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Original article

Efficacy of a solvent-free crude extract of lemongrass *(Cymbopogon citratus)* and sugar apple *(Annona squamosa)* as an insect repellent

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ABSTRACT

Essential oils of sugar apple and lemongrass are highly insect repellent ingredients due to their relatively low toxicity, comparable efficacy, and customer approval. Experimental research assessed the larvicidal ability of solvent-free combined crude extract of lemongrass and sugar apple under varied time intervals (5 minutes, 10 minutes, 15 minutes and 20 minutes). Findings showed that commercial spray is more effective against mosquito larvae versus the combined crude extract of lemongrass and sugar apple. However, combined lemongrass and sugar apple extract divulged potential larvicidal activity against dengue-causing mosquito vectors and need to be reformulated for greater concentrations to equate commercial mosquito spray effectiveness.

KEYWORDS: *sugar apple, solvent-free, crude extract, efficacy, insect repellent*

1 INTRODUCTION

The occurrence of dengue year after year is alarming as it is a significant threat to all humanity (WHO, 2013). It is widespread in the Philippines and is considered one of the eight pervasive infectious diseases (DOH, 2011). Reportedly, in 2016, large dengue outbreaks were reported worldwide. More than 375,000 suspected dengue cases were reported in the Western Pacific region, and almost half were in the Philippines. The Department of Health declared 43,770 cases, 250 deaths were reported, 84 fatalities less than the 334 deaths in the same period in 2016. (DOH) declared a national dengue epidemic when the recorded cases from January to May 2019 reached 146,062 (almost twice the number of cases recorded in the same period of 2018). Out of 17 regions, seven exceeded the epidemic threshold, while three exceeded the alert threshold for dengue. Four dengue serotypes infect the Philippines (Capeding et al., 2011; Salda et al., 2005) transmitted by Aedes aegypti L. and Ae. albopictus Skuse (Diptera: Culicidae) (Edillo et al 2012).

Environmental risk factors and inconsistent preventive practices (Borja, 20017) and urbanization, increasing population, scarce public health set-up, poor solid waste management, and lack of an effective mosquito surveillance system contribute to the growing dengue challenge (Betty et al. 2011; Edillo, 2012). The key to eradicate mosquitoes is to kill the larvae before they turn into adult biting mosquitoes causing dengue. This could be done by altering their surroundings or through insect repellent (Batabyal et al., 2007; Dua et al., 2009) in all forms.

Thus, mosquito larvae control and personal protection from mosquito bites are currently the most important measures to reduce mosquito-related diseases (Das et al., 2003). Plants' essential oils, in general, have been recognized as a vital natural resource of insecticides and insect repellents. Many essential oils have also been documented to exhibit acute toxic effects on insects, including mosquitos (Pavela, 2008). Essential oils of sugar apple, cedrene and caryophyllene (Joy et al., 1997) and lemongrass citral and geramoil contents are highly insect repellent ingredients due to their relatively low toxicity, comparable efficacy, and customer approval (Katz et al., 2008).

Nevertheless, repellents based on chemical insecticides help reduce and prevent mosquito vectors. On the other hand, chemical insecticides containing (DEET) Diethyltoluamide are unsafe for humans, particularly children, since they may cause skin irritation, hot sensation rashes, or allergy (Das et al. 2003). In recent years, there was an increase in public concern about the safety of many chemical products that instigated a renewed interest in using natural products of plant origin for mosquito vector management (Pavela, 2008).

Lemongrass is a perennial monocotyledonous grass that can grow up to 6 feet in height and 4 feet in width. It grows in clusters. It has long, slender, drooping bright green leaves measuring from 1.3-2.5cm in width and 3feet in length. Leaves are simple with entire margins. Flowers grow on spikes with long inflorescence ranging from 30 to 60cm. The floral arrangement of this scented grass gives it the name ‘Cymbopogon.’ Cymbopogon citratus is a common inhabitant of Southeast Asia

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(Kumar et al., 2010; Viabhav et al., 2013) and its greater abundance is found basically in the Philippines. It can be used in preparations of topical skin creams and the manufacture of plant-based oral drugs. It has been used to treat fever, cough, elephantiasis, flu, leprosy, malaria and digestive problems, among others, reported (Bankole, 2004).

