	0	No	
2%	Garlic	5	9-11	0	0	5	100	No	
	Lemon Grass	5	7-10	5	3-5	0	0	No	
	Fortune Bogorchid	5	8-11	4	3-5	1	20	No	
	Cinnamon	5	8-10	5	4-5	0	0	No	
3%	Garlic	5	7-10	0	0	5	100	Congestion in treated skin area	
	Lemon Grass	5	9-11	4	2-4	1	20	No	
	Fortune Bogorchid	5	8-10	3	1-3	2	40	No	
	Cinnamon	5	8-11	4	2-5	1	20	No	
4%	Garlic					Not test			
	Lemon Grass	5	8-11	2	1-3	3	60	No	
	Fortune Bogorchid	5	8-10	2	1-3	3	60	No	
	Cinnamon	5	8-11	3	2-4	2	60	No	

DISCUSSION

All of essential oils extracted from garlic, lemon grass, fortune bogorchid and cinnamon showed different levels of anti-parasitic effects on chicken chigger, and thus providing pharmacological basis for their therapeutic uses in external parasitic diseases, which have been recorded in traditional herbalism (Chevallier, 1999; Do, 2005a, b, c, d; Le and Nguyen, 1999 a, b, c). Even there has been a number of studies investigated the anti-parasitic effect on both of external and internal parasites (Athanasiadou et al., 2007; Fernandes and Freitas, 2007; Githiori et al., 2006; Kaaya et al., 1995; Nadkarni, 2002; Nguyen et al., 2014; Nguyen et al., 2015a; Nguyen et al., 2015b; Nguyen and Miyamoto, 2014; Pirali-Kheirabadi and Razzaghi-Abyaneh, 2007; Pirali-Kheirabadi and Teixeira da Silva, 2011; Ribeiro et al., 2007), our study represents the first research that tested the medicine plant effects with chiggers infested on chicken. Our study found that among all of tested plants, garlic is the most promissory candidate for the treatment of this disease. Researcher have reported the remarkably high potency of garlic in bacterial treatment (Nguyen et al., 2015a; Nguyen et al., 2016), and our study further confirmed the high pharmacological property of this plant. However, we also observed that when applying at high concentrations (starting from 3%), garlic oil might induce the side-effect. Researcher have reported that within the concentrations in which drugs or plants/ plants preparation showed the appropriate treatment, lower concentrations are always recommended to limit the side-effects with host animals. (Nguyen et al., 2014). We therefore suggested that the application of garlic at concentrations of 1% or 2% are the most advantageous therapy to treat parasitic chigger disease on chicken, because they can terminate the disease while still show no harmful effect on the host chicken. With the abundant of garlic in Vietnam and the simple in oil extraction process, we believe that garlic oil might serve as the cheap and effective therapy to control this disease. However, further studies are required to additionally clarify garlic pharmacological properties in parasitic chiggers infested on chicken, with the prospect of applying this medicinal plant as an alternative therapy to replace synthesized drugs in the control of this disease.

CONCLUSION

All of essential oils extracted from garlic, lemon grass, fortune bogorchid and cinnamon showed anti-parasitic effects on chicken chigger, but only the oil from garlic can terminate the disease in all of tested chicken. The results suggested that garlic oil is the most promissory candidate for the treatment of this disease. The concentrations of 1% and 2 % of garlic oil should be used because they showed 100% of treatment efficacy but exerted no side-effects. Follow-up study should be focus on garlic oil to further access its potential.





