Not all *Tawa-tawas* are Alike: A Morphological, Molecular Genetic, Phytochemical, and Anti-thrombocytopenic Profiling of Different *Euphorbia hirta* Linn. plants from the Philippines

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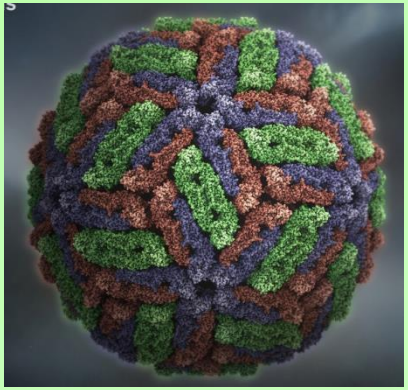
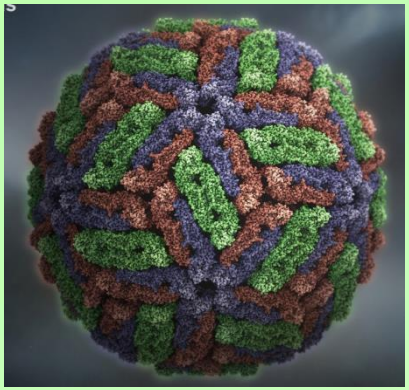
Dengue as a Health Threat



- **Dengue has remained to be a national epidemic.**
  - **One of the top 50 causes of death in the Philippines (22nd) 3,406 deaths /100,000 population**
    http://www.worldlifeexpectancy.com/country-health-profile/philippines
  - **Total of 117,658 cases from Jan-Sep 2013, mostly affecting children (DOH 2013)**
  - **CFR= 0.81%**
- **Infectious agent: Dengue Virus serotypes 1-4 transmitted by *Aedes* mosquito bite**
- **Dengue virus associated diseases: Dengue Fever and Dengue Hemorrhagic Fever, Dengue Shock Syndrome**
- **Symptoms: high fever followed by symptoms that may include rashes, fatigue, headache, joint aches, nausea, and vomiting.**

Issues in Dengue Therapy

- **Vaccine is not widely available.**
- **Current disease management is based on symptomatic intervention.**
- **When symptoms of thrombocytopenia occurs, blood transfusion is recommended (below: 150,000/uL).**
  www.ouhsc.edu/platelets/platelets/platelets%20intro.html
- **Herbal intervention is gaining popularity in local communities with reports of beneficial effect from taua-taua decoction, papaya leaf extract, camote tops.**
- **Taua-taua commercial products like tea and health drink are currently marketed with inadequate safety studies.**

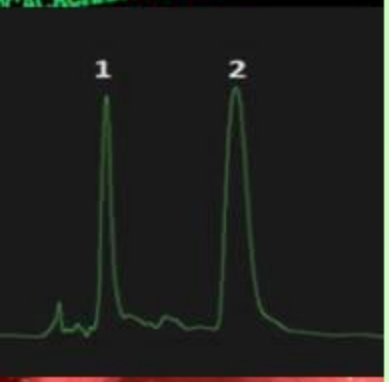


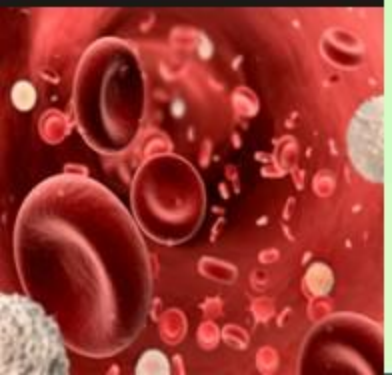
Issues in Dengue Therapy

- **The platelet-promoting activity of *E. hirta* has been demonstrated in ethanol-induced thrombocytopenic rat model and in Sprague-Dawley rats (Kumar et al.,2009; Patil et al., 2009; Apostol et al., 2009)**

- **There are no scientific studies on toxicity and characterization of the different taua-taua varieties and their respective bioactivities.**

- **Communities can be misled that taua-taua collected anywhere are equally beneficial against dengue.**

