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Microbiological risk characterization of select complementary alternative therapies for dengue fever in the Philippines

Lucilyn Maratas*, Dana Joanne Von Trono

Department of Biological Sciences, Mindanao State University-Iligan Institute of Technology, Philippines

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Abstract

Dengue fever causes mortality and morbidity across the world and in the Philippines, there is widespread use of herbal-based medicines and medicinal plants to treat this infection. The research study was primarily undertaken to conduct a general microbiological evaluation of the local complementary alternative therapies (CAT) for dengue fever, in order to determine its safety for human use and consumption. A preliminary question-based survey was conducted to assess the awareness and the commonly practiced CAT of the study population. 63% of the population reported to be aware of the potential of herbal plants for supplementary therapy particularly against dengue-induced thrombocytopenia. The general safety of three most commonly used herbal plants, *Euphorbia hirta*, *Carica papaya* and *Ipomea batatas*, (in forms of decoction and infusion) were assessed through employing heterotrophic plate counts (HPC) and antimicrobial screening. HPC evaluation showed varied levels of contamination ranging from >1000 to 3.0 x 10^2 CFU/ml, which could be attributed to unstandardized preparation procedures. All decoctions and infusions exhibited negative antimicrobial properties against *Salmonella typhi, Escherichia coli* and *Staphylococcus aureus* , hence, is advantageous as orally administered therapies must contain diminutive amount of antibacterial activity property in the intention that it will not invade other normal human cells except for transient and pathogenic bacterial cells.

***Corresponding Author:** Lucilyn Maratas ✉ lucilyn.lahoylahoy@g.msuiit.edu.ph

Introduction

Dengue fever is an acute infectious disease that threatens human health worldwide (Huang *et al.,* 2017). However, there are no effective drugs that exist to treat dengue infection and the current common treatment is limited to fluid therapy and supportive care (Guzman *et al.,* 2010). As a consequence, the search for for new anti-dengue agents from medicinal plants have assumed more urgency than in the past. The demand for plant-based medicines is growing as they are generally considered to be safer, non-toxic and less harmful than synthetic drugs (Abd Kadir *et al.,* 2013).

The most commonly accepted local complementary alternative therapy (CAT) for dengue fever in the Philippines makes use of plant decoctions of *Euphorbia hirta, Carica papaya* and *Ipomea batatas*. There have been studies conducted that emphasized the pharmacological activities of these plants (Patil *et al.*, 2009, Karishna *et al.*, 2008). However, despite its popularity amongst the masses, the Department of Health of the Philippines (DOH) does not recommend the usage of these CAT due to its lack of scientific basis and possible toxic effect in the other body organs. Even with the precaution from the government, there have been reports of unprecedented rise of interest as well as increasing usage of CAT as mitigation method towards dengue fever. This may probably expose consumers ill health effects due to indiscriminate and long term use of these therapies. Thus, this research generally intends to assess the microbiological safety of selected local CAT for human use.

Materials and methods

Assessment of Awareness and Usage of Complementary Alternative Therapies (CAT) against Dengue Fever 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 awareness as well as usage of CAT being practiced by the respondents in mitigating dengue fever.

Collection of Plant Samples Commonly Used as CAT against Dengue Fever

The most commonly used plants for alternative therapy against dengue fever were also evaluated in this study: *Euphorbia hirta, Carica papaya* and *Ipomea batatas*. Plant samples were collected from various areas in Iligan City between eight to ten in the morning to minimize the accumulation of moisture in the plants as recommended by the World Health Organization (WHO) "Guidelines on Good Agricultural and Collection Practices for Medicinal Plants" (2003), as high moisture may increase contamination. Different plant parts were collected and placed in sterile plastic bags.

Processing of Plant Samples for Various Extraction Procedures

After harvesting the plant samples, the selected plant parts of each representative plant samples were processed directly and subjected to three different extraction procedures. After which, the freshly prepared test samples were then analyzed for microbiological evaluation.

Decoction: A ratio of 1:4 (plant sample to distilled water) was boiled until the volume was brought down to 25% its original volume (International Centre for Science and High Technology- United Nations Industrial Development Organization (ICS-UNIDO), 2008).

