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Microbiological quality assessment of select commercial mosquito repellents in the Philippines

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Abstract

Dengue fever is a major health concern in the Philippines with its morbidity and mortality. Repellents represent an important tactic in preventing the spread of this disease, however, even with the stamp of approval from the World Health Organization, concerns regarding possible toxic reactions have been raised. Thus, the need for a study on the safety evaluation of mosquito repellents. Based on the survey conducted in this study, 50% of the study population used mosquito repellents as a form of self-protection. Investigation on microbiological contamination and antibacterial activities of the select commercially available mosquito products (CAAMP) were done. Bacterial load of the products ranged from (1.0×10^1 to 6.0×10^1 cfu/ml) and antimicrobial screening through the modified Kirby- Bauer Method revealed that conventional and citronella-based repellents showed no antimicrobial activity against *Salmonella typhi*, *Escherichia coli* and *Staphylococcus aureus*. However some bio-repellents revealed a minimal antibacterial activity against the bacterial strains (6.1mm-13mm). Caution should be taken when using such products and excessive administration should be avoided.

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Introduction

Dengue fever is the most prevalent mosquito borne viral disease and is considered of topmost public health concern in tropical and subtropical countries (Kumar *et al.*, 2017). Repellent technologies represent fundamental aspect of preventing the spread of dengue (Norris and Coats, 2017) and commercially-available anti-mosquito products (CAAMP) are the forefront in vector-controlling agents (Kumar *et al.*, 2012) which are intended in preventing the vector from landing on and biting the human skin (Sributra *et al.*, 2011). Two types of mosquito repellents have been accepted as part of the integrated dengue prevention measure by the World Health Organization - the conventional synthetic repellent and the bio-repellents (WHO Prevention and Control Measure, 2012). There have been reports of unprecedented rise of interest as well increasing usage of CAAMP as mitigation methods towards dengue. This may probably expose consumers to certain ill-health effects due to indiscriminate and long term use of these products. Thus, this research generally intends to assess the safety of selected mosquito repellents for human use.

Materials and methods

Assessment of the usage of dengue prevention methods among the study population

Three hundred tertiary students of the Mindanao State University-Iligan Institute of Technology (MSU-IIT) were recruited to fill-in a questionnaire-based survey to assess the mitigation methods practiced by the respondents towards Dengue Fever (DF). The questionnaire also provided a comprehensive checklist of commercially available anti-mosquito products to evaluate the commonly used products by the respondents.

Sample collection of the commercially available anti-mosquito products (CAAMP)

The CAAMP products to be tested for consumer safety was determined based on the following criteria: (1) products topically applied and (ii) commonly purchased by the study population based on the results garnered from the checklist of CAAMP provided in the survey. Consequently, six commercially available mosquito repellent products (one conventional and five bio-repellents) were purchased for safety evaluation. Conventional repellents contain the chemically active compounds such as N, N-diethyl-meta-toluamide (DEET) and picaridin. Bio repellents are those with active components from essential oil and natural oil of plants (Maia and Morre, 2011).

Determination of microbial load of selected mosquito repellents

Heterotrophic plate count was employed to examine the level of microbial contamination on the identified test samples. The presence of sufficient number of certain microorganisms could be harmful to the consumers as it increases the possibility of having pathogenic contaminants which may pose adverse health effects on the users such as foodborne or skin diseases (Rajeh *et al.*, 2012).

Ten milliliters of each product sample was added with 90mL phosphate buffer (pH 7.2). One ml of each sample fluids was pipetted to nutrient agar (NA) plates using the spread plate method. Three replicated were employed. After 24 hour-incubation at ambient room temperature, viable isolated colonies were counted by employing grid-line pattern (Owens, 1996).

Antimicrobial activity screening of the selected mosquito repellents

Modified Kirby-Bauer Disc Diffusion Method was employed to determine the antibacterial potency of the commercial repellents to the following bacterial species: *Staphylococcus aureus*, *Escherichia coli* and *Salmonella typhi* (Cavalieri *et al.*, 2005).

Furthermore, the phenotypic reactions of the extracts were compared to that of common antibiotics which served as positive controls: tetracycline, gentamycin, chloramphenicol, ampicillin, amoxicillin, ciprofloxacin, cefoxitin, clindamycin, erythryomycin, and vanconmycin. Distilled water as used as negative control.

Statistical analysis

The data obtained from this study were expressed as mean standard deviation and subjected to statistical analysis of variance to assess whether there is a significant difference on the variables tested.

