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Efficacy of *Piper aduncum* Extract against the Adult Housefly (*Musca domestica*)

Kong Chiou Mee, Sallehudin Sulaiman, Hidayatulfathi Othman

Department of Biomedical Science, Faculty of Allied Health Sciences, Universiti Kebangsaan Malaysia, Jalan Raja Muda Abdul Aziz, 50300 Kuala Lumpur

Abstract

The efficacy of *Piper aduncum* extract was evaluated under laboratory conditions for its adulticidal activity against the housefly *Musca domestica* WHO 213 strain and the wild strain from Chow Kit, Kuala Lumpur. By topical application, the LC_{50} and LC_{90} of *P. aduncum* extract on WHO 213 males were 6,151 ppm (= 6.2 µg/fly) and 13,353 ppm (= 13.3 µg/fly), with 11,036 ppm (= 11.0 µg/fly) and 35,252 ppm (35.3 µg/fly) for WHO 213 females, respectively. The LC_{50} and LC_{90} of *P. aduncum* extract on wild-strain Chow Kit males were higher, at 9,981 ppm (= 10.0 µg/fly) and 28,898 ppm (= 28.9 µg/fly), while the wild-strain Chow Kit females were 23,821 ppm (= 23.8 µg/fly) and 50,502 ppm (= 50.5 µg/fly), respectively. Resigen® insecticide yielded much lower LC_{50} and LC_{90} values, of 3.55 ppm (= 0.004 µg/fly) and 7.88 ppm (= 0.008 µg/fly), for the WHO 213 adult males and 5.13 ppm (= 0.005 µg/fly) and 10.85 ppm (= 0.011 µg/fly), for the WHO 213 adult females, respectively. Resigen® also showed much lower LC_{50} and LC_{90} values for the adult Chow Kit males, of 4.45 ppm (= 0.004 µg/fly) and 10.35 ppm (= 0.010 µg/fly), and adult Chow Kit females, of 5.23 ppm (= 0.005 µg/fly) and 11.07 ppm (= 0.011 µg/fly), respectively. There were significant differences between the effects of *P. aduncum* extract on WHO 213 and wild Chow Kit ($p < 0.05$), and between *P. aduncum* extract and Resigen® for both WHO 213 and Chow Kit wild strain ($p < 0.05$). *P. aduncum* extract, although less effective than the commercial product Resigen®. However, it is abundante in the Tropics, and could be utilized for adult housefly control.

Keywords: *Musca domestica, Piper aduncum,* topical application

Introduction

Musca domestica Linnaeus (Diptera: Muscidae) act as important mechanical carriers of pathogenic bacteria, such as *Shigella* sp, *Vibrio cholerae*, *Escherichia coli*, *Staphylococcus aureus*, and *Salmonella* sp [1]. In Malaysia, several bacterial species have been isolated from houseflies, including *Acinetobacter* sp, *Bacillus* sp, *Enterobacter* sp, *Proteus* sp, *Escherichia* sp, and *Klebsiella* sp [2]. In another study in the Chow Kit area of Kuala Lumpur, 18 species of bacteria were isolated from houseflies [3]. In a recent study, it has been shown that houseflies may transmit *E. coli* 0157: H7 (EHEC) [4], which is highly associated with hemolytic uremic syndrome.

Several surveys have shown that insecticide

Correspondence:
Sallehudin Sulaiman,
E-mail: <salsul@medic.ukm.my>

resistance among houseflies is wide spread and increasing [5-7]. Pyrethroids were initially highly effective in housefly control. Unfortunately, very high levels of resistance to this class of insecticides have developed worldwide, due to their intensive use over the past 30 years [5, 8-10]. The development of insecticide resistance in houseflies has resulted in failure to control housefly populations, in the areas of agriculture and public health [11]. Considerable efforts have been made to synthesize an alternative to overcome this problem. Botanical products have become more prominent in assessing current and future pest control alternatives, [12].

Over the past two decades, surveys of plant families [13-15] have discovered sources of new botanical insecticides, which could possibly meet some of this demand. The Piperaceae family has many promising phytochemicals with insecticidal activity [16]. *Piper aduncum* L, also known as "matico", is a member of the Family Piperaceae. It has a wide range of traditional uses and its essential oil is a well-known insecticide, molluscicide, and antibacterial [17]. Previous studies have revealed *P. aduncum* extract to be very efficacious against *Aedes aegypti* larvae [18,19], and the parasitic protozoan, *Leishmania amazonensis* [20]. However, no study has been conducted on houseflies. The aim of present study was to determine the efficacy of *P. aduncum* plant extract against adult houseflies in the laboratory.