The resulting decoction was then filtered and aseptically transferred to sterile glass bottles for storage prior to usage.

Infusion:

Commercial tea products of *Euphorbia* and *Carica* were purchased for safety evaluation. The tea was prepared by soaking the tea bag (2 grams) in 240ml hot distilled water, as per package direction, in a sterile covered glass bottle for 5 minutes (Acevedo, 2010). The sample was allowed to cool down for about 3-5 minutes and then analyzed for microbiological evaluation testing.

Determination of Microbial Load of Selected Mosquito Repellents and Different Plant Extracts (United States Pharmacopia, 2009)

Heterotropic 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 90 ml phosphate buffer (pH 7.2). One milliliter of each sample fluids was pipetted to nutrient agar (NA) plates using spread plate count method. Three replicates were made. After 24-hour incubation at ambient room temperature, viable isolated colonies were counted by employing grid-line pattern.

Antimicrobial Activity Screening of the Different Plant Extracts (Cavalieri et al., 2005)

Modified Kirby-Bauer Disc Diffusion Method was employed to determine the antimicrobial potency of the herbal extracts to the following bacterial species: *Staphylococcus aureus*, *Escherichia coli* and *Salmonella typhi*. To test its susceptibility, 0.1ml of bacterial suspension (0.5 McFarland Turbidity Standard) was swabbed on top of Mueller Hinton Agar (MHA). The plates were then left at ambient temperature to dry for five minutes. Sterile filter paper discs were aseptically placed on top of the plates which were then added with 0.01ml of test samples. 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, erythromycin, and vancomycin. Distilled water was used a negative control. The plates with test samples were then allowed to be incubated at ambient temperature for 18 hours. After which, the diameter of the zones of inhibition (clearing zones) produced by each antimicrobial-impregnated disc were measured in millimetres.

Statistical analysis

The data obtained from this study were expressed as mean +standard deviation (SD) and subjected to analysis of variance to assess whether there is a significant difference on the variables tested. Data were subjected to two-way ANOVA followed by Tukey-Kramer's Multiple Comparison Test. The mean difference was considered significant if $p<0.05$ as adapted in the study of Hussain and Ananthan (2012).

Results and discussion

Awareness and Usage of Locally Known Complementary and Alternative Therapies (CAT) by the Study Population

There is a growing interest of alternative therapies from natural products in the Philippines as it offers lower cost of treatment against dengue fever.

Table 1. Commonly Used Local Herbal Plants as Complementary Alternative Therapies against Dengue Fever Among the Study Population.

Herbal plants	Number of respondents		
	E. hirta n=276	*C. papaya* n=13	*I. batatas* n=11
Plant parts			
Whole plant	64	0	3
Leaves	182	13	6
Stems	30	7	0
Roots	74	3	2
Fruit	0	1	0
Herbal preparations			
Decoctions	261	13	11
Tea	12	2	0
Capsule	3	0	0

These local folkloric claims of dengue cure from various plant decoctions spurred the interest of the Department of Science and Technology (DOST) in promoting researches in identification, characterization, and evaluation of anti-dengue activity of selected Philippine plants (Philippine Council for Health Research and Development (PCHRD), 2012). According to anecdotal reports, various plant decoctions, can alleviate the symptoms manifested by dengue fever patients, particularly, thrombocytopenia (Aragones, 2010). Yet there is no clear explanation with regard to the therapeutic claim of these herbal plants.

Table 2. Microbial counts of various herbal preparations of selected CAT.

Test samples	Microbial count (CFU/ML)*
Decoctions	1.5×10^2
DPL	>1000
DCW	>1000
DCL	>1000
DCS	>1000
DTW	>1000
DTL	>1000
DTS	>1000
DTR	>1000
Infusions	
RPT	3.0×10^2
RTT	>1000

Legend:
DPL-*Carica* leaves decoction DTW-*Euphorbia* whole plant decoction DTS-*Euphorbia* stem decoction
DCL-*Ipomea* leaves decoction DTR-*Euphorbia* roots decoction RPT-*Carica* leaf tea
DCS-*Ipomea* stem decoction DTL-*Euphorbia l*eaves decoction RTT-*Euphorbia* tea
* Colony Counts after 24 hours of incubation at ambient room temperature.

