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Mosquito Repellence of *Astrolochii hepii*, *Cymbopogon citratus* and *Ocimum gratissimum* Extracts and Mixtures

T. *Kazembe[1] and D. Chauruka[2]

[1]Department of Science and Mathematics Education, Faculty of Education, University of Zimbabwe, P. O. Box MP167, Mount Pleasant, Harare, Zimbabwe.

[2]Ministry of Higher and Tertiary Education, Corner 4th Street and Samora Machel Avenue, Causeway, Harare, Zimbabwe.

Corresponding author Email: kazembet@rocketmail.com

ABSTRACT

The repellences of *Astrolochii hepii*, *Cymbopogon citratus* and *Ocimum gratissimum* extracts and mixtures were studied using a block design experiment involving volunteers' hands in cage with hungry laboratoty bred malaria-free Aedes aegypti mosquitoes at the Ministry of Health Research Institute in Harare, Zimbabwe in 2010 using DEET as standard. *Ocimum gratissimum* gave 100% repellence for 0.5h; *Astrolochii hepii* for i.0h and *Cymbopogon citratus* for 1.5h. The mixtures: *Ocimum gratissimum/ Astrolochii hepii* gave 100% repellence for 1.5h; *Ocimum gratissimum/ Cymbopogon citratus* for 2.5h and *Astrolochii hepii/ Cymbopogon citratus* for 3.5. This indicated that constituents of mixtures of extracts reinforced each other to repel mosquitoes.

Key words: *Astrolochii hepii; Cymbopogon citratus; Ocimum gratissimum;* extracts, extract mixtures.

INTRODUCTION

The burden of malaria, a scourge that kills around two million people (mostly pregnant women and children under five years) mostly in southern Africa is worsened by mosquito resistance to mosquitocides and malaria parasites' resistance to antimalarial drugs [1]. The resistances dictate that new antimalarial drugs and effective mosquito repellents and mosquitocides be developed for personal use, and replacing the synthetic control agents with the more environmentally friendly botanical compounds [2, 3].

Personal protection is particularly important because most mosquito control efforts have been directed towards indoor spraying, bed-netting, and eradication of mosquito breeding sites. These measures are important, but they give protection only to people who are indoors. Other people, for example those working in the fields or tending livestock or performing odd duties outside the house or having outdoor leisure on a warm evening also need protection as they go about their outdoor activities. People in the different malaria endemic regions of the world have always employed mosquito repellent plants to drive mosquitoes away by burning plant parts to generate smoke that kills or repel mosquitoes. Some have hung mosquito repelling plants in their dwelling places or they have kept potted plants to repel mosquitoes [4 - 6].

Wanton destruction of vegetation, coupled with over-harvesting of useful plants, has dwindled the populations of plants that may be used in specialist activities such as malaria control. There is need to develop ways to economically use the remaining plants for the benefit of humanity. One step towards that goal is documentation of the efficacies of the repellent and/or mosquitocidal plants. Such documentation will create a database that may be used to develop mosquito repellents/mosquitocides through use of the best aspects of selected plants in the database. This report highlights some of our efforts towards that goal.

RESEARCH DESIGN

Information about mosquito repelling plants was obtained from inhabitants of an area about 100 km south of Harare by interviewing the inhabitants of the area using the interview guide (Appendix 1). Most interviewees talked about the efficacies of three plants: *Astrolochii hepii, Cymbopogon citratus* and *Ocimum gratissimum.* These were subsequently chosen for evaluation, collected and identified at the Government Herbarium in Harare, chopped to small pieces and dried in the shade for 1 week, then ground to fine powders using mortar and pestle. Literature search

had revealed that in a similar study in Nigeria, *Ocimum gratismum* had exhibited some knock down effects [7]. In a similar study using *Ocimum americanum* in our laboratory no knock down effect was observed. It was, thus tempting to find out if Zimbabwean grown *Ocimum gratissimum* could exhibit some knock down effect. Hence, instead of carrying out the repellence experiments with solutions of the oils, the oils were applied neat. The oils from the three plants in this study were applied neat to facilitate comparison of results.