Sugar apple is a robust, half-evergreen bush or little tree achieving 8 m (26 ft). Mostly, blooming and fruit-bearing begin when the tree is 2-3 years old. Sugar-Apple (Annona squamosa) is widespread throughout the world's tropical regions. The leaves containing borneol allegedly had the effect of insecticides (Duke, 1992). Extracting the leaves and seeds of sugar apple has efficacy against some insects, especially vector diseases mosquito (Chorge, 2000). Phytochemical examinations of the plant have demonstrated that they have a wide assortment of components, such as acetogenins which were hostile to malarial, cytotoxic and immunosuppressive exercises. The number of alkaloids accounted for from this plant is within various classes, e.g., aporphine and benzoquinazoline. Squamocenin, annotemoyin, reticulatain-2, squamocin-I, squamocin-B, squamocin, motrilin, squamostatin-D, squamostatin-E, cherimolin-1, and cherimolin-2 were all extracted from the ethyl liquor concentrate of A. squamosa L. Other researchers found squamocenin, Annotemoyin, and reticulatain-2 (Pareek et al., 2011). With these initial study results, the above-mentioned chemical constituents in this plant reinforce the plant being used for different therapeutic qualities (Gajalakshmi et al., 2012).

Hence the study investigated the efficacy of the combined extract of essential oils found in lemongrass and sugar apple against mosquito larvae versus chemical-based mosquito spray. The results of this study are projected to provide information on biological insecticide development, thus formulating organic-based insect repellent to eradicate mosquito larvae before they turn into dengue-causing mosquitoes, by which abundance is widely evident in every locality.

2 MATERIALS AND METHODS

Plant and Solvent Material. The study focused on lemongrass and sugar apple leaves utilizing a sole solvent, mainly distilled water.

Sample Collection. Two plants with therapeutic properties were collected locally from the same point of plant origin between 8 am to 10 am. When exposed to mosquito larvae, fresh and tender leaves of identified plants were used for crude extraction for the larvicidal effect. For the larvae sample, a bug of the chamber was filled with water and left uncovered until there was a noticeable number of mosquito larvae for three sets of replication. Larvae were sorted out and only strong and healthy 3rd -4th instar larvae were selected for assays. The intentionally grown larvae were transferred to a clear glass after three weeks of a strain containing 15 ml water with 50 samples in each group. The fresh leaves are equally weighed, amounting to 50 grams each, and pounded together to extract the essential oils found in lemongrass and sugar apple. A sufficient quantity of water was added to obtain a 30 ml crude extract equal to the commercially sold mosquito repellent spray volume. The macerated extract was filtered twice to avoid possible mix-in leaf residue. The extraction procedure was based on Tiwari et al. (2011). Each group of larvae was exposed to a series of treatments. The first group was treated with 30 ml combined extract of lemongrass and sugar apple. The second group was treated with commercially sold mosquito spray for children with 30 ml volume. Each group of larvae was exposed for 20 minutes, and the repellent efficiency was recorded every 5 minutes. During the entire laboratory procedures, the researcher observed measures to ensure safety. Laboratory gowns, gloves, and masks were worn at all times.

Preparation of Plant Extract. The leaves of the selected plants were taken from the plants and then washed with running tap water to remove dust. The plant samples were air-dried for 5 to 6 hours. A sufficient amount of distilled water was added to obtain a 30 ml crude extract equal to the commercially sold mosquito repellent spray volume. The macerated extract was filtered twice to avoid possible mix-in leaf residue. The extraction procedure was based on Tiwari et al. (2011). The first group (T0) was treated with 30 ml commercially sold mosquito spray for children. The second group (T1) was treated with 30 ml combined extract of lemongrass and sugar apple.