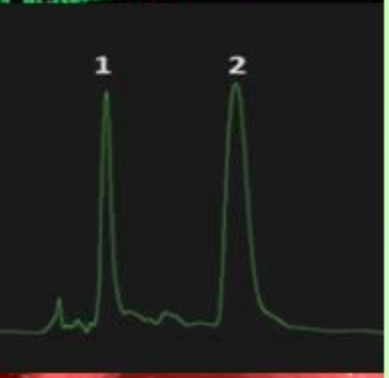


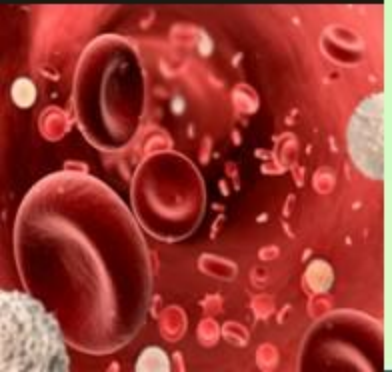


Objectives

This study aimed to characterize taua-taua samples from different areas in the Philippines using the following parameters:

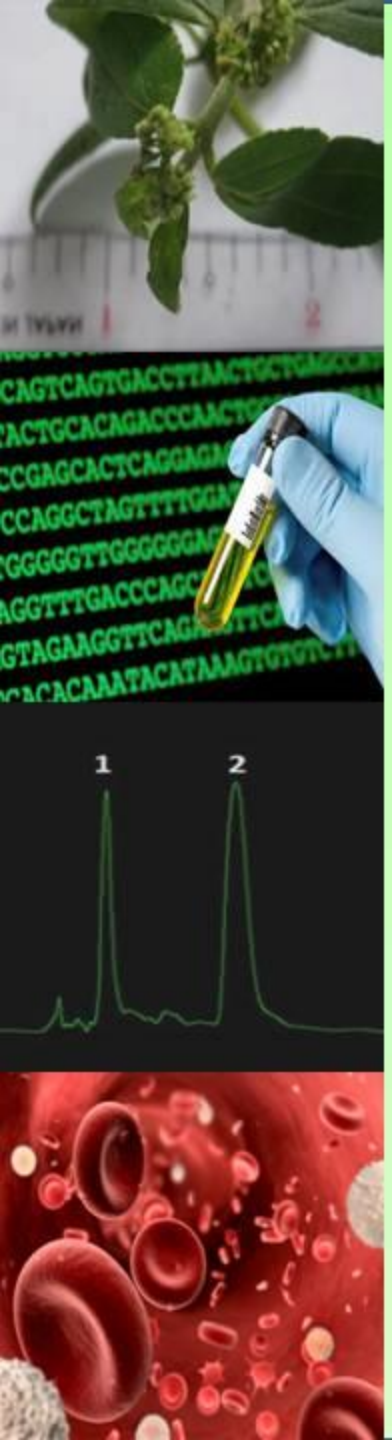
- ❑ **Morphologic**
- ❑ **Molecular Genetic**
- ❑ **HPLC Profile**
- ❑ **Anti-thrombocytopenic Activity**