Materials and methods

Plant collection

P. aduncum leaves were collected from a secondary jungle in Gombak, 13 miles east of Kuala Lumpur (3.29°U; 101.63°T). Botanists at the Universiti Kebangsaan Malaysia (National University of Malaysia), Bangi, identified and confirmed the species.

Isolation of essential oil

Leaf samples were washed and shade-dried at room temperature. The dried leaves were ground into small pieces to enhance extraction yield. Essential oils were obtained by hydro-distillation of dried leaves for 8 hours in a Clevenger-type apparatus. The oil layer was separated, dried over anhydrous sodium sulphate, and stored in an aluminum-foil-sealed glass ampoule under refrigeration at 8°C, until required for analysis. Stock solutions were then prepared by dissolving one gram of plant extract in acetone (Fisher Scientific Malaysia, Pte, Ltd), 1% Tween 80 (Fisher Scientific Malaysia, Pte, Ltd) and distilled water. Stock solutions were then diluted to the required concentrations.

Housefly collection

The houseflies used were of two strains: WHO 213 strain (obtained from the Institute of Medical Research) and a wild population caught from dump sites in Chow Kit, Kuala Lumpur. The houseflies were reared in the insectarium of the Universiti Kebangsaan Malaysia at 25-27°C, and 55-60% relative humidity.

Adulticidal bioassay

An adulticidal bioassay was performed by topical application [21], with slight modification. Using a Gilson pipette, one microliter of different concentrations of plant extract were applied to the dorsal thorax of 3- to 6-day-old flies after anesthetization by chilling at -10°C for one minute per fly. Treated houseflies were placed in paper cups, which were supplied with sugar cubes. Knockdown effect after one hour, and mortality after 24 hours, were counted after treatment. A total of 6 replicates of 25 flies were used for each concentration. The results were then compared with the positive control, Resigen®, supplied by Bayer Cropscience Malaysia Pte Ltd. All tests were run at 26 ± 2°C.

Statistical analysis

Bioassay data were pooled and analyzed by standard probit analysis [22] to obtain LC_{50} and LC_{90}. LC_{50} and LC_{90} are concentrations in ppm that cause 50 and 90% mortality, with 95% confidence interval. LC_{50} and LC_{90} at 1 hour and 24 hours were then compared statistically using one-way ANOVA.

Results

The LC_{50} and LC_{90} values for *P. aduncum* extract against adult houseflies after treatment for one hour are shown in Table 1. Knockdown is defined as the initial effect, such as morbid or unusual behavior due to alterations in a specific physiological process or processes that take place upon contact with the toxicant. Female houseflies from Chow Kit showed low susceptibility towards *P. aduncum* extract, with higher concentrations for LC_{50} (31,153.61 ppm) and LC_{90} (63,292.01 ppm) values after 1 hour exposure. Meanwhile, male Chow-Kit strain houseflies showed higher susceptibility towards *P. aduncum* extract, since lower concentrations were recorded for the LC_{50} (18,434.43 ppm) and LC_{90} (49,641.05 ppm) values, respectively. After 24 hours, higher LC_{50} (23,820.78 ppm) and LC_{90} (50,501.84 ppm) values were obtained for the Chow Kit females, and lower LC_{50} (9,981.39 ppm) and LC_{90} (28,898.01 ppm) values for the Chow Kit males (Table 2).