Results and discussion

Usage of commercially available anti-mosquito products

In this study, all of the three hundred tertiary students recruited to be part of the study have reported to have used a variety of anti-mosquito products. The most preferred form CAAMP are the lotion type repellents as affirmed by 252 respondents. The least preferred repellent formulation was anti-mosquito sprays (24/300). A work of Wang and Gu (2007) revealed that repellent lotions produce significantly higher transdermal permeation of chemicals than repellent sprays due mainly to its low viscosity and intimate contact with the stratum corneum. Furthermore the presence of other excipients and additives in the lotion preparation such as emulsifiers and surfactants could facilitate the diffusion and permeation of chemicals across the skin layers. However, despite the efficacy of lotion repellents against mosquito bites, its routine users is at a risk in bio accumulation of chemicals upon longer exposure.

Table 1. Microbial counts of the commercially available mosquito repellents.

Test samples	Microbial count (cfu/ml) at 24 hours
A_D	1.0×10^1
B_C	2.0×10^1
B_N	4.0×10^1
B_L	6.0×10^1
B_E	2.0×10^1
B_A	2.0×10^2

Legend:

A_D - with DEET repellent, B_L - lemongrass-based repellent

B_C - citronella-based repellent, B_E - eucalyptus-based repellent

B_N - neem-based repellent, B_A - andiroba-based repellent

The study further revealed that the study population preferred anti-mosquito products that are conventional ones (264/300), with 231 individuals routinely purchasing commercially available DEET-based repellent.

This is in conformity with DEET being recognized to be the most effective protection against mosquito bites as it produce a broader spectrum of activity and effectively repels most insect vectors for a longer period of time (Phasomkusolsil and Soonwera, 2011). Although studies have shown that DEET may expose the general public to varying health consequences upon long periods of exposure, it is also important to note that DEET has a low toxicity to animals, especially via the dermal route (Center of Disease Control-Agency for Toxic Substances and Disease Registry, 2004).

Picaridin or Icaridin was introduced to the US market in 2005 to address some of the complaints of the consumers on DEET-based products. A significantly low percentage of the study population favoured using DEET-free repellents (33/300). Picaridin-based products are odourless, non-greasy, reportedly could not cause any damage to plastics and fabrics and have a relatively low toxicity for acute dermal exposure and ocular exposure (EPA, 2005). However, it has been proven to have a lowered efficacy compared to DEET (Antwi *et al.*, 2008).

Only 36 respondents (12%) preferred anti-mosquito bio-repellents. The significantly small fraction of the population who preferred this type of formulation could be attributed to lesser media promotion of such products as these are relatively new in the market and the inaccessibility of these products to the marginalized customers (International Federation of Organic Agriculture Movement, 2003). The bio-repellents identified by the respondents have active ingredients from citronella, neem, lemongrass, eucalyptus and andiroba plants. Studies have revealed that the general public have started to gain interest in these all organic products as they are commonly perceived as "safe" in comparison to those long established synthetic repellents and due to its low cost (Maia and Moore, 2011; Mittal and Subbarao, 2003). However, some studies of bio-repellents reported it to be less efficacious as compared to DEET and offer short-lived protection (Kongkaew *et al.*, 2011; Phasomkusolsil and Soonwera, 2011).

Table 2. Diameter of zones of inhibition formed by selected mosquito repellents.

Test samples	Zones of inhibition (in mm)		
	S. typhi	*E. coli*	*S. aureus*
A. Controls			
Tetracycline	15.3 ± 1.2	19.5 ± 1.05	25.4 ± 2.4
Chloramphenicol	32.3 ± 12.5	23.7 ± 4.2	27.0 ± 3.1
Gentamycin	NT	19.0 ± 1.6	20.4 ± 2.3
Ampicillin	NT	-	24.2 ± 5.3
Amoxicillin	NT	21.8 ± 5.8	NT
Ciprofloxacin	NT	28.7 ± 2.5	NT
Cefoxitin	NT	NT	27.7 ± 7.8
Vancomycin	NT	NT	24.1 ± 2.6
Erythromycin	NT	NT	25.6 ± 6.3
Distilled water	-	-	-
B. Mosquito repellents	*S. typhi*	*E. coli*	*S. aureus*
A_D	neg	neg	neg
B_C	neg	neg	neg
B_N	9.9 ± 7.3	12.7 ± 1.05	9.8 ± 5.1
B_L	10.0 ± 7.5	neg	6.1 ± 4.6
B_E	9.2 ± 5.5	neg	neg
B_A	neg	13.0 ± 2.4	10.6 ± 4.9

Legend:

A_D - with DEET repellent, B_L - lemongrass-based repellent

B_C - citronella-based repellent, B_E - eucalyptus-based repellent

B_N - neem-based repellent, B_A - andiroba-based repellent

NT- Not Tested, Neg negative.