Results from the survey conducted in this study revealed that more than half of the study population (189/300; 63%) were aware of or have used alternative/herbal treatments against dengue fever. The knowledge of the plants' curative effect could have emanated from testimonies of elders which have been handed down from generation. Additionally, its widespread use could have begun upon its commercialization in the market as pharmaceutical companies adapted herbal medicine (Miano *et al.*, 2011).

The commonly utilized herbal plants as reported by the study population is presented on Table 1.

The most common herbal plant reported (276/300; 92%) against dengue fever was *Euphorbia hirta*. Its popularity could have been due to the increasing reports regarding its efficacy towards dengue infection. Additionally, it has been featured in many health news reports; hence, this may have gained the interest of the consumers. In this study, 182 individuals (66%) preferred to utilize the *Euphorbia* leaves, 74 respondents (27%) claimed to use the roots, 64 (23%) reported the use of the whole plant and only 30 individuals (11%) reported preference for the stems.

With the increasing awareness of the general public regarding the plant's potential as supplementary therapy against dengue symptoms such as thrombocytopenia, commercialized products of *Euphorbia* are now available as herbal teas and encapsulated supplements.

Only a low percentage of the respondents reported to have used *Euphorbia* tea (12/276; 4%) and capsule (3/276; 1%). Despite the increasing anecdotal reports of its efficacy, DOH did not make any official recommendation regarding its usage due to its lack of scientific basis and possible toxicity due to over dosage.

Table 3. Diameter of zones of inhibition formed by decoction, infusion, and ethanolic extracts of *Euphorbia hirta, Carica papaya* and *Ipomea batatas* on Kirby-Bauer Test.

Test samples	Zones of inhibition (in mm)		
	S. typhi	*E. Coli*	*S. aureus*
Controls			
Tetracycline	17.1 + 3.22	16.6 + 2.13	32.7 + 1.22
Chloramphenicol	37.6 + 4.72	23.2 + 1.56	31.3 + 2.60
Gentamycin	NT	19.2 + 0.67	21.9 + 2.15
Ampicillin	NT	-	28.9 + 4.59
Amoxicillin	NT	13.7 + 3.87	NT
Ciprofloxacin	NT	31.1 + 3.95	NT
Cefoxitin	NT	NT	35.2 + 7.8
Vancomycin	NT	NT	35.0 + 2.45
Erythromycin	NT	NT	19.7 + 1.58
Distilled Water	neg	neg	neg
Extractions of *Euphorbia*			
Decoctions			
Whole plant	neg	neg	neg
Leaves	neg	neg	neg
Stem	neg	neg	neg
Roots	neg	neg	neg
Infusion	neg	neg	neg
Extractions of *Carica*			
Decoctions	neg	neg	neg
Infusion	neg	neg	neg
Extractions of *Ipomea*			
Decoctions			
Whole plant	neg	neg	neg
Leaves	neg	neg	neg
Stem	neg	neg	neg
Infusion	neg	neg	neg

Legend:

NT - not tested

Neg- no inhibition zone.

Thirteen respondents (4%) have identified *Carica papaya* as another herbal treatment used against dengue. This subgroup has reported decoction as the method of preparation and only two individuals claimed to have used commercialized *Carica* tea. A study in Malaysia conducted by Sathasivam *et al.* (2004) revealed that there was a significant increase in the thrombocyte count of mice upon administration of papaya leaf suspension. However, indiscriminate use of *Carica* may possibly expose the general public to toxic and allergic reactions (Raintree Nutrition, 2012).

Some respondents have also identified *Ipomea batatas* (11 individuals) as possible therapy against the symptoms of dengue fever. However, this plant has not gained wide patronage among locals as CAT. Additionally, there were not significant and accounted researches regarding the matter. Despite the growing patronage of CAT among the locals, the DOH does not recommend any of its usage, until further research.