The WHO 213 male and female houseflies proved more susceptible to *P. aduncum* extract than the Chow Kit wild strain. The LC_{50} and LC_{90} values for the WHO 213 males were 9,428.42 ppm and 20,771.87 ppm after 1 hour's exposure, and 6,151.46 ppm and 13,353.09 ppm after 24 hours, respectively. The LC_{50} and LC_{90} for the WHO 213 females were 17,663.86 ppm and 52,387.06 ppm after 1 hour's exposure and 11,035.98 ppm and 35,252.35 ppm after 24 hours, respectively. Resigen® treatment showed much higher susceptibility than *P. aduncum* extract, for both housefly strains. The LC_{50} and LC_{90} for Chow Kit females after 1 hour's treatment with Resigen® were 7.4898 ppm and 16.8486 ppm, and at 24 hours' post-treatment, 5.2284 ppm and 11.0659 ppm, respectively. The Chow Kit males after 1 hour's treatment with Resigen® had LC_{50} and LC_{90} values of 6.9523 ppm and 14.0505 ppm, respectively, and 4.4532 ppm and 10.3493 ppm at 24 hours' post-treatment. The male WHO 213 houseflies had lower LC_{50} and LC_{90} values than the female houseflies after 1 hour and 24 hours post-treatment with Resigen®. The LC_{50} and LC_{90} values for the male WHO 213 were 4.9582 ppm and 12.2924 ppm after 1 hour's exposure to Resigen®, and 3.5509 ppm and 7.8830 ppm 24 hours' post-treatment, respectively. The female WHO 213 at 1 hour post-treatment with Resigen® indicated LC_{50} and LC_{90} values of 6.7772 ppm and 14.1425 ppm, and at 24 hours, 5.1273 ppm and 10.8513 ppm, respectively. There was a significant difference in the effect of *P. aduncum* extract

Table 1 LC_{50} and LC_{90} values of *P. aduncum* extract against adult houseflies after 1 hour.

Strain & gender	*P. aduncum* extract (ppm)				Resigen® (ppm)			
	LC_{50}	95% CI	LC_{90}	95% CI	LC_{50}	95% CI	LC_{90}	95% CI
WHO 213 Male	9,428.417[bcd]	8,714.08 - 10,200.27	20,771.87[bcd]	18,578.48 - 23,745.73	4.95852[bcd]	0.4655 - 52.4405	12.2924	0.0720 - 2,576.18
WHO 213 Female	17,663.86[ad]	16,024.64 - 19,584.99	52,387.06[a]	44,213.56 - 64,704.86	6.7772[a]	0.6594 - 71.0719	14.1425	0.1097 - 2,058.66
Chow Kit Male	18,434.43[ad]	16,816.10 - 20,310.05	49,641.05[a]	42,479.31 - 60,254.46	6.9523[a]	1.3114 - 38.4347	14.0505	0.4533 - 552.3011
Chow Kit Female	31,153.61[abc]	26,424.01 - 36,732.30	63,292.01[a]	46,090.59 - 89,105.68	7.4898[a]	1.7027 - 40.9507	16.8486	0.5301 - 1,484.3410

[a] - Significant difference ($p < 0.05$) compared with male WHO 213 houseflies.
[b] - Significant difference ($p < 0.05$) compared with female WHO 213 houseflies.
[c] - Significant difference ($p < 0.05$) compared with male Chow Kit houseflies.
[d] - Significant difference ($p < 0.05$) compared with female Chow Kit houseflies.

Table 2 LC_{50} and LC_{90} values of *P. aduncum* extract against adult houseflies after 24 hours.

Strain & gender	*P. aduncum* extract (ppm)				Resigen® (ppm)			
	LC_{50}	95% CI	LC_{90}	95% CI	LC_{50}	95% CI	LC_{90}	95% CI
WHO 213 Male	6,151.463[bcd]	5,640.293 - 6,683.197	13,353.09[bcd]	11,924.73 - 15,344.31	3.5509[bcd]	3.1744 - 3.8908	7.8830	6.9541 - 9.3955
WHO 213 Female	11,035.98[ad]	7,509.575 - 16,225.01	35,252.35[a]	18,017.60 - 70,057.25	5.1273[ac]	0.5928 - 44.3692	10.8513	0.1578 - 832.5186
Chow Kit Male	9,981.39[ad]	6,064.447 - 16,428.18	28,898.01[ad]	12,627.41 - 66,595.99	4.4532[ab]	0.8029 - 23.5416	10.3493	0.3901 - 404.1643
Chow Kit Female	23,820.78 [abc]	19,477.74 - 29,043.69	50,501.84[ac]	37,075.07 - 69,816.40	5.2284[a]	4.8386 - 5.6542	11.0659	9.7052 - 13.1912

[a] - Significant difference ($p < 0.05$) compared with male WHO 213 houseflies.
[b] - Significant difference ($p < 0.05$) compared with female WHO 213 houseflies.
[c] - Significant difference ($p < 0.05$) compared with male Chow Kit houseflies.
[d] - Significant difference ($p < 0.05$) compared with female Chow Kit houseflies.

on WHO 213 and wild Chow Kit *M. domestica* ($p < 0.05$), and also a significant difference between *P. aduncum* extract and Resigen® for both WHO 213 and Chow Kit wild strain ($p < 0.05$).