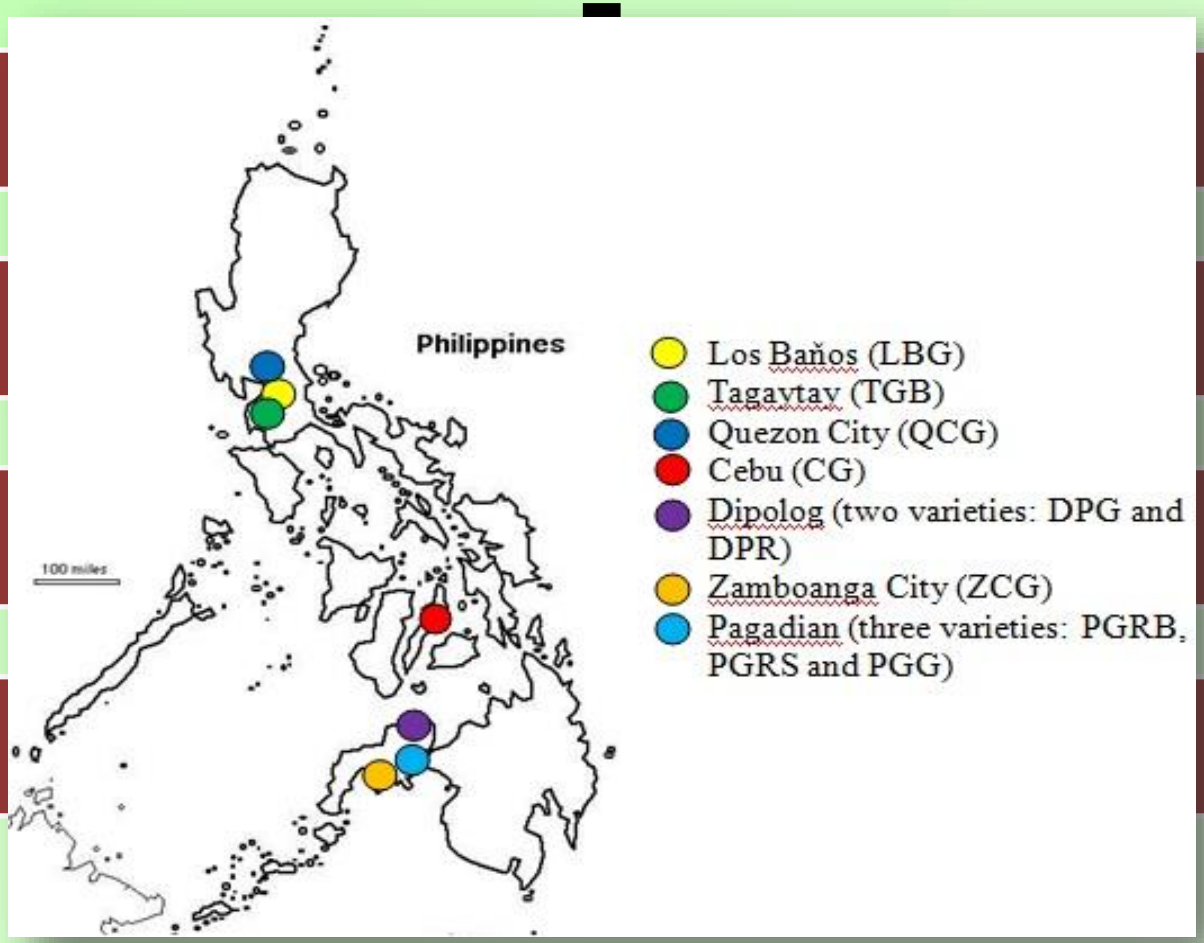
Significance of the Study

- **Provide clarification to the different communities and health practitioners regarding taua-taua therapeutic issues.**
- **Provide guidance to concerned parties on identification of suitable taua-taua varieties that can be propagated in communities.**
- **Provide baseline information for future safety studies and policies on taua-taua.**



General Methodology

Plant Collection



MORPHOLOGICAL ANALYSIS

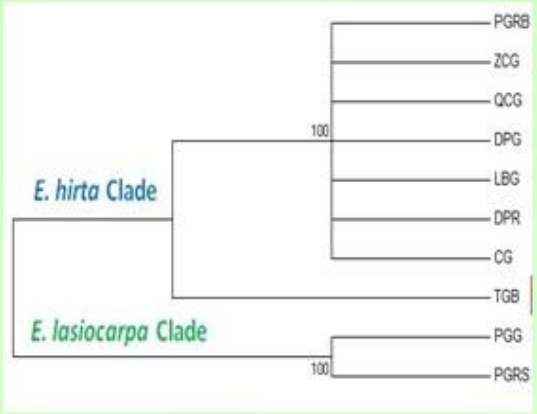
Morphologic Characterization

- leaf margin
- leaf shape
- leaf base
- leaf apex
- presence of inflorescences
- trichome visibility

Scoring

Samples	Leaf Margin		Leaf Shape		Leaf Base			Leaf Apex		Presence of Inflorescences		Trichome Visibility		
	Serrate	Entire	Ovate	Elliptic	Oblique	Rounded	Cordate	Acute	Rounded	Presence	Absence	Leaves	Stem	L&S
PGRB	1	0	1	0	1	0	0	1	0	1	0	0	0	1
ZCG	1	0	1	0	1	0	0	1	0	1	0	0	0	1
QCG	1	0	1	0	1	0	0	1	0	1	0	0	0	1