Heterotrophic plate counts of CAAMP

With the widespread patronage of CAAMP, regulatory measures must also be put in place to ensure that it is safe for human usage (Health Canada, 2005). Assessment of microbial counts is necessary for maintenance of safety of these products as high microbial load could lead to biodegradation of the product and hence may increase risk of infection of consumers (Orus and Leranzo, 2005; Hugbo *et al.*, 2003). The standard microbiological limit utilized in this research study was in conformity with the United States Pharmacopeia Chapter 61: the acceptable microbiological limits indicated in the quality regulation for topical products, in this case mosquito repellents, are between 10^2 to 10^3 colony-forming units/mL (cfu/mL) for pathogenic and nonpathogenic bacteria respectively (Detmer, 2005).

All test samples were far from sterile and had heterotrophic plate counts of 1.0 x 10^1 to 4.0 x 10^1 cfu/ml. The presence of microbial contaminants in the products could be primarily due to the aqueous form of the products since water is a common ingredient in this type of preparation (Muhammed, 2011). It is know that aqueous preparations are more susceptible to contamination compared to oily products such as creams and lotions, as it can support microbial growth (Scholtyseek, 2004).

Furthermore, the bio-repellents included in the microbial assessment yielded higher microbial counts as compared to the conventional repellents. In fact, the highest contamination was exhibited from B_L (lemongrass-based) repellent and in contrast to this, the lowest microbial count was from the A_D (a conventional repellent). Upon comparison of the mean bacterial counts, B_N (neem-based) and B_L (lemograss-based) repellents were shown to have significant difference to that of the conventional repellent ($p<0.01$).

The active components present in bio-repellents are mainly essential oils from various plants that were reported to have significant repellent activity against mosquitoes. However, it is widely known that products of plant origin may harbor higher microbial load (Khurana *et al.*, 2010), hence the presence of contamination could be primarily due to existing microflora. These botanical repellents are generally exempted from regulatory approval (EPA "Active Ingredients Found in Insect Repellents", 2009) as the exemption was made in order to facilitate the rapid development and commercialization of products based on these materials. Thus, these botanical repellents may have not undergone thorough quality assessments before its release to the market. On the other hand, the presence of contaminants in the conventional repellents could also be attributed to the cross-contamination during the manufacturing process: unsanitary conditions in the area, non-sterile equipment and facilities (Suvarna *et al.,* 2011).

Antimicrobial spectrum of the selected CAAMP

The two-way ANOVA revealed that there was a significant difference between the zones of inhibition exhibited by each product ($p<0.0001$). Based on the results presented in Table 2, the products that exhibited clearing zones were all bio-repellents. This could be due to the presence of antimicrobial compounds in the essential oil that serve as active ingredients for bio-repellents.

On the other hand, no clearing zones were observed in A_D (DEET-based) and B_C (citronella-based) repellents. These results seemed duly as it still does comply to the claimed effect of these products since they were merely advertised as an effective insect repellent with no claimed antimicrobial effect. The zero antimicrobial activity of these products was highly advantageous as it does not suppress the growth of residential bacteria.

The two-way ANOVA also revealed that the products have comparable antimicrobial activities against each tested strain ($p=0.3836$). Hence, connote the probability that it can inhibit the growth of the human residential flora other than the transient or pathogenic micro-organisms.

However, there was a relative disparity between the mean diameters of the clearing zones observed. Generally, antimicrobial activity of the products have greater impact on gram-negative bacterial strains (*E. coli*>*S. typhi* > *S. aureus*). These results could be attributed to the difference of their cell wall structures (El-Mahmood *et al.*, 2010). Despite these results, mean comparison of the diameter of clearing zones revealed that all the products have non-comparable antimicrobial activity to those of standard antibiotics ($p>0.05$). This may imply that the products may only have mild inhibitory activities. Exposure to antimicrobial agents (at low dose for a long period) can damage skin, leading to a change in microbial flora, and an increase shedding of the original protective bacterial flora of the skin leading to an increased risk of transmission of pathogenic microorganisms (Scientific Committee on Emerging and Newly Identified Health Risks, 2009).

It is thus advisable to reserve essential oils with strong antimicrobial activities for when they are needed to avoid their indiscriminate or excessive use.

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