Discussion

Many plant extracts have shown potential insecticidal activity against houseflies. Studies in Ceylon (now Sri Lanka) using *Cinnamomum zeylanicum* bark and *Cymbopogon citratus* oils showed good knockdown and mortality against adult *M. domestica,* with LD_{50} of 1.37 µg/fly and 1.71 µg/fly, respectively; *C. zeylanicum* leaf and *Cymbopogon nardus* oils had LD_{50} of 2.75 µg/fly and 3.10 µg/fly, respectively [23]. Studies using *Piper betle* leaf oil against adult *M. domestica* indicated LC_{50} 10.3 and 8.7 mg/dm³ after 24 and 48 hours' exposure, respectively; *C. nardus* oil, used as a standard, showed LC_{50} values of 26.5 and 24.2 mg/dm³ after 24 and 48 hours' exposure, respectively [24]. Studies in Thailand, using topical applications of eucalyptol against houseflies and blowflies showed that male *M. domestica* were more susceptible than females, with LD_{50} values of 118 and 177 µg/fly, respectively. A higher LD_{50} for *Chrysomya megacephala* was obtained; 197 µg/fly for males and 221 µg/fly for females [25].

The present study also indicated that males of both WHO 213 and Chow Kit strains were more susceptible than females. Studies in India, using *Campsis grandiflora* aqueous leaf extract against *M. domestica* adults, showed 80% mortality with 5.75 µg/fly topical application [26]. Studies in Ghana on the toxicity of *Griffonia simplicifolia* seed extract and *Zanthoxylum xanthoxyloides* root extract were effective against *M. domestica* adults. The LD_{50} for 24-hour topical application of *G. simplicifolia* seed extract and *Z. xanthoxyloides* root extract were 0.28 and 0.35 µg, respectively [27]. Studies in Argentina using topical applications of 12 essential oils against *M. domestica* adults found that *Citrus sinensis* was the most potent insecticide (LC_{50} = 3.9 mg/dm³), and by GC/MS analysis, limonene (92.47%), linalool (1.43%) and ß-myrcene (0.88%) were the principal components of the essential oil of *C. sinensis* [28]. Although the commercial product Resigen®, indicated higher adulticidal efficacy than *P. aduncum* extract, constant and long-term usage of this commercial synthetic insecticide would likely result in resistance problems. It is timely that a natural product, such as *P. aduncum* extract, could be utilized or combined with synthetic insecticide for use in housefly control programs.