INFORMATION ON THE THREE PLANTS USED IN THIS STUDY

Astrolochii hepii

Multiple fluffy flowers grow from the joints near the root and droop until they are nearly buried in the ground or in their dried leaves. Multiple much branched stems grow from the woody rootstock. The root is a horizontal rhizome with numerous long, slender roots below it. The plants taste and smell aromatic, resembling a mixture of valerian and camphor. The plants grow in warm regions of the world in rich soil in shady woods. Aristolochia species have been used as birthing herbs since the middle ages all over the world. They were also used in early America together with quinine against fevers and snakebites. The species has been used in medicine as emmenagogue, emetic, to aid parturition, against fever, malaria, and leaves have been used as mosquito repellents. Aristolochic acid has been implicated in kidney failure, nephlopathy and subsequent urinary cancer. Too large doses of aristolochia cause nausea, gripping pains in the bowels, vomiting, and dysenteric tenesmus, irritation of the gastrointestinal tract and of the kidneys. Coma and death may ensue from respiratory paralysis [8-10].

Cymbopogon citrates

Cymbopogon citratus is a toll perennial grass that grows in warm temperate and tropical regions of the world. Its common names include lemon grass, lemongrass, barbed wire grass, silky heads, citronella grass, fever grass, and many others. The plant is native to India and tropical Asia and widely used in Asian cusine as a powder or used as fresh in teas, soups and curries, or added to poultry, beef, fish, and seafood. It is often used as tea in African countries such as Togo and the Democratic Republic of Congo and Latin American countries such as Mexico. In India, it is used both as a medical herb and in perfumery. Brazilians consume it as a tea for anxiety but a study did not prove its efficacy against anxiety. Instead, the tea caused a recurrence of contact dermatitis in one case. In Zimbabwe and surrounding countries the plant is used to relieve coughs and colds and to repel mosquitoes. In Ayuverdic medicine, lemon grass is used as a traditional “soup” to relieve coughs and nasal congestion [11]. Lemon grass is used as a pesticide and preservative and has antifungal properties [12].

Ocimum gratismum

Plants of the Lamiaceae have been used for centuries in folk medicine to treat different diseases such as upper respiratory tract infection, diarrhoea, headache, fever, ophthalmic, skin disease and pneumonia. The plants have been described as antibacterial and antifungal [13]. The plants belong to a group of plants known as spices, erect but usually not higher than 1 m tall and found throughout the tropics and warm temperate regions, mostly in Africa and India. The plants are used for a variety of purposes such as culinary in salads, soups, pastas, and jelly in many parts of the world.

The leaves are used as a general tonic and anti-diarrhoea agent, for treatment of conjunctivitis, applied as a lotion for skin infections and a decoction is drunk or gaggled for bronchitis. The dried leaves are snuffed to alleviate headaches and fevers.

Phytochemical studies and bioassays revealed that the antibiotic activity of the plant was due to the phytochemical constituents of the plant and bioassays revealed that the antibacterial activity corresponded with the traditional uses of the plant in different parts of the world [14].

EXTRACTION OF *CYMBOPOGON CITRATUS* ESSENTIAL OIL

The essential oil was extracted by dry distillation of *Cymbopogon citrus* powder (50g). The round bottomed flask containing the sample was heated by Bunsen burner, collecting the distillate and separating the oil and aqueous phases using a separating funnel, yielding 4.6g of the oil.

EXTRACTION OF *ASTROLOCHII HEPII* AND *OCIMUM GRATISSIMUM*

Accurately weighed 50g samples of the powders of each of the two plants were extracted with EtOAc over 24 hours with agitation, decanted, filtered and the EtOAc removed at the rotavap at 40°C. The recovered EtOAc was poured back into the extraction flask and the plant residues were extracted for a further 24 hours with agitation, decanted, filtered and the solvent recovered at the rotavap. A third extraction with EtOAc revealed that extraction had exhausted. The extracts were combined, re-dissolved in MeOH to homogenize, and the MeOH removed at the rotavap, yielding 3.1g of an off brown gum from *Astrolochii heppii*, and 3.3g from *Ocimum gratissmum*. The gum was kept in the fridge until they were used to prepare test solutions for the experiments.

The plant residues were similarly exhaustively extracted with MeOH over 24 hours (x 3) recovering the solvent using the rotavap at 50°C. The MeOH extracts were combined, re-dissolved in MeOH to homogenize, and the MeOH removed at the rotavap, yielding 6.7g from *Astrolochii hepii* and 6.15g from *Ocimum gratissmum*, as oils. The recovered oils were kept in the fridge until they were used to prepare the test solutions.

DOSE FINDING EXPERIMENTS

O. gratissimum

The dose finding experiment gave 2.5mg as the minimum dose for *O. gratissimum.* Borne [15] reported knockdown and paralyzing effect of *O. gratissimum* in Nigeria. There was no indication of the knockdown or paralyzing effect of the extract in the present study.