REFERENCES

1. Athanasiadou, S., Githiori, J., Kyriazakis, I. *Medicinal plants for helminth parasite control: facts and fiction. Animal 1: 1392-1400, 2007.*
2. Boseret, G., Losson, B., Mainil, J.G., Thiry, E., Saegerman, C. *Zoonoses in pet birds: a review and perspective. Vet Res. 44: 36, 2013.*
3. Brien, D.J. *Treatment of psoroptic mange with reference to epidemiology and history. Veterinary Parasitology 83: 177-185, 1999.*
4. Chevallier, A. *Duoc Thao Toan Thu [The Encyclopedia of Medicinal Plants]. A DK Publishing House, UK. 1999. Translated to Vietnamese by Kim Dan Nguyen. Ho Chi Minh publishing house, Vietnam. 2006. Allium sativum (Liliaceae). In Chevallier, A. (Eds.), pp: 78-79.*
5. Chu, T.T., Murano, T., Uno, Y., Usui, T., Yamaguchi, T. *Molecular epidemiological characterization of poultry red mite, Dermanyssus gallinae, in Japan. J Vet Med Sci. 77:1397-1403, 2015.*
6. Clark, A.M., Stephen, F.B., Cawley, G.D., Bellworthy, S.J., Groves, B.A. *Resistance of the sheep scab mite Psoroptes ovis to propetamphos. Veterinary Record: 139 and 451, 1996.*

7. Currie, B.J., Harumal, P., Mckinnon, M., Walton, S.F. First documentation of in vivo and in vitro ivermectin resistance in *Sarcoptes scabiei*. *Clinical Infectious Diseases* 39: 8212, 2004.
8. Do, T.L. 2005 (a). *Nhung cay thuoc va vi thuoc thuong dung tai Vietnam*. [Book in Vietnamese]. Vietnam Medical publishing house, Hanoi, Vietnam. Man tuoi. In: Do TL (Eds.), pp. 252-253.
9. Do, T.L. 2005 (b). *Nhung cay thuoc va vi thuoc thuong dung tai Vietnam*. [Book in Vietnamese]. Vietnam Medical publishing house, Hanoi, Vietnam. Que. In: Do TL (Eds.), pp. 857-858.
10. Do, T.L. 2005 (c). *Nhung cay thuoc va vi thuoc thuong dung tai Vietnam*. [Book in Vietnamese]. Vietnam Medical publishing house, Hanoi, Vietnam. Sa. In: Do TL (Eds.), pp. 688-689.
11. Do, T.L. 2005 (d). *Nhung cay thuoc va vi thuoc thuong dung tai Vietnam*. [Book in Vietnamese]. Vietnam Medical publishing house, Hanoi, Vietnam. Toi. In: Do TL (Eds.), pp. 181-182.
12. Fernandes, F.F. and Freita, E.P.S. Acaricidal activity of an oleoresinous extract from *Copaifera reticulata* (Leguminosae: Caesalpinioideae) against larvae of the Souther Cattle tick, *Rhipicephalus (Boophilus) microplus* (Acari: Ixididae). *Vet. Parasitol.* 147: 150-154, 2007.
13. Geerts, S. and Gryseels, B. Drug resistance in human helminthes: current situation and lessons from livestock. *Clinical Microbiology Review.* 13: 207-222, 2000.
14. Githiori, J.B., Athanasiadou, S., Thamsborg, S.M. Use of plants in novel approaches for control of gastrointestinal helminthes in livestock with emphasis on small ruminants. *Vet. Parasitol.* 139, 308-320, 2006.
15. Halley, B.A., Vanden Heuvel, W.J.A., Wislocki, P.G. Environmental effects of the usage of avermectines in livestock. *Veterinary Parasitology* 48: 109-125, 1993.
16. Kaaya, G.P., Mwangi, E.N., Malanza, M.M. Acaricidal activity of margaritaria discoedea plant extracts against the ticks *Rhipicephalus appendiculatus* and *hyaloma varigatum*. *Int. J. Acarol.* 21: 123-129, 1995.
17. Kaplan, R.M and Nielsen, M.K. Drug resistance in equine parasites: an emerging global problem. *J. Equine Vet. Sci.* 29: 285-295, 2010.
18. Le, V.T. and Nguyen, G.C. 1999 (a). *Selected Medicinal plants in Vietnam, Volume I. Vietnam National Institute of Materia Medica Publications. Science and Technology Publishing house, Hanoi, Vietnam. Cinnamomum cassia Blume – Lauraceae.* In: Le Van Truyen and Nguyen Gia Chan (Eds.), pp. 193-199.
19. Le, V.T. and Nguyen, G.C. 1999 (b). *Selected Medicinal plants in Vietnam, Volume I. Vietnam National Institute of Materia Medica Publications. Science and Technology Publishing house, Hanoi, Vietnam. Cymbopogon sp. – Poaceae.* In: Le Van Truyen and Nguyen Gia Chan (Eds.), pp. 271-275.
20. Le, V.T. and Nguyen, G.C. 1999 (c). *Selected Medicinal plants in Vietnam, Volume I. Vietnam National Institute of Materia Medica Publications. Science and Technology Publishing house, Hanoi, Vietnam. Eupatorium fortune Turcz - Asteraceae.* In: Le Van Truyen and Nguyen Gia Chan (Eds.), pp. 342-345.
21. Madzimure, J., Nyahangare, E.T., Hamudikuwanda, H., Hove, T., Stevenson, P.C., Belmain, S.R., Mvurni, B.M. Acaricidal efficacy against cattle ticks and acute oral toxicity of *Lippia javanica* (Burn F.) spreng. *Tropical Animal Health and Production.* 43: 481-489, 2011.
22. Moyo, B. and Masika, P.J. Tick control methods used by resource-limited farmers and the effect of ticks on cattle in rural areas of the Eastern Cape Province, South African. *Tropical Animal Health and Production.* 41: 517-523, 2009.
23. Nadkarni, K.M. *Indian Materia Medica, Vol-II, 2nd Edn. Bombay Popular Prakashan, Mumbai.* 237-239, 2002.