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References

1. Greenberg B. Flies and disease (Vol. II). Biology and disease transmission. New Jersey: Princeton University Press; 1973.
2. Sulaiman S, Aziz AH, Hashim Y. Abdul Rahim S. Isolations of enteropathogenic bacteria from some cyclorrhaphan flies in Malaysia. Malays Appl Biol. 1988; 17:129-33.
3. Sulaiman S, Othman MZ, Aziz AH. Isolation of enteric pathogens from synanthropic flies trapped in downtown Kuala Lumpur. J Vector Ecol. 2000; 25:90-3.
4. Iwasa M, Makino S, Asakura H, Kobori H, Kaljser B. Detection of *Escherichia coli* O157: H7 from *Musca domestica* (Diptera: Muscidae) at a cattle farm in Japan. J Med Entomol. 1999; 36**:**108-12.
5. Scott JG, Alefantis TG, Kaufman PE, Rutz DA. Insecticide resistance in house flies from caged-layer poultry facilities. Pest Manage Sci. 2000;56:147-53.
6. Kaufman PE, Scott JG, Rutz DA. Monitoring insecticide resistance in house flies from New York dairies. Pest Manage Sci. 2001;57:514-21.
7. Kristensen M, Andrew GS, Jorgen BJ. The status and development of insecticide resistance in Danish populations of the house fly, *Musca domestica* L. Pest Manage Sci. 2001;57**:**82-9.
8. Hoton DL, Shepherd DC, Nolan Jr MP, Ottens RJ, Joyce JA. House fly (Diptera: Muscidae) resistance to permethrin in a Georgia caged layer poultry operation. J Agr Entomol. 1985; 2**:**196-9.
9. Chapman PA, Learmount J, Morris AW, McGreevy PB. The current status of insecticide resistance in *Musca domestica* in England and Wales and the implications for housefly control in intensive animal units. Pest Sci. 1993;39**:**225-35.
10. Pap L, Farkas R. Monitoring of resistance of insecticides in housefly (*Musca domestica*) populations in Hungary. Pestic Sci. 1994;40**:**245-58.
11. Chobei I. Control of insecticide resistance in a field population of houseflies, *Musca domestica*, by releasing susceptible flies. Res Popul Ecol.1987;29**:**129-46.
12. National Research Council. The future role of pesticides in U.S. agriculture. Committee on the future role of pesticides in U.S. agriculture, board on agriculture and natural resources and board on environmental studies and toxicology, Commission on Life Sciences, National Academy of Sciences, Washington DC; 2000.
13. Lydon J, Duke SO. The potential of pesticides from plants. Herbs Spices Medicinal Plants. 1989;4**:**-41.
14. Isman MB. Botanical insecticides. Pestic Outlook.1994;5**:** 26-30.
15. MacKinnon S, Chauret D, Wang M, Mata R, Pereda-Miranda R, Jiminez A, *et al*. In: Hedin PA, Hollingworth RM, Masler EP, Miyamoto J, Thompson DG, editors. Botanicals from the Piperaceae and Meliaceae of the American Neotropics: phytochemistry. Washington DC: Chemical Society; 1997. p. 49-57.
16. Arnason JT, Durst T, Philoge`ne BJR. Prospection d'insecticides phytochimiques de plantes tempe´re´es et tropicales communes ou rares. In: Regnault-Roger C, Philoge`ne BJR, Vincent C, editors. Biopesticides d'origine ve´ge´ tale. Editions TEC and DOC, Paris 2002. p. 37-51.
17. Pohlit AM, Pinto ACS, Mause R. *Piper aduncum* L: pluripotente plant and important phytochemical substance source. Rev Fitos. 2006;2**:**7.
18. Hidayatulfathi O, Sallehudin S, Ibrahim J, Azizol AK. Evaluation of methanol extracts of some Malaysian plants for larvicidal activities. Trop Biomed. 2003;20:153-7.
19. Hidayatulfathi O, Sallehudin S, Ibrahim J. Adulticidal activity of some Malaysian plant

extracts against *Aedes aegypti* Linnaeus. Trop Biomed. 2004;21:61-7.

20. Torres-Santo EC, Moreira DL, Kaplan MAC, Meirelles MN, Rossi-Bergmann B. Selective effect of 2′,6′-dihydroxy-4′-methoxychalcone isolated from *Piper aduncum* on *Leishmania amazonensis*. Am Microbiol. 1999;43:1234-41.
21. World Health Organization. Insecticide resistance and vector control. Geneva: WHO; 1970.
22. Finney DJ. Probit analysis. Cambridge: University Press; 1971.
23. Samarasekera, Radhika, Kalhari, Kosmulalage S, Weerasinghe, Indira S. Insecticidal activity of essential oils of *Ceylon cinnamomum* and *Cymbopogon* species against *Musca domestica*. J Essent Oil Res. 2006;18:352-4.
24. Mohottalage S, Tabacchi R, Guerin PM. Components from Sri Lankan *Piper betle* L. leaf oil and their analogues showing toxicity against the housefly, *Musca domestica*. Flavour Frag J. 2007;22:130-8.
25. Sukontason KL, Boonchu N, Sukontason K, Choochote W. Effects of eucalyptol on house fly (Diptera: Muscidae) and blow fly (Diptera: Calliphoridae). Rev Inst Med Trop Sao Paulo. 2004;46:97-101.
26. Pandey K. Pesticidal and insecticidal effect of *Campsis grandiflora* (Thumb). J Pharm Allied Sci. 2006;3:274-7.
27. Bisseleua HBD, Gbewonyo SWK, Obeng-Ofori D. Toxicity, growth regulatory and repellent activities of medicinal plant extracts on *Musca domestica* L. (Diptera: Muscidae). Afr J Biotechnol. 2008;7:4635-42.
28. Palacios SM, Bertoni A, Rossi Y, Santander R, Urzua A. Efficacy of essential oils from edible plants as insecticides against the house fly, *Musca domestica* L. Molecules. 2009;14: 1938-47.