Astrolochii hepii

The dose finding experiment indicated that the minimum required dose for *A. hepii* was 4.5 mg, i. e. almost twice as much of *A. hepii* is required to repel mosquitoes as *O. gratissimum* or more than 4 times that of Peaceful Sleep. Peaceful sleep is a commercial repellent conaining 11.9 g of DEET in 34 g of formulation. The minimum dose for Peaceful Sleep was 1.0 mg.

Cymbopogon citratus

The minimum dose for *Cymbopogon citratus* was 5.0 mg

Repellence of the extracts

Mosquito repellence tests were carried out in a Hand in Cage Experiment as described by Lukwa et al [16], using the methanol extracts of the three plants.

Table 1. Repellences of extracts and extract mixtures

Time/hours	0	0.5	1	1.5	2	2.5	3	3.5	4	4.5	5	5.5	6
O. gratissimum	100	100	90	84	38	10	8	-	-	-	-	-	-
A.hepii	100	100	100	96	84	46	32	2	-	-	-	-	-
C. citratus	100	100	100	100	86	58	52	4	-	-	-	-	-
O.g/A.h	100	100	100	100	92	91	64	57	-	-	-	-	-
O.g/C.c	100	100	100	100	100	100	97	73	35	-	-	-	-
A.h/C.c	100	100	100	100	100	100	100	98	87	80	74	70	64
DEET	100	100	100	100	100	100	100	100	100	100	100	100	100
Control	0	0	0	0	0	0	0	0	0	0	0	0	0

Key

O.g/A.h = *Ocimum gratissimum/ Astrolochii hepii*

O.g/C.c = *Ocimum gratissimum/Cymbopogon citratus*

A.h/C.c = *Astrolochii hepii/Cymbopogon citratus*

A desirable repellent is one that gives 100% protection all the time. However, the repellences of plant-derived repellents rarely exceed 2 hours. Oshaghi [17] argued that plant repellence above 60% would be considered good and that below 60% would be considered poor. We have chosen a repellence of 70% as effective and that below 70% as ineffective in discussions in this report.

O. gratissimum extract was 100% effective for at least 0.5 hour and is at least 84% effective up to 1.5 hours post application. There after its repellence fell rapidly to reach 38% within 2.0 hours post application and to 8% by 3.0 hours post application.

Cymbopogon citratus

Cymbopogon citratus was 100% effective up to 1.5 hours post application, was still 86% 2hours post application, falling to bellow 70% after 2.0 hours post application, and rapidly to 52% by 3.0 post application.

Astrolochii hepii

Astrolochii hepii was 100% effective up to 1.0 hour, was still 96% effective after 1.5hours and 84% after 2.0 hours, but fell rapidly to 46% by 2.5 h post application.

DEET is 100% efficient over the whole test period of 6.0 hours and MeOH is 0.0% all the way.

Ocimum gratissimum/ Astrolochii hepii mixture is 100% effective for 1.5 hours. It was still 91% 2.5h post application, but fell to 64% by 3.0 hours post application.

Ocimum gratissimum/Cymbopogon citratus mixture was 100% effective 2.5 hours post application, was still 97% effective 3.0 hours post application, but fell to 73% effective 3.5 hours post application and then rapidly fell to 35% by 4.0 hours post application.

Astrolochii hepii/Cymbopogon citratus is 100% effective for 3.0 hours post application and fell slowly to 70% effective 5.5 hours post application.

On the basis of the 70% cut-off, *Ocimum gratissimum* was effective for 1.5hours; *Astrolochii hepii* and *Cymbopogon citratus* were each effective for 2.0 hours. *Ocimum gratissimum/ Astrolochii hepii* was effective for 2.5 hours; *Ocimum gratissimum/Cymbopogon* citratus was effective for 3.5 hours and *Astrolochii hepii/Cymbopogon citratus* was effective for 5.5 hours. Thus, all mixtures were more effective that their component extracts. The effectiveness does not appear to have been simply additive. Considering synergistic effects would appear appropriate, but this conclusion must be reached through further experimentation.

The important thing is that a case appears to have been made for the creation of a database for repellence of plants, documenting information that may be useful for development of insect repellents. The realization that mixtures of high repellent plants appear to give mixtures with high repellence is a welcome observation, for a choice of high repelling plants will likely give high repelling mixtures, as long as the constituents do not antagonize each other. Situations have been met where constituents of different plants are antagonistic, giving rise to lower repellences/or lower efficacies in the case of drug interactions [9, 18].

