

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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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

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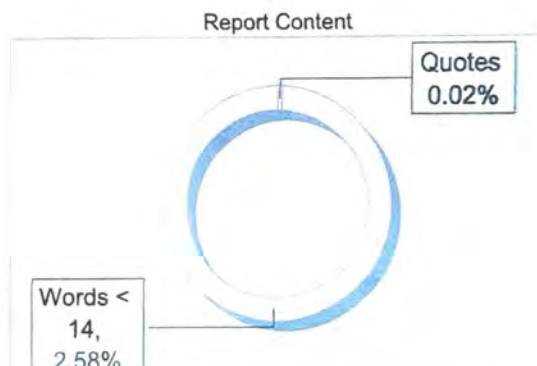
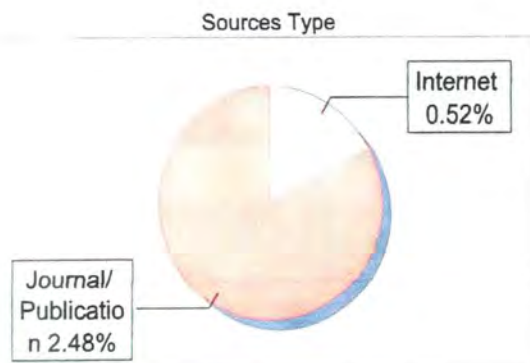
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PREFACE

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 phytochemicals 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 preliminary 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 ^1H -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.

List of Figures

Fig. 1: *Heracleum nepalense* plant.

Fig 2: *Zingiber montanum* plant.

Fig. 3: Isoprene unit.

Fig 4: Processing of dry sample of *Heracleum nepalense*. **A.** *Heracleum nepalense* flower, **B.** *Heracleum nepalense* fruits freshly harvested (on top) and shed dried (at the bottom).

Fig. 5: Processing of *Zingiber montanum* plant. **A.** live plant (September, 2020) **B.** Dried plant with intact rhizome **C.** Harvested rhizome. **D.** chopped and dried rhizome.

Fig. 6: Herbarium submitted in the Botanical Survey of India: **a.** *Artemisia vulgaris*, **b.** *Zingiber montanum*, **c.** *Zanthoxylum oxyphyllum* and **d.** *Heracleum nepalense*

Fig. 7: Larvicidal activity of *H. nepalense* fruit extracts after **A.** 24hrs, **B.** 48hrs and **C.** 72hrs of exposure. **D.** Larvicidal bioassay with dead *Ae. albopictus* larvae.

Fig. 8: Larval bioassay; **A, B** and **C** Larval mortality of *Cx. quinquefasciatus* after 24 48 and 72hrs of exposure on diethyl ether extract of *H. nepalense* fruit extract, respectively ($p < 0.05$). **D** larval mortality of *Cx. quinquefasciatus* after 24 hrs of exposure on different concentration of temephos ($p < 0.05$). **E.** Live *Cx. quinquefasciatus* larvae **F** *Cx. quinquefasciatus* larval death after exposure to diethyl ether extract.

Fig. 9: **A** Pupicidal activity of diethyl ether extract of *Heracleum nepalense* fruits against *Culex quinquefasciatus* after 24hrs exposure. **B** *Cx. quinquefasciatus* pupal death after exposure to diethyl ether extract.

Fig. 10: **A.** Toxicity assay on non-target organisms. **B** larval mortality of *T. splendens* after 24hrs of exposure on diethyl ether extract of *H. nepalense* ($p < 0.05$).

Fig. 11: GC-MS analysis of diethyl ether extract of *H. nepalense*

Fig. 12: GC-MS analysis of methanol extract of *H. nepalense*.

Fig. 13: Isolation of bioactive ingredient through column chromatography.

Fig. 14: GC-MS analysis of Fraction (21-25). **I** Bergapten, **II** Isopimpinellin, **III** Heraclenin, **IV** Byakangelicol.

Fig. 15: Mass spectra of Isolated compound (bergapten).

Fig. 16: ^1H NMR of active compound isolated from the fruit of *Heracleum nepalense*.

Fig. 17: ^{13}C NMR of active compound isolated from the fruit of *Heracleum nepalense*.

Fig. 18 **A-** Female mosquitoes feed on blood meal. **B-** Blood-fed mosquitoes inside netted cage. Oviposition deterrent activity of hexane extract of *Zingiber montanum* against **C** and **E-** *Culex quinquefasciatus* and **D** and **F-** *Aedes albopictus*. Cn=Control; T=Temephos(1ppm.). all concentrations are in mg/L

Fig 19: Ovicidal activity of n-hexane extract of *Zingiber montanum* rhizome **A-** Untreated, **B-** and **C-** Treated eggs of *Aedes albopictus*. **D-** Untreated, **E-** and **F-** Treated egg rafts of *Cx. quinquefasciatus*. Ovicidal activity of *Z. montanum* against **G-** *Ae. albopictus* and **H-** *Cx. quinquefasciatus*.