Treatment Efficacy. The larvicidal activity test was carried out for 20 minutes and efficiency was recorded every 5 minutes. The recording is based on the number of larvae that floated on the water surface. Pre-testing on individual extract was conducted to affirm efficacy; on the other hand, it was found out that individual representation of the extract rendered slow release of its larvicidal activity and delayed the larvae's mortality rate. Hence, the two extracts were combined to obtain a full larvicidal release against the mosquito larvae. Percent mortality was computed by counting the dead mosquito larvae employing an average weighted mean. The mortality rate was calculated using the paired t-test at a 0.05 level of significance to determine the larvicidal activity of the combined extract of lemongrass and sugar apple against the commercially sold mosquito spray in each sample.

3 RESULTS AND DISCUSSION

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Table 1 shows the mortality rate of the combined lemongrass and sugar apple extract against the commercial mosquito spray. It is noted that commercial mosquitoes are effective in the first 5 minutes due to more potent larvicidal activity. Basically, it contains only high purity and high-quality citronella oil and lemon eucalyptus oil. Vegetable extracts and pure substances may manifest their toxic effects upon mosquitos in various ways; reproduction and fertility suppression, mortality or intoxication with trypsin inhibitors, toxicity, mortality and growth inhibition (Jbilou et al., 2006). Therefore, the combined extract of lemon grass and sugar apple may partially replace DEET-based sprays to control mosquito larvae before they develop into causing dengue mosquitos. This organic-based larvicidal formulation would greatly help locals, especially those under the marginal status in society, as they can hardly afford to buy commercially sold chemicals in the market.

Table 1. Mortality rate of larvae with the combined extract of lemongrass and sugar apple versus commercial mosquito spray in 5 minute intervals.

Treatment	Repellent efficiency every 5 minutes interval							
50% Lemongrass and 50% sugar apple extract	5 minutes	%	10 minutes	%	15 minutes	%	20 minutes	%
	43	29	49	33	42	28	16	10
Commercial mosquito repellent spray	60	40	78	52	12	8	--	--

The larvicidal activity of the combined lemongrass and sugar apple extract against the commercial insecticide was only significant after 15 minutes, with a computed value of -3.560 at a 0.05 p-value. The result denotes that the formulated combined extract exhibited a remarkable larvicidal activity (Azhari, 2012). Larvicidal component on plant essential oils in lemongrass and sugar apple effectively controls mosquito larvae.

Table 2. Significance level of the combined lemongrass and sugar apple extract versus commercial mosquito spray.

Treatment	Significance level at 0.05 value					
50% Lemongrass and 50% sugar apple extract	5 minutes	Not Significant	10 minutes	Not Significant	15 minutes	
	2.181		1.386		-3.560	Significant
Commercial mosquito repellent spray	1.034	Not Significant	0.892	Not Significant	-1.233	Significant

effect compared to the commercial mosquito repellent spray; it is noted that commercial mosquito repellent spray showed significance after 15 minutes as the computed value is -1.233, meaning less than the p-value (0.05).

Therefore, both treatments illustrated an effect in killing mosquito larvae but statistically, commercial mosquito repellent spray showed a higher significance level close to the p-value. It proved that the crude combined extract of lemongrass and sugar apple showed prospective evidence in exterminating mosquito larvae. The biological activities of these plant extracts may be due to various phytochemical classes in the plant. Indeed, these compounds may jointly or independently produce spray exposure to mosquito larvae. Therefore, the commercial spray is very effective, but the treatment solution also manifests potential efficacy. Larvicidal component on plant essential oils in lemongrass and sugar apple effectively controls mosquito larvae. However, they worked after some time and need to be reformulated for greater concentrations to equate commercial mosquito spray effectiveness.

4 CONCLUSIONS AND RECOMMENDATION

Results obtained from this study revealed that commercial spray is more effective against mosquito larvae versus the combined crude extract of lemongrass and sugar apple. However, combined lemongrass and sugar apple extract divulged potential larvicidal activity against dengue-causing mosquito vectors. It exhibited an equally observable significant effect with the commercial

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