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REFERENCES

1. Rivero, A., Vezilier, J., Weill, M., Read, A.F., Gandon, S. (2010). Insectcide control of vector-borne diseases: when is insecticide restance a problem? PLOS Pathog. O. Pii. E1001000.
2. Isman, M. B. (2006). Botanical insecticides, deterrents, and repellents in modern agriculture and an increasingly regulated world. Annu. Rev. Entomol. 51: 45-66.
3. Mullal, K., Jebanenesan, A. And Pushpanathan, T. (2008). Mosquitocidal and repellent activity of the leaf extract of Citrullus vulgaris (Cucurbitaceae) against the malarial vector, Anopheles stephensi. European Review for Medical and Pharmacological Science 12:1-7.
4. Seyoum, A., Palsson, K., Kung'a, S., Kabiru, E. W., Lwande, W., Killeen, G. F.,Hassanali, A. and Knols, B. G. (2002). Traditional use of mosquito-repellent plants in western Kenya and their evaluation in semi-field experimental huts against Anopheles gambiae: ethnobotanical studies and application by thermal expulsion and direct burning. *Trans R Soc Trop Med Hyg*. 96(3): 225-31.
5. Painter, T. (2010). Fresh Clips: Plants that Repel Mosquitoes. http://www. Herb companion.com/ gardening/fresh-clips-herbs-to-repel-insects.aspx# ixzz1x1 wb2csH
6. Maia, M; Moore,S. J. (2011). Plant-based insect repellents: a review of their efficacy, development and testing. Malaria Journal 10 (suppl 1): S11 doi:10.1186/1475-2875-10-S1-S11
7. Oparaocha, E. J., Iwu, I. and Ahanaku, J. E. (2010). Preliminary study on mosquito repellent and mosquitocidal activities of Ocimum gratissimum (L) grown in eastern Nigeria. J. Vector Borne Dis. 47: 45-50.
8. Kazembe, T. & Munyarari, E. (2006) Effect of Aristolochia petersiana on the efficacy of fansidar, The Central African Journal of Medicine, Vol.52, Nos ½, pp 11-16.
9. Kazembe T, (2011). Mosquito Repellents, Mosquitocides and Malaria Cure Agents of Plant Origin. Lap- Lambert Academic Publishers.
10. Annie's Remedy. (2012). Aristolochia spp. Copyright 2005-2013. Retrieved 11 June 2012.
11. Wikipedia (2012). Cymbopogon. Wikipedia, the free encyclopedia. Wikipedia Foundation, Inc. Retrieved http://en.wikipedia.org/w/index.php?title=Cymbopogon&oldid=496247084

12. Shadab, Q., Hanif, M. & Chaudhary, F. M. (1992). Antifungal activity by lemongrass essential oils. Pak. J. Sci. India. Res. 35:246-249.
13. Mbata, T. I. and Saika, A. (2012). Antibacterial Activity of Essential Oil from Ocimum gratissimum on Liste ria monocytogene. Insect Journal of Food Safety .V (7):15-19.
14. Nweze, E. I. and Eze, E. E. (20090. Justification for the use of Ocimum gratissimum L in herbal medicine and its interaction with disc. Antibiotics. BMC Complementary and Alternative Medicine (9): 37 doi:10.1186/1472-6882-9-37 http://www.biomedcentral.com/1472-6882/9/37
15. Borne, J. V. (2010). Preliminary study on mosquito repellent and mosquitocidal activities of Ocimum gratissimum (L) grown in Eastern Nigeria. Imo State, Nigeria. p45-50.
16. Lukwa, N., Nyazema, N. Z., Curtis, C. F., Mwaiko, G. L., Chandiwana, S. K. (1999). People's Perception about Malaria Transimission and Control in a locality in Zimbabwe. *Central African Journal Of Medicine* 45 (3): 64-68.
17. Oshaghi, M. A. et al. (2003). Repellent Effects of Extracts and Essential Oils of *Citrus limon* (Rutaceae) and *Melissa officinalis* (Labiatae) Against Main Malaria Vector, *Anopheles stephensi* (Diptera: Culicidae), Iranian Journal of Public Health 32 (4).
18. Lukwa, N., Adebayo, A., Kalenda, D. T., Boet, C. and Bart, G. J. (2004) Guidelines for Studies on Plant Based Vector Control Agents in Traditional Medicinal Plants and Malaria, CRC Press. Boca Raton, Florida.

Appendix 1
Interview Guide

1. Is malaria a problem in your area?
2. Do you know when the problem started?
3. What measures are in place to protect people from the disease?
4. How would you compare the efficacy of herbal medicines to that of modern medicines?
5. What do you think is the cause of the differencies?
6. What can be done to improve the efficacy of herbal medicines?

Thank you very much for your time and valued information.