DECLARATION

I declare that the thesis entitled '**Screening and characterization of extracts from few sub-Himalayan plants having mosquitocidal properties against different developmental stages of *Aedes* sp. and *Culex* sp.**' has been presented by me under the guidance of **Dr. Dhiraj Saha**, Professor, Department of Zoology, University of North Bengal. No part of this thesis has formed the basis for the award of any degree or fellowship previously.

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UNIVERSITY OF NORTH BENGAL

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Certificate

I certify that Abhisekh Subba, M.Sc. has prepared the thesis entitled "**Screening and characterization of extracts from few sub-Himalayan plants having mosquitocidal properties against different developmental stages of *Aedes* sp. and *Culex sp.***" For the award of Ph.D. degree of the University of North Bengal under my guidance

He has carried out the work in the Department of Zoology, University of North Bengal

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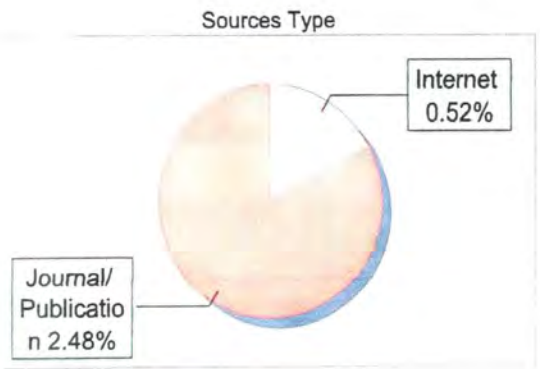

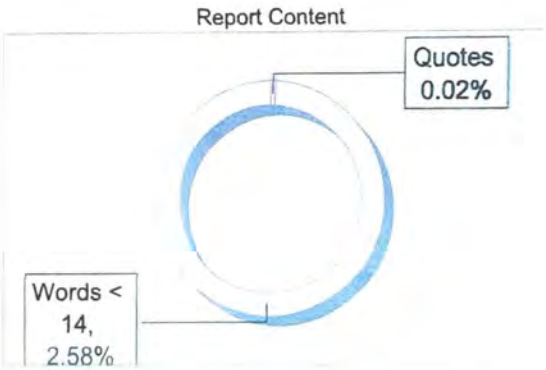

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Abhishek Sutra
20/8/24

PREFACE

Mosquito borne diseases are one of the major public health issues in the world. More than half of the world population are in the risk of contracting one or the other form of mosquito borne diseases. One reason for this is due to the vector competence of mosquitoes and their higher adaptability in different kind of climatic conditions. Moreover, in an effort of eradicating these diseases several methods have been applied. Out of which chemical control of vector through synthetic insecticide is by far the most effective and cheap. However, unscientific use of these compounds has a cost of its own. Environmental pollution, toxicity to non-target organisms and the most important is the development of resistance in mosquito vectors. Insecticide resistance is a phenomenon which is manifested by the anthropogenic interference.

In light of these challenges, many researchers have dedicated their life's research in combating insecticidal resistance in mosquitoes. Use of phytochemicals is one of the oldest practice which had lost its lustre after the advent of fast acting synthetic insecticides. Plants provides a complex of phytochemiclas that will not only serve as botanical insecticide but by virtue of their chemical nature and origin they pose negligible amount of harm to the environment. Besides, plant biosynthesize several analogous compounds which will aid in combating resistance through structural differences. Therefore, my work entitled as **'SCREENING AND CHARACTERIZATION OF EXTRACTS FROM FEW SUB-HIMALAYAN PLANTS HAVING MOSQUITOCIDAL PROPERTIES AGAINST DIFFERENT DEVELOPMENTAL STAGES OF AEDES SP AND CULEX SP**.' is dedicated in finding the plants that are effective in controlling mosquito vectors mainly, *Aedes sp.* and *Culex sp.*

The sub-Himalayan region of West Bengal basically comprises of Darjeeling and Kalimpong districts. These areas are rich in floral diversity which are the main source of raw materials for many local traditional practitioners. Therefore, plants with medicinal value from

this area were screened for their mosquitocidal activity. About 27 different medicinally important plants were collected. Plant parts were macerated using organic solvents and the extracts obtained were tested on different life-stages of mosquito. To understand the chemical nature of the compounds present in all the extracts GC-MS was used as a preliminarily step for chemical characterization. Then, the active extract was column chromatographed and the bioactive compounds were isolated via bioassay-guided fractionation. Finally, analytical tools such as ^{1}H-NMR, ^{13}C-NMR and HRMS were used to characterize the isolated bioactive compound.

Therefore, this study comprises screening of different plants from the sub-Himalayan region of West Bengal against *Culex sp* and *Aedes sp.* Isolation of the bioactive compounds and their chemical characterization. The findings of this study is totally laboratory based and the toxicity level of the extracts as well as the isolated compound is most likely to vary if it is tested in the field trials. However, our result suggests that the bioactivity of the extract and the compound is highly potent against mosquitoes we recommend semi-field and field trials of these compounds before its introduction as a full-fledged botanical insecticides.

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Abbreviations:

ASE: Accelerated Solvent Extraction.

UAE: Ultrasound Assisted Extraction.

^{13}C NMR: ^{13}C Nuclear magnetic Resonance

^{1}H NMR: Proton Nuclear magnetic resonance

DDT: Dichloro diphenyl trichloroethane

DMSCB: cis-1,2-Bis-[(E)-3,4-dimethoxystyryl]cyclobutane

ER: Effective Repellency

GCMS: Gas Chromatography-Mass Spectrometry

HITSS: High-Throughput Screening System

HPLC: High Performance Liquid Chromatography

HRMS-ToF: High Resolution Mass Spectrometry-Time of Flight

KDT: Knock Down Time

KDT_{50}: Knock Down time causing 50% mortality

LC_{50}: Lethal Concentration causing 50% mortality

LC_{90}: Lethal Concentration causing 90% mortality

LC-HRMS: Liquid Chromatography- High Resolution Mass Spectrometry

MAE: Microwave Assisted Extraction

NCVBDC: National Centre for Vector-Borne Disease Control

OAI: Oviposition Activity Index

Ppm: parts per million

PSF: Predator Safety Factor

SAI: Spatial Activity Index

SFE: Supercritical Fluid Extraction.

SI: Suitability Index

TLC: Thin Layer Chromatography

USEPA: United States Environmental Protection Agency

WHO: World Health Organization