Cluster Analysis Using MEGA Software



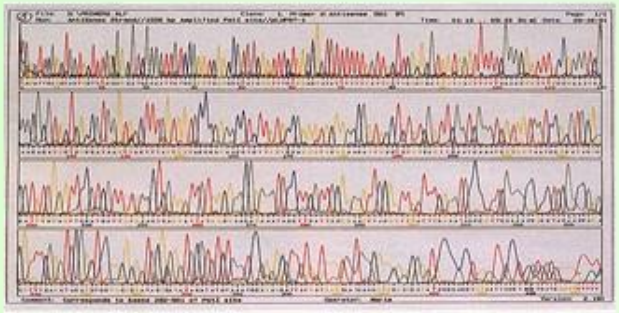
Phylogenetic Tree Reconstruction

character states.

MOLECULAR GENETIC ANALYSIS

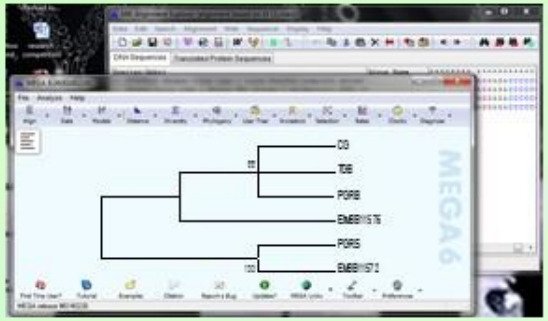
DNA Extraction

Polymerase Chain Reaction of *rbcL* Gene



DNA Sequencing

Phylogenetic Tree Reconstruction



Morphological Analysis with Integration of Genetic Information

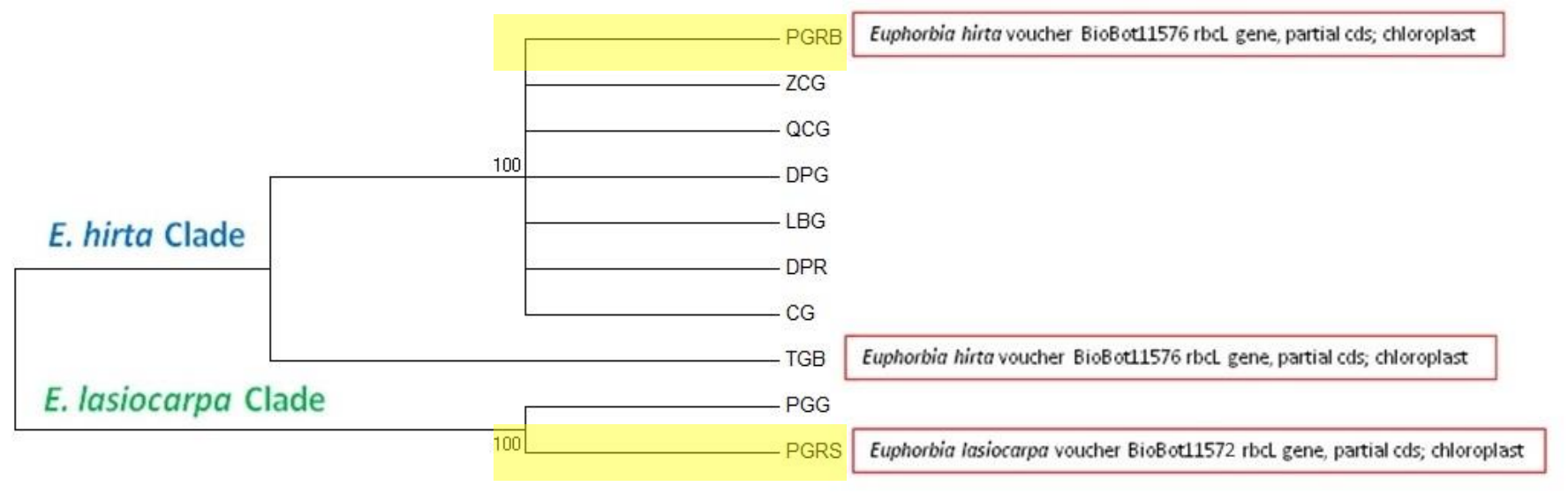
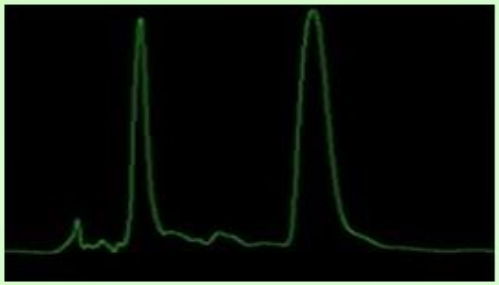