Microbiological Quality Assessment of Complementary Alternative Therapies against Dengue Fever

The frequency of dengue fever in the Philippines has exponentially escalated and the use of alternative therapies have increased markedly. Given the toxic potential of alternative medicines, evaluating the microbiological quality of CAT against dengue fever is of utmost importance.

Heterotropic Plate Count of Select Complementary Alternative Therapy

In this study, two common herbal preparations, decoction and commercial tea, were subjected to microbiological analysis to determine its contamination. Table 2 presents the quantitative bacterial counts of the samples tested. Microbial count contaminant evaluation of the decoctions exhibited greater than 1000 cfu/ml. On the contrary, decoction from the papaya leaves (DPL) presented a lower microbial count compared to other decoction samples.

Several factors may have attributed to the high microbial contamination in herbal decoctions. Tradionally, local users usually collect these plant materials around or in bushes hear human habitation such as in backyards or vacant lots. Increased microbial risks come when plants grown in soil contaminated with human or animal manure or heavy metals, which are main source of disease-causing pathogens. Cross-contamination should also be considered (Kunle *et al.,* 2012). This method of herbal preparation is usually carried out in homes with no standardized procedures and lack of hygienic and sanitary conditions.

Though heat from boiling may lessen or kill large number of microorganisms, spore-forming bacteria and fungi are not effectively eliminated by boiling water.

Microbial quality assessment of two commercial herbal teas revealed that commercial *Euphorbia* tea (RTT) presented a higher contamination (>1000 cfu/ml) compared to commercial *Carica* leaf tea (RPT; 3.0 x 10^2 cfu/ml). Primary origin of contamination could be from the contaminated irrigation water or biological contaminants such as human excrement, animal manure and feces used as fertilizers. Important parameters in tea preservation are dry, cool, dark and inert storage areas which are viable conditions for microbial contamination. Additional contamination may also occur during post-processing such as drying of raw materials and poor hygiene and sanitation practices during harvesting, sorting, packaging and transporting plant materials were possible routes for contamination (Kunle *et al.*, 2012).

Therefore, based on general assessment fo CAT, homemade herbal preparations such as decoction and herbal infusions have high contamination as are usually prepared at homes or by a herbalist without any set of regulations for quality and safety of such preparations.

Antimicrobial Spectrum of CAT used against Dengue Fever

Table 3 presents the inhibitory activity of the different preparations of the select CAT. Antibacterial tests of *Euphorbia's* herbal tea and decoctions yielded negative results against the test bacterial strains.

This was beneficial since these preparations were being ingested and a positive antibacterial activity in beverage or food may possibly be harmful as it could inhibit not just foreign microbial cells but the growth of normal cells, as well (Elena *et al.*, 1993) Despite these results, it is still highly advisable to avoid indiscriminate or excessive use of this herbal plant as it may probably expose an individual to toxic and allergic reactions.

All the extraction types (both decoction and infusion) of *Carica* leaves yielded negative results in the antibacterial test as shown in Table 4. Studies of Leite *et al*., (2005) and Vieira *et al*., (2001) coincide with the present results wherein no antibacterial activities were exhibited in decoctions, aqueous, acetone and ethanolic extract of *Carica* leaves. In contrast to these studies cited, a review on the nutritional and pharmacological value of *Carica* leaves have shown positive antimicrobial properties of broad spectrum of microorganisms (Krishna *et al*., 2008).

The difference could probably be due to insufficient quantity of active compounds in the extract. However, negative results in the inhibitive properties of this plant could be advantageous if its preparation will be taken orally such as decoctions and tea since it will not facilitate any major changes in the normal flora, particularly on the gastrointestinal tract.

The *Ipomea* leaves and stems were screened for its antibacterial activity against the three microbial strains. No zones of inhibition were seen on the decoctions of respective plant parts. Hence, this is advantageous since it may not cause any alteration in the gut microflora of humans. Nevertheless, caution should still be considered when taking such preparation types.

Based on the preliminary safety evaluation of the selected CAT, it is therefore, empirical that caution should be taken in using these products and therapies until further research. Excessive and long-period usage of these therapies is not advisable.

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