24. Nguyen, T.H. and Miyamoto, A. 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 Plants Research*. 3: 284-289, 2014.
25. Nguyen, T.H., Nguyen, V.T, Bui, T.T., Miyamoto, A. A study about anthelmintic effect of *Punica gramatum* L bark on veterinary endo-parasites. *Asian Journal of Pharmacology Clinical Research*. 7: 148-152, 2014.
26. Nguyen, T.N. A study on the chigger diseases on pastured chicken raised in Cam Pha city, Quang Ning prefecture – the prevention and treatment. Master thesis in Thai Nguyen University of Agriculture and Forestry, Thai Nguyen, Vietnam, 2015.
27. Nguyen, V.T, Nguyen, T.H, Bui, T.T, Miyamoto, A. The effect of garlic (*Allium sativum* L.) bulbs and ginger (*Zingiber officinale*) rhizomes extracts on *Escherichia coli* and *Salmonella spp* isolated from duck diarrhea syndrome. *International Journal of Medical Research and Pharmaceutical Sciences*. 2: 8-12, 2015 (a).
28. Nguyen, V.T., Nguyen, T.H, Dao, T.H. and Miyamoto, A. The effect of garlic (*Allium sativum* L.) bulbs and pepper (*Piper nigrum* L.) seeds ethanol extracts on piglet diarrhea. *International Journal of Medical Research and Pharmaceutical Sciences*. 3: 23-28, 2016.
29. Nguyen, V.T., Nguyen, T.H., Dam, Q.T. and Miyamoto, A. Study on the inhibition effects of some vietnamese traditional medicinal plants on egg hatching and larval movement of goat haemonchus contortus. *International Journal of Medicinal Plants Research*. 4: 309-313. 2015 (b).
30. Nong, X., Fang, C.L., Wang, J.J., Gu, X.P., Yang, D.Y., Liu, T.F., Fu, Y., Zhang, R.H., Zheng, W.P., Peng, X.R., Wang, S.X., Yang, G.Y. Acaricidal activity of extract from *Eupatorium adenophorum* against the *Psoroptes cuniculi* and *Sarcoptes scabiei* in vitro. *Veterinary Parasitology*. 187: 345-349, 2012.
31. Nong, X., Ren, Y.J., Wang, J.H., Fang, C.L., Xie, Y., Yang, D.Y., Liu, T.F., Chen, L., Zhou, X., Gu, X.B., Zheng, W.P., Peng, X.R., Wang, S.X., Lai, S.J., Yang, G.Y. Clinical efficacy of extract from *Eupatorium adenophorum* against the scab mite, *Psoroptes cuniculi*. *Veterinary Parasitology*. 147: 199-203, 2013.
32. Pampiglione, S., Pampiglione, G., Pagani, M., Rivasi, F. [Article in Italian]. [Persistent scalp infestation by *Dermanyssus gallinae* in an Emilian country-woman]. *Parassitologia*. 43:113-5, 2001.
33. Pirali-Kheirabadi, K.H, Teixeira da Silva J.A. In-vitro assessment of the acaricidal properties of *Artemisia annua* and *Zataria multiflora* essential oils to control cattle ticks. *Iranian J. Parasitol* 6: 58-65, 2011.
34. Pirali-Kheirabadi, K.H. and Razzaghi-Abyaneh, M. Biological activities of chamomile (*Matricaria chamomile*) flowers' extract against the survival and egg laying of the cattle fever ticks (*Acari Ixodidae*). *J. Zhejiang University Science B*. 8: 693-696, 2007.
35. Ribeiro, V.L.S, Toigo, E., Bordignon, S.A.L, Gonc, K., Poser Alves, G.V. Acaricidal properties of extracts from the aerial parts of *Hypericum polyanthemum* on the cattle ticks *Boophilus microplus*. *Veterinary Parasitology*. 147: 199-203, 2007.
36. Terada, Y., Murayama, N., Ikemura, H., Morita, T., Nagata, M. *Sarcoptes scabiei* var. *canis* refractory to Ivermectin treatment in two dogs. *Veterinary Dermatology* 21: 608-612, 2010.
37. William, S., Botros, S., Ismail, M., Fraghally, A., Day, T.A., Bennet, J.I. Praziquantel-induced regimental damage in vitro is diminished in schistosomes derived from praziquantel-resistant infections. *Parasitology* 122. 63-66, 2001