***Figure 2*. Maximum parsimony analysis of taua-taua specimens using the 14 morphological character states. Texts in red boxes indicate identities based on a BLAST search of *rbcL* sequence data. Bootstrap values >50 indicate robust branching.**

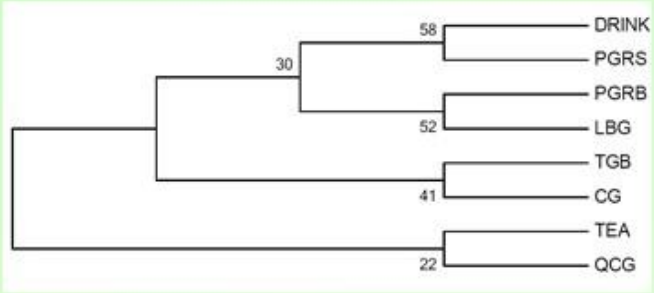
High Performance Liquid Chromatography Profile Analysis

Extraction

High Performance Liquid Chromatography



Scoring and Cluster Analysis using MEGA Software



Tree Construction

HPLC Profiles of Different Taua-taua Samples

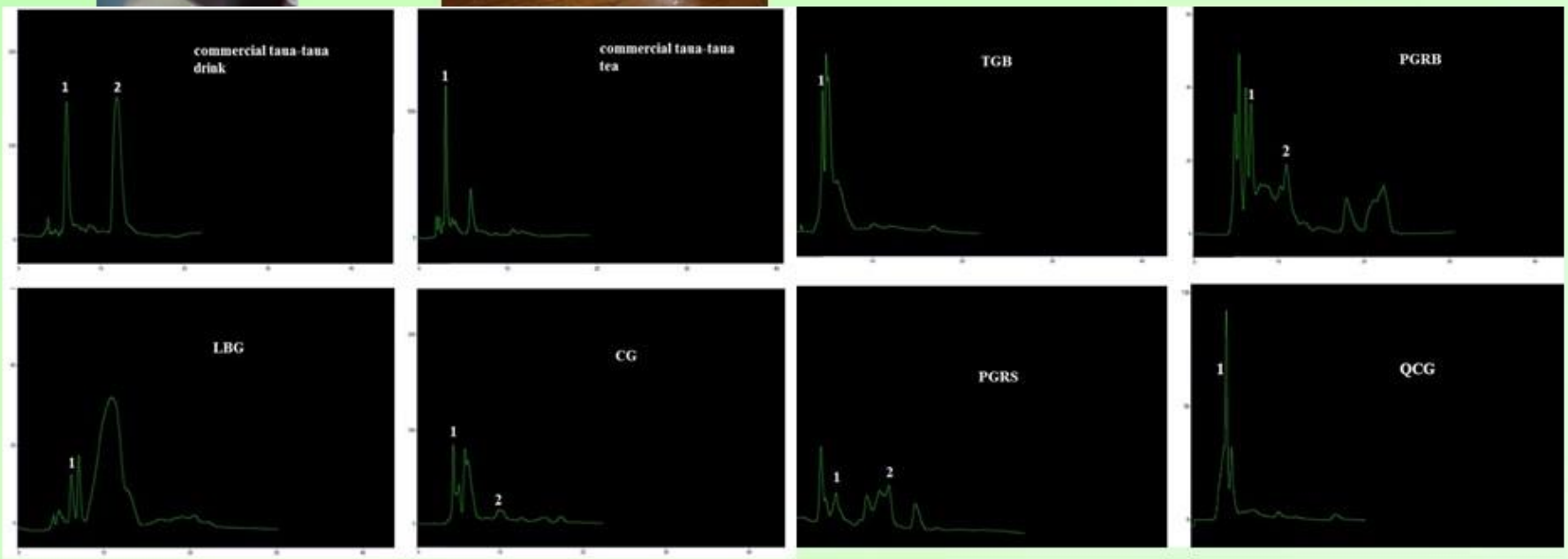


Figure 4. HPLC profiles of commercially available taua-taua drink, taua-taua tea, and methanolic extracts of LBG, CG, TGB, PGRB, PGRS and QCG. Peaks corresponding to gallic acid (1) and catechin (2) are shown.