Fig. 20: Larvicidal bioassay against *Aedes albopictus* larvae after **A-**24hrs, **B-**48hrs and **C-**72hrs. **D-** Control group and **E-**treated group (n-hexane at 100ppm)

Fig. 21: Larvicidal bioassay of *Z. montanum* extract against *Cx. quinquefasciatus* after **A-**24hrs, **B-**48hrs and **C-**72hrs. **D-** Control group. **E-***Culex quinquefasciatus* larvae after treatment in 100ppm of n-hexane extract of *Zingiber montanum* rhizome.

Fig. 22: Pupicidal activity of *Z. montanum* rhizome extracts, against **A.** *Ae. albopictus* and **B.** *Cx. quinquefasciatus* pupae.

Fig. 23: Contact irritant activity of *Z. montanum* rhizome extract against *Cx. quinquefasciatus*.

Fig. 24: Spatial Repellency bioassay

Fig.25: **A.** Adulticidal Bioassay; **B.** Knockdown

Fig. 26: **A.** Larvicidal activity of n-hexane extract of *Zingiber montanum* rhizome against *Toxorhynchites splendens* after 24 hours of exposure, **B.** *Tx. splendens* larvae.

Fig. 27: GC-MS analysis of n-hexane extract of *Zingiber montanum* rhizome.

Fig. 28: GC-MS analysis of diethyl ether extract of *Zingiber montanum* rhizome extract

Fig. 29: GC-MS analysis of acetone extract of *Zingiber montanum* rhizome.

Fig. 30: GC-MS analysis of methanol extract of *Zingiber montanum* rhizome.

Fig. 31: Larvicidal activity of DMSCB isolated from *Zingiber montanum* rhizome. **A.** and **B.** Treated larvae; **C.** and **D.** Mortality percentage of treated mosquito larvae.

Fig. 32: Mass-spectra of bioactive phenylbutanoid isolated from *Z. montanum* rhizome in LC-HRMS Q-Tof Micro.

Fig. 33: **A.** ^1H NMR Spectra **B.** ^{13}C NMR Spectra **C.** Structure of Bioactive phenylbutanoid, cis-1,2-Bis-[(E)-3,4-dimethoxystyryl]cyclobutane, isolated from the rhizome of *Z. montanum*.

List of tables:

Table 1: Oviposition deterrent activity of plants against mosquitoes

Table 2: Ovicidal larvicidal, pupicidal and adulticidal activities of phytochemicals against different species of medically important mosquitoes

Table 3: Irritant and repellent activity of plants against mosquitoes

Table 4: Larvicidal activity of *Heracleum nepalense* fruit extract against *Aedes albopictus*

Table 5: Larvicidal activity of *Heracleum nepalense* fruit extract against *Culex quinquefasciatus*. (World Health Organisation (WHO) guidelines 2005)

Table 6: Pupicidal activity of *Heracleum nepalense* fruit extract against *Aedes albopictus*.

Table 7: Pupicidal activity of *Heracleum nepalense* activity against *Culex quinquefasciatus*.

Table 8: Larvicidal activity of Diethyl ether extract of *Heracleum nepalense* against *Toxorhynchites sp*

Table 9: Phytochemical Screening of different solvent extracts of *H. nepalense* through biochemical tests

Table 10: Oviposition deterrent activity of *Zingiber montanum* against *Aedes albopictus*

Table 11: Oviposition deterrent activity of *Zingiber montanum* against *Culex quinquefasciatus*

Table 12: Ovicidal activity of *Zingiber montanum* against *Aedes albopictus*

Table 13: Ovicidal activity of *Zingiber montanum* against *Culex quinquefasciatus*

Table 14: Larvicidal activity of extracts of *Zingiber montanum* against *Aedes albopictus*.

Table 15: Larvicidal activity of *Zingiber montanum* rhizome extract against *Culex quinquefasciatus*.

Table 16: Pupicidal activity of *Zingiber montanum* rhizome extracts against *Aedes albopictus*.

Table 17: Pupicidal activity of *Zingiber montanum* rhizome extracts against *Culex quinquefasciatus*.

Table 18: Spatial repellency of n-hexane *Zingiber montanum* rhizome extract against *Aedes albopictus*.

Table 19: Spatial repellency of n-hexane *Zingiber montanum* rhizome extract against *Culex quinquefasciatus*.

Table 20: Non target toxicity bioassay of n-hexane extract of *Zingiber montanum* rhizome against *Toxorhynchites splendens* after 24 hours of exposure.

Table 21: Phytochemical screening of all the extracts of *Zingiber montanum* rhizome through biochemical test:

Table 22: LC₅₀, LC₉₀, LC₉₉ values of fraction 28 isolated from *Z. montanum* against *Ae. albopictus* larvae.

Table 23: LC₅₀, LC₉₀, LC₉₉ values of fraction 28 isolated from *Z. montanum* against *Culex quinquefasciatus*.

Abbreviations:

ASE: Accelerated Solvent Extraction.

UAE: Ultrasound Assisted Extraction.

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

^1H 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

KDT₅₀: Knock Down time causing 50% mortality

LC₅₀: Lethal Concentration causing 50% mortality

LC₉₀: 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