AUTHOR BIBLIOGRAPHY

	<p>Nguyen Thi Kim Lan, PhD, DVM</p> <p>Professor, lecturer in Faculty of Animal Husbandry and Veterinary Medicine, Thai Nguyen University of Agriculture and Forestry, Vietnam. website profile: http://mysite.tuaf.edu.vn/nguyenthikimlan Email: nguyenthikimlan@tuaf.edu.vn</p>
	<p>Pham Dieu Thuy, PhD, DVM</p> <p>Lecturer in Faculty of Animal Husbandry and Veterinary Medicine, Thai Nguyen University of Agriculture and Forestry, Vietnam. Website profile: http://mysite.tuaf.edu.vn/phamdieuthuy Email: phamdieuthuy@tuaf.edu.vn</p>
	<p>Dao Thi Huong, DVM</p> <p>Veterinarian in PDS Dog Professional Research Center, Faculty of Veterinary Medicine, Vietnam National University of Agriculture, Vietnam. Email: huong.dvm.pds.center.2006@gmail.com. Website of affiliation (PDS Dog Professional Research Center): http://www.huanluyenchopds.com</p>
	<p>Dao Van Cuong, DVM, MSc</p> <p>Lecturer in Faculty of Animal Husbandry and Veterinary Medicine, Thai Nguyen University of Agriculture and Forestry, Vietnam. PhD student in Department of Veterinary Pharmacology, Joint Faculty of Veterinary Medicine, Kagoshima University, Japan. Website profile: http://mysite.tuaf.edu.vn/daovancuong Email: cuongdhnl@gmail.com</p>