Anti-thrombocytopenic Activity Assay

3 x  Control

3 x  LBG

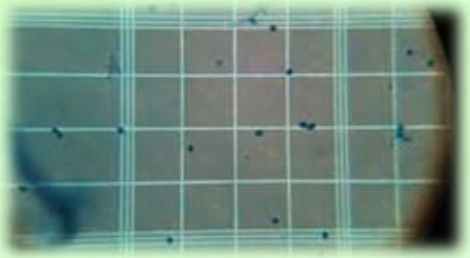
3 x  QCG

Blood Extraction and Basline Platelet Counting

Cyclophosphamide Treatment

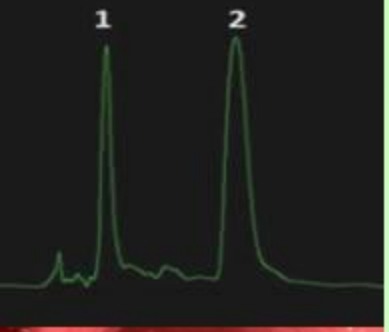
Treatment with Taua-taua Extract (for LGB/QCG) or Solvent (for control)

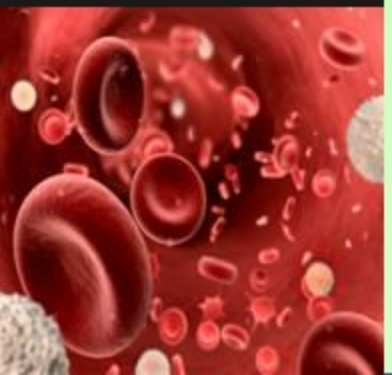
Saphenous Vein Blood Extraction



Platelet Counting through Hemocytometry

Termination





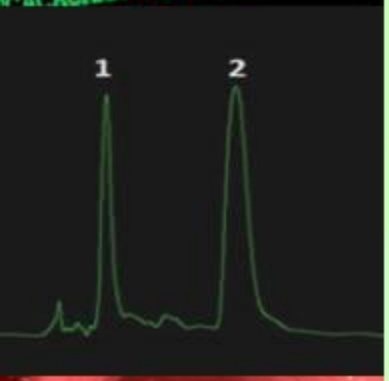
Conclusions

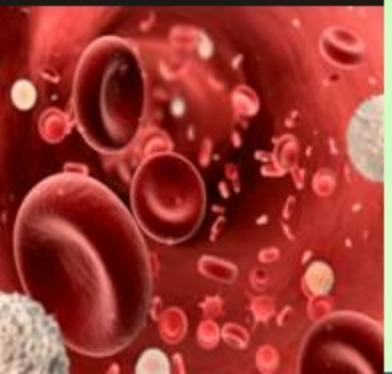
- **There are two clades of putative taua-taua in a typical field collection area where these plants grow: the *E. hirta* clade and the *E. lasiocarpa/E. prostrata* clade. A morphological characterization scheme was determined and validated by rbcl sequence analysis; thus, would help in correct field identification of taua-taua.**

- **The HPLC analysis of the phytochemical signatures of these plants revealed that taua-taua samples of the same species do not necessarily produce the same relevant compounds. Commercial products of taua-taua can be mislabled or adulterated with non- E.hirta species.**

- **The anti-thrombocytopenic activity of taua-taua extracts could vary in terms of pattern with corresponding effects on survival. QCG proved to be better than LBG with more stable anti-trombocytophenic activity.**

- **Prolonged intake of taua-taua more than 6 days should be discouraged as it could a have toxic principle that is responsible for the mice mortalities in the in-vivo animal**





Recommendations

- **Caution must be taken when promoting the health benefits of taua-taua in that not all taua-taua produce the same compounds, even though they appear to be morphologically similar.**

- **Further studies must be done to confirm the toxicity of the extract.**

- **Antiviral activities of the extract may also be explored to elucidate claims on its therapeutic effects.